Association between vitamin D status and diabetes mellitus in a multiethnic adult population

Submitted.

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**Background:** Vitamin D deficiency is associated with a higher prevalence of diabetes mellitus type 2 (T2DM). Both vitamin D deficiency and T2DM are highly prevalent within non-western immigrant populations in the Netherlands. This raises the question of whether a causal association exists between vitamin D deficiency and diabetes in these groups.

**Objective:** Our aim was to assess the association between vitamin D status and diabetes within a multiethnic population in the Netherlands (52°N).

**Design:** A cross-sectional study of a random sample stratified according to gender and ethnicity; 487 adults (aged 18-65 years) from ten general practices participated. Diabetes mellitus was defined as self-reported diabetes mellitus, or fasting plasma glucose ≥7.0 mmol/l. Serum 25-hydroxyvitamin D (25(OH)D) was categorized into deficiency (serum 25(OH)D <25 nmol/l), insufficiency (serum 25(OH)D ≥25; <50 nmol/l) and sufficiency (serum 25(OH)D ≥50 nmol/l).

**Results:** Vitamin D deficiency (34%), insufficiency (40%) and diabetes (12%) were highly prevalent. After adjustment for age, gender, BMI, season and ethnic group, diabetes was positively, but not significantly, associated with vitamin D deficiency (Odds Ratio =1.70; 95% Confidence Interval 0.60 – 4.81) and vitamin D insufficiency (OR =2.13; 95% CI 0.79 – 5.77).

**Conclusions:** There appears to be an association between low serum 25(OH)D concentrations and diabetes in this adult multiethnic population. Longitudinal and intervention studies should be performed to assess the effect of vitamin D status on the incidence of diabetes within various ethnic adult populations.
Introduction

Both vitamin D deficiency and diabetes mellitus type 2 are highly prevalent within non-western immigrant populations. The high prevalence of vitamin D deficiency within these groups might be due to a diet low in vitamin D and calcium, darker skin, insufficient exposure to direct sunlight or covering of the skin. The high prevalence of diabetes in these populations might be due to environmental factors, high body mass index, genetic predisposition, or an insufficient intrauterine situation leading to increased risk when growing up in a wealthy environment (the fetal origins-hypothesis).

Vitamin D is hydroxylated into 25-hydroxyvitamin D (25(OH)D) in the liver. Serum 25(OH)D is the major circulatory form (storage form) of vitamin D in the body. Vitamin D status is assessed by measurement of serum 25(OH)D concentration. The active form of vitamin D results from a second hydroxylation into 1,25-dihydroxyvitamin D (1,25(OH)₂D) in the kidneys. Vitamin D deficiency may lead to secondary hyperparathyroidism, impaired bone mineralization, rickets, osteomalacia, myopathy (muscle weakness) and impaired physical functioning. Additionally, vitamin D deficiency has been associated with a higher prevalence of diabetes mellitus type 1 and type 2. As such, the high prevalence of diabetes in non-western groups might, in part, be the result of vitamin D deficiency. Increasing serum 25-hydroxyvitamin D (25(OH)D) concentration might therefore be appropriate to prevent diabetes mellitus.

Several mechanisms might be responsible for an association between vitamin D and diabetes mellitus type 2: vitamin D deficiency impairs insulin synthesis and secretion, 1,25-dihydroxyvitamin D stimulates the insulin response, and 25-hydroxyvitamin D is positively correlated with insulin sensitivity. However, most of these studies have been performed among western populations; those among non-western populations were performed in New Zealand and the United States, among ethnic groups other than those that immigrated to the Netherlands. We performed a study to assess the association between serum 25-hydroxyvitamin D (25(OH)D) and diabetes in an adult multiethnic population in the Netherlands.

Subjects and methods

A cross-sectional study was performed among multiple ethnic groups in the Netherlands (latitude 52°N). A random sample of 2,397 individuals (aged 18-65 years) was drawn from patient files of ten general practices in four large cities (Amersfoort, Amsterdam, Haarlem and The Hague). Almost all residents of the Netherlands are registered in a general practitioner’s patient file, making this a suitable means of drawing a representative population sample.
The sample was stratified according to gender and ethnicity: indigenous Dutch, Turkish, Moroccan, Surinamese Creole, Surinamese South Asian and sub-Saharan African (each category of equal size). Subjects who had received vitamin D treatment within six months (oral) or one year (injection) of the study starting, or who were unable to participate due to their mental condition, were excluded. Women who reported being pregnant were excluded from the analysis.

The subjects were invited to participate in the study through a letter from their general practitioner written in Dutch and – for non-Dutch ethnic groups – also in Turkish, English or French. Several actions were taken to increase response rates: a reminder by telephone (or by mail when the telephone number was not known); a 15 euro remuneration for participants who completed all measurements; a press release to local newspapers; posters in the waiting rooms of participating general practices; and a personal recommendation by the general practitioners to individuals in the sample who came for consultation for other reasons.

The study protocol was approved by the Medical Ethics Committee of the Haaglanden Medical Centre (Medisch Centrum Haaglanden) and all participants gave written informed consent.

Data collection

Data collection took place between September 2003 and June 2005; each participant was measured once within this period. The sample was divided evenly over the four seasons. Participants were asked to complete a questionnaire relating to general characteristics, exposure to sunlight and dietary habits. To calculate body mass index (BMI, kg/m²), height and weight were measured in the general practice. Blood samples – fasting if obtained in the morning – were taken, and plasma glucose analyzed using the glucose hexokinase method. A part of the blood sample was centrifuged, and the serum kept frozen at -20°C until used for further analysis. Serum 25-hydroxyvitamin D (serum 25(OH)D) was measured in duplicate by radioimmunoassay (Diasorin, Stillwater, MN, USA). The inter-assay coefficient of variation was 15% at 30 nmol/l and 10% at 60 nmol/l.

In selecting the sample, ethnicity was based on the general practitioner’s judgment as each individual’s country of birth – and those of his/her parents – were not known. For the analysis, however, ethnic group was assigned according to the participant’s country of birth and those of both parents as provided in the questionnaire. Only if both parents were born in the Netherlands was the participant considered to be indigenous Dutch. Some participants (9%) appeared to be from ethnic groups other than the six selected for the study. Ethnic groups were combined according to skin type: Western (e.g. indigenous Dutch); Turkish and North African (e.g. Moroccan); Asian (e.g. Surinamese South Asian); and Black (e.g. Surinamese Creole and sub-Saharan African). The Surinamese South Asian population originated in India and had been living in Suriname for three or four generations prior to immigrating to the Netherlands.
Vitamin D deficiency was defined as serum 25(OH)D of less than 25 nmol/l, vitamin D insufficiency as serum 25(OH)D greater than or equal to 25 and less than 50 nmol/l, and vitamin D sufficiency as serum 25(OH)D greater than or equal to 50 nmol/l. Higher thresholds are sometimes recommended, but these are mainly based on studies performed among the elderly, and the thresholds might be age-dependent.

The questionnaire included two questions about diabetes mellitus: the first whether the participant had diabetes mellitus; the second whether they used medication for their diabetes. If both questions were answered positively, we assigned them as having diabetes mellitus. In another study among a Dutch multiethnic population, the agreement between self-reported diabetes and data from medical records was observed to be substantial to almost perfect. Because a part of the population would have undiagnosed diabetes, which cannot be assessed using a questionnaire, we also defined all participants with fasting glucose ≥7.0 mmol/l as having diabetes, in accordance with the criteria for epidemiological studies of the World Health Organisation.

**Statistical analysis**

Associations of gender, age category and ethnic group with vitamin D deficiency, vitamin D insufficiency and diabetes were assessed using univariate logistic regression. The association between vitamin D status and diabetes was assessed using univariate and multivariate logistic regression, with adjustments for age, gender, BMI, season and ethnic group. The adjustment for season was necessary because diabetes is not variable and a mean serum 25(OH)D concentration over the year would be more suitable. Vitamin D was included as a categorical variable (vitamin D deficiency, vitamin D insufficiency and vitamin D sufficiency) because the association between serum 25(OH)D and diabetes is non-linear. We tested for effect modification using interaction terms for gender, age and ethnic group, but none of these were significant.

The association between vitamin D and fasting glucose was tested among non-diabetic participants (n=430) using linear regression with fasting glucose as outcome variable and the three categories of serum 25(OH)D as independent variable; with adjustment for age, gender, ethnic group and BMI. Gender, age and ethnic group did not modify the effect. SPSS version 15.0 was used for all analyses.
Results

Of the 2,397 people invited, 677 (28%) participated. 1,027 non-participants did not respond and proved uncontactable; some non-respondents were not known at the address held in the general practice files (ghost patients) and therefore could not be considered to be part of the initial sample. As such, the actual participation rate is somewhere between 28% (677/2,397) and 49% (677/(2,397-1,027)). Furthermore, we excluded data from 190 participants: 170 because relevant data was missing (serum 25(OH)D in 58 cases, fasting glucose in 109, ethnic group in 3), 17 women were pregnant and three subjects appeared to be outside the age range of 18-65 years. Thus, data from 487 respondents (182 men and 305 women) were available for analysis.

Gender, age and ethnic group distribution of the participants is presented in Table 1. The mean age was 41. Overweight (BMI 25-30 kg/m²) and obesity (BMI ≥30 kg/m²) were highly prevalent: 38% and 25% respectively. Only 8 participants (2%) had been living in the Netherlands for less than two years (data not shown). Prevalence of vitamin D deficiency, vitamin D insufficiency and diabetes according to gender, age category and ethnic group is also presented in Table 1. The median serum 25(OH)D concentration was 32 nmol/l; the lowest quintile was <20 nmol/l and the highest >57 nmol/l. Thirty-eight participants (8%) had diabetes according to the questionnaire; we classified an additional 19 participants as having diabetes based on a fasting glucose concentration ≥7.0 mmol/l, for a total of 57 diabetic participants (12%). Diabetes was most prevalent in the oldest age category (50-65 years) and among the non-western ethnic groups. Vitamin D deficiency and insufficiency were more prevalent in the non-western ethnic groups than in the western group. The majority of the western group was vitamin D sufficient (78%), whereas a minority of the Turkish/North African (18%), Asian (9%) and Black (16%) groups were vitamin D sufficient. Vitamin D sufficiency was more prevalent among the oldest age group (35%) than among the younger groups (24%).

Prevalence of diabetes was lowest (6%) among the participants with sufficient serum 25(OH)D concentrations (Table 2). For vitamin D deficient and vitamin D insufficient participants, the prevalence of diabetes was similar (13% and 14% respectively). Vitamin D deficiency and insufficiency were significantly associated with a higher prevalence of diabetes in the unadjusted analysis, as well as in the age, gender, BMI and season-adjusted analyses. Adjusting for ethnic group still showed a higher prevalence of diabetes in the vitamin D deficient (OR=1.70, 95% CI 0.60 – 4.81) and vitamin D insufficient groups (OR=2.13, 95% CI 0.79 – 5.77), but these associations were no longer significant.
Table 1. Serum 25(OH)D status and diabetes according to gender, age and ethnic group of the multiethnic study population (18-65 years)

<table>
<thead>
<tr>
<th></th>
<th>Vitamin D deficiency</th>
<th>Vitamin D insufficiency</th>
<th>Vitamin D sufficiency</th>
<th>Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% (n)</td>
<td>% (n)</td>
<td>% (n)</td>
<td>% (n)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men (reference group)</td>
<td>182</td>
<td>30 (54)</td>
<td>44 (80)</td>
<td>26 (48)</td>
</tr>
<tr>
<td>Women</td>
<td>305</td>
<td>36 (110)</td>
<td>37 (113)</td>
<td>27 (82)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-34 years (reference group)</td>
<td>158</td>
<td>37 (58)</td>
<td>39 (62)</td>
<td>24 (38)</td>
</tr>
<tr>
<td>35-49 years</td>
<td>212</td>
<td>34 (72)</td>
<td>42 (89)</td>
<td>24 (51)</td>
</tr>
<tr>
<td>50-65 years</td>
<td>117</td>
<td>29 (34)</td>
<td>36 (42)</td>
<td>35 (41)*</td>
</tr>
<tr>
<td>Ethnic group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Western (reference group)</td>
<td>87</td>
<td>6 (5)</td>
<td>16 (14)</td>
<td>78 (68)</td>
</tr>
<tr>
<td>Turkish/North African</td>
<td>186</td>
<td>36 (67)***</td>
<td>46 (85)***</td>
<td>18 (34)***</td>
</tr>
<tr>
<td>Asian</td>
<td>92</td>
<td>51 (47)***</td>
<td>40 (37)**</td>
<td>9 (8)***</td>
</tr>
<tr>
<td>Black</td>
<td>122</td>
<td>37 (45)***</td>
<td>47 (57)***</td>
<td>16 (20)***</td>
</tr>
<tr>
<td>Total</td>
<td>487</td>
<td>34 (164)</td>
<td>40 (193)</td>
<td>27 (130)</td>
</tr>
</tbody>
</table>

1 serum 25(OH)D < 25 nmol/l
2 serum 25(OH)D ≥ 25 nmol/l and < 50 nmol/l
3 serum 25(OH)D ≥ 50 nmol/l
4 (self-reported diabetes and self-reported use of medication for diabetes) and/or fasting glucose ≥ 7 mmol/l
5 Western: Dutch and other; Turkish/North African: Turkish, Moroccan and other; Asian: Surinamese South Asian and other; Black: Surinamese Creole, sub-Sahara African and other

* p < 0.050
** p < 0.010
*** p < 0.001

Table 2. Odds ratios for the association between serum 25-hydroxyvitamin D (nmol/l) and diabetes, in a multiethnic population (18-65 years) in the Netherlands.

<table>
<thead>
<tr>
<th>25(OH)D</th>
<th>N</th>
<th>Diabetes % (n)</th>
<th>Crude OR (95% CI)</th>
<th>OR (95% CI) 1</th>
<th>OR (95% CI) 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 25 nmol/l</td>
<td>164</td>
<td>13 (22)%</td>
<td>2.36 (1.02 - 5.50)</td>
<td>3.38 (1.32 - 8.69)</td>
<td>1.70 (0.60 - 4.81)</td>
</tr>
<tr>
<td>25-49 nmol/l</td>
<td>193</td>
<td>14 (27)%</td>
<td>2.48 (1.09 - 5.65)</td>
<td>3.59 (1.44 - 8.98)</td>
<td>2.13 (0.79 - 5.77)</td>
</tr>
<tr>
<td>≥ 50 nmol/l  (reference group)</td>
<td>130</td>
<td>6 (8)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

1 due to a non-linear association in the analysis we categorised serum 25(OH)D according to the thresholds for deficiency and insufficiency by Lips; 2 (self-reported diabetes and self-reported use of medication for diabetes) and/or fasting glucose ≥ 7 mmol/l 3 adjusted for age, gender, BMI and season 4 adjusted for age, gender, BMI, season and ethnic group

* p < 0.050
The mean fasting glucose concentrations among non-diabetic participants was similar in the three serum 25(OH)D categories, both with and without adjustment for age, gender, BMI and ethnic group (Table 3).

Table 3. Association between serum 25-hydroxyvitamin D (nmol/l) \(^1\) and fasting glucose in a non-diabetic multiethnic population (18-65 years) in the Netherlands.

<table>
<thead>
<tr>
<th>25(OH)D N</th>
<th>Mean fasting glucose concentration mmol/l (95% CI)</th>
<th>Adjusted (^2) mean fasting glucose concentration mmol/l (95% CI)</th>
<th>Adjusted (^3) mean fasting glucose concentration mmol/l (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 25 nmol/l</td>
<td>142 5.1 (5.0 – 5.2)</td>
<td>5.2 (5.1 – 5.3)</td>
<td>5.2 (5.1 – 5.3)</td>
</tr>
<tr>
<td>25-49 nmol/l</td>
<td>166 5.1 (5.0 – 5.2)</td>
<td>5.2 (5.1 – 5.3)</td>
<td>5.2 (5.1 – 5.3)</td>
</tr>
<tr>
<td>≥ 50 nmol/l</td>
<td>122 5.1 (5.0 – 5.2)</td>
<td>5.2 (5.1 – 5.3)</td>
<td>5.1 (5.0 – 5.2)</td>
</tr>
</tbody>
</table>

\(^1\) due to a non-linear association in the analysis we categorised serum 25(OH)D according to the thresholds for deficiency and insufficiency by Lips\(^15\)

\(^2\) adjusted for age, gender and BMI

\(^3\) adjusted for age, gender, BMI and ethnic group

Discussion

In this relatively young (mean age 41) multiethnic population, diabetes (12%) and vitamin D deficiency (34%) were both highly prevalent, especially in the non-western groups. Vitamin D deficiency (serum 25(OH)D <25 nmol/l) and vitamin D insufficiency (serum 25(OH)D ≥25; <50 nmol/l) were associated with a higher prevalence of diabetes mellitus as compared with a sufficient vitamin D status (serum 25(OH)D ≥50 nmol/l). However, these associations, adjusted for age, gender, BMI, season and ethnic group, were not significant. Among those with vitamin D deficiency or insufficiency, the prevalence of diabetes was similar, though the adjusted associations tended to be stronger for the insufficient groups. Fasting plasma glucose among non-diabetics was not associated with serum vitamin D status.

In other, larger, studies, the associations between serum 25(OH)D and diabetes have been similar to our results, i.e. a higher prevalence of diabetes among individuals with lower serum 25(OH)D concentrations, although the associations are not always significant.\(^27\)\(^33\)\(^35\) The lack of significance in our study might indicate there is no association between vitamin D and diabetes in a multiethnic adult population. However, the magnitude of the association (Odds Ratios of 1.70 and 2.13) suggests an association. There are several possible explanations for the lack of significance.

First, our study was cross-sectional while diabetes is a chronic disease; it would have been better to relate diabetes to vitamin D status over preceding years.

Second, there were large ethnic differences in vitamin D status: the majority of the western group were vitamin D sufficient (78%), but only a minority of the non-western populations
were vitamin D sufficient (9%-18%). Therefore, comparing subjects with high and low serum 25(OH)D concentrations largely coincides with comparing western and non-western subjects. A favourable effect of vitamin D can probably only be assessed when the vitamin D status within each ethnic group varies sufficiently.

Third, the vitamin D-status of the majority of our total population was poor, only 27% having a serum 25(OH)D concentration ≥ 50 nmol/l. It is possible that the threshold to find a significant association between serum 25(OH)D and diabetes should be higher than 50 nmol/l. For instance, in a randomized clinical trial among 81 South Asian women with insulin resistance and vitamin D deficiency in New Zealand, optimal serum 25(OH)D concentrations for reducing insulin resistance were shown to be 80-119 nmol/l. In addition, a significantly lower prevalence of hyperglycemia among 8,421 US adults was found comparing serum 25(OH)D >96.4 nmol/l to the reference category of ≤48.4 nmol/l, but not when comparing lower concentrations of serum 25(OH)D to the reference category. However, a significant association between low serum 25(OH)D and diabetes among non-Hispanic whites and Mexican Americans was found in the comparison of serum 25(OH)D ≥44 nmol/l with <44 nmol/l. A significant association between low serum 25(OH)D and diabetes among Finnish men was found at a threshold of about 55 nmol/l in a pooled analysis of two cohort studies, but among women the association at this threshold was inverse and not significant. Thus, literature is inconclusive on the need for a higher threshold.

Fourth, the ethnic groups are heterogeneous. An indication for different effects of vitamin D in different groups has been observed for the vitamin D-endocrine system and bone health in American blacks. Scragg et al. performed stratified analyses according to ethnic group and observed different associations between serum 25(OH)D and diabetes among non-Hispanic blacks, non-Hispanic whites and Mexican Americans. The estimate for the association among non-Hispanic blacks was even inverse compared to the protective effect among the other ethnic groups. Unfortunately, our study was too small to permit a stratified analysis according to ethnic group.

We did not observe significant associations between serum 25(OH)D and fasting glucose in the non-diabetic population. However, Need et al. observed higher fasting glucose associated with lower serum 25(OH)D concentrations in postmenopausal women, with the highest fasting glucose concentrations at serum 25(OH)D concentrations below 40 nmol/l. Using the threshold of 40 nmol/l in our study did not result in a significant association, even when a selection of the oldest half of the population (>40 years) was used (data not shown).

This study was performed in a population in which, to our knowledge, the association between vitamin D and diabetes has not yet been reported. Because the present study was cross-sectional, we cannot draw conclusions about the causal direction of any relationship. The low participation rate (28-49%) could have introduced selection bias. However, we have no indications that a particular subgroup of possible vitamin D – diabetes combinations had a lower response rate compared to others, making a selection bias improbable.
We have no information about the type of diabetes mellitus, but assume that the majority of diabetes patients in our study population would have type 2 diabetes. This is because: 1) from participants with self-reported diabetes (questionnaire) about 90% will have type 2, as 90% of patients with diagnosed diabetes have diabetes type 2,41 and 2) of the participant added due to fasting glucose levels, almost all will have type 2 as we assume that almost every patient with type 1 diabetes will be diagnosed earlier in the course of the disease.

In this adult multiethnic population, vitamin D deficiency and insufficiency seemed to be associated with a higher prevalence of diabetes after adjustment for age, gender, BMI, season and ethnic group, although statistical significance is lacking. As the vitamin D status of the study population was poor, higher serum 25(OH)D concentrations might be required to find a preventive effect on diabetes. As these higher serum 25(OH)D concentrations are rare among non-western immigrant groups in the Netherlands, longitudinal intervention studies should be performed among these groups.

Acknowledgements

We are grateful to the participating general practitioners, their assistants and the interviewers, for their efforts in collecting the data. We also thank A. van Dijk for his statistical advice. The authors’ responsibilities were as follows – All authors: study design and reviewing the manuscript; IMM: data collection, data analysis, writing of manuscript. None of the authors had any financial or personal conflict of interest.
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