SHORT COMMUNICATION

Lower prediagnostic serum 25-hydroxyvitamin D concentration is associated with higher risk of insulin-requiring diabetes: a nested case–control study

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Abstract

Aims/hypothesis Low serum 25-hydroxyvitamin D [25 (OH)D] concentration may increase risk of insulin-requiring diabetes.

Methods A nested case–control study was performed using serum collected during 2002–2008 from military service members. One thousand subjects subsequently developed insulin-requiring diabetes. A healthy control was individually matched to each case on blood-draw date (±2 days), age (±3 months), length of service (±30 days) and sex. The median elapsed time between serum collection and first diagnosis of diabetes was 1 year (range 1 month to 10 years). Statistical analysis used matched pairs and conditional logistic regression.

Results ORs for insulin-requiring diabetes by quintile of serum 25(OH)D, from lowest to highest, were 3.5 (95% CI 2.0, 6.0), 2.5 (1.5, 4.2), 0.8 (0.4, 1.4), 1.1 (0.6, 2.8) and 1.0 (reference) (p_{trend} <0.001). The quintiles (based on fifths using serum 25(OH)D concentration in the controls) of serum 25(OH)D in nmol/l, were <43 (median 28), 43–59 (median 52), 60–77 (median 70), 78–99 (median 88) and ≥100 (median 128).

Conclusions/interpretation Individuals with lower serum 25(OH)D concentrations had higher risk of insulin-requiring diabetes than those with higher concentrations. A 3.5-fold lower risk was associated with a serum 25 (OH)D concentration ≥60 nmol/l.

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Introduction

There are approximately 40,000 new cases of type 1 diabetes in the USA annually, including an estimated 15,600 in children [1] and 24,400 in adults [2]. The overall annual incidence rate in the US population is approximately 13 per 100,000 [2], similar to the rate of 12 per 100,000 person-years in US military service members [3]. Annual age-standardised incidence rates of type 1 diabetes vary from a low in the tropics of 0.5 per 100,000 (in Venezuela, latitude 9° N) to a high of 60 per 100,000 in Finland (60° N) [4, 5].

An association has been proposed between vitamin D deficiency and higher risk of type 1 diabetes [5–7]. The EURO-DIAB case–control study reported that children who did not receive a vitamin D supplement during infancy had 1.5 times the risk of type 1 diabetes as those who received a vitamin D supplement [7]. That study had information only on the use of vitamin D supplements, not serum 25-hydroxyvitamin D

[25(OH)D] concentrations. Hypponen et al reported that children in a prospective cohort study who received <2,000 IU/day of vitamin D through vitamin D supplements had 4.5 times higher risk than those with intake ≥2,000 IU/day [6]. These studies depended on self-reported questionnaires, which may have limited their validity. Two ordinary case–control studies found that serum 25(OH)D was inversely associated with risk of insulin-requiring diabetes [8, 9], but a third study found no association [10].

Methods

Study population This was a nested case–control study of prediagnostic serum from military service members on active duty who were screened by a US Department of Defense serological surveillance programme. One-thousand consecutive new cases of insulin-requiring diabetes and 1,000 healthy controls were identified. Controls were individually matched to cases according to date that the blood sample was drawn (±2 days), age (±3 months), length of military service (±30 days), sex and whether the control was on active duty when the case was diagnosed. Serum samples were collected and frozen during 2002–2008. Cases were ascertained during 2002–2011. The 3-year-longer interval for case and control ascertainment allowed time to elapse between the date that blood was collected and date of occurrence of a case. The median interval between collection of the blood sample and diagnosis was 1 year and the interquartile range was also 1 year. The longest interval was 10 years and the shortest was <12 months. Procedures were in accordance with Department of Defense Directive 6490.2 and Instruction 6490.3 [11]. The samples were frozen in 0.5-ml portions and stored in the Department of Defense Serum Repository. All data were stored in the Defense Medical Surveillance System [11]. This was an exempt protocol of anonymised data, so informed consent was not required.

This study was conducted in accordance with the ethical standards of the relevant Department of Defense Institutional Review Board and the Helsinki Declaration of 1975, as revised in 1983, and approval was obtained from an Institutional Review Board on 5 November 2009.

Case ascertainment and definition Cases were ascertained from a database that included care in all Department of Defense hospitals and clinics and civilian facilities serving military service members worldwide. A case was defined as an active-duty military service member diagnosed with insulin-requiring diabetes (ICD 9-CM codes 250.0x–250.9x, where x=1 or 3; see www.icd9data.com/2007/Volume1/240-279/250-259/250/default.htm). The case definition required: (1) being hospitalised with a discharge diagnosis of insulin-requiring diabetes certified by a physician;

(2) having three or more clinic or outpatient medical care visits with a primary diagnosis by a physician of insulin-requiring diabetes during a 12-month period; or (3) having seven or more outpatient medical care visits with a primary diagnosis of insulin-requiring diabetes by a physician. The most recent serum sample preceding the date of diagnosis was obtained.

Laboratory procedures Blood samples were collected in plain tubes and allowed to clot. Serum was separated from cells by centrifugation at approximately 1,400 g for 10 min. Serum was divided into 0.5 ml volumes in polypropylene cryogenic vials, frozen at –70°C and stored in freezers equipped with temperature alarms. Serum was analysed by a 25(OH)D analysis laboratory (Heartland Laboratories, Ames, IA, USA), using a US Food and Drug Administration-approved direct, competitive chemiluminescence immunoassay, the DiaSorin LIAISON Assay (DiaSorin, Stillwater, MN, USA) [12]. The inter- and intra-assay coefficients of variation were 12.4% and 5.4%, respectively [12]. Measurements were validated according to the Vitamin D External Quality Assessment Scheme. The laboratory had no knowledge of case or control status of the samples.

Statistical analysis Matched-pairs ORs were calculated as the ratio of discordant pairs. Statistical significance was calculated using the McNemar test. Differences in means and medians were tested using the Mann–Whitney U test.

Quintiles of serum 25(OH)D were determined based on the distribution of 25(OH)D concentrations in the controls. Analyses were performed using SAS-PC, version 9.1 (SAS Institute, Cary, NC, USA). The criterion for statistical significance was $p \leq 0.05$, two-tailed.

Results

The risk of insulin-requiring diabetes was 3.5 times higher in individuals with the lowest 25(OH)D concentration compared with those with the highest (Fig. 1). ORs by quintile of serum 25(OH)D concentration, from lowest to highest, were 3.5 (95% CI 2.0, 6.0), 2.5 (1.5, 4.2), 0.8 (0.4, 1.4), 1.1 (0.6, 2.8) and 1.0 (reference), respectively (p_{trend} <0.001) (Fig. 1). The quintiles of serum 25(OH)D, in nmol/l, were <43 (median 28), 43–59 (median 52), 60–77 (median 70), 78–99 (median 88) and ≥100 (median 128). African-Americans were more likely to be cases than individuals of other races (Table 1). The mean serum 25(OH)D concentration was substantially lower in individuals who developed diabetes (62.2 nmol/l) than in healthy controls (72.5 nmol/l) (p<0.0001) (Table 2). The association was present within all three racial groups (Table 2).

After adjustment for race, the ORs for the lowest vs highest quintile of 25(OH)D, were 1.9 (95% CI 1.4, 2.7),
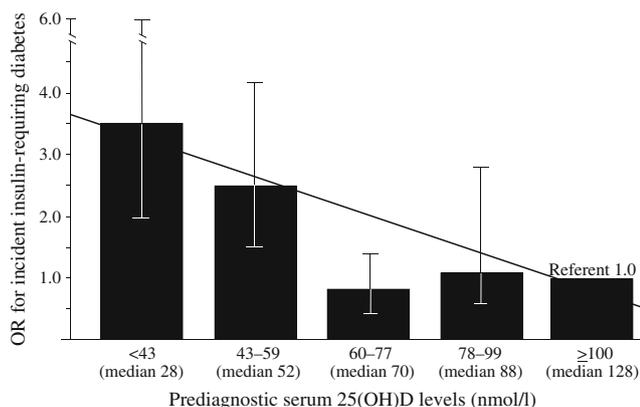
Fig. 1 ORs and 95% CIs for insulin-requiring diabetes by prediagnostic serum 25(OH)D concentration, active-duty military service members, USA, 2002–2011. Data were generated using McNemar matched-pairs analysis in which healthy controls were individually matched to cases by the date the blood sample was drawn (±2 days), age (±3 months), sex and length of military service (±30 days). Cases and controls in each pair were on active duty when the case was diagnosed. The number of discordant pairs, from lowest to highest quintile (except the reference quintile defined as having an OR of 1.0), were 52/15, 50/20, 33/26 and 34/32. The regression equation was: $y=-0.026x+3.6$; p for linear trend <0.001

1.7 (1.3, 2.3), 1.1 (0.8, 1.6), 1.2 (0.9, 1.6) and 1.0 (reference) ($p<0.0001$ for top vs bottom quintile). The OR of insulin-requiring diabetes for black compared with white subjects was 1.6 (1.2, 2.0) ($p<0.001$).

Discussion

Individuals with a low serum 25(OH)D concentration had a substantially higher risk of developing insulin-requiring

Table 1 Characteristics of the study population of 1,000 insulin-requiring diabetes cases and 1,000 matched controls, active-duty US military service members, 2002–2011

Characteristic	Cases		Controls	
	n	%	n	%
Race[a]				
White	561	56.1	669	66.9
Black	276	27.6	163	16.3
Other	163	16.3	168	16.8
Age in years[b]				
17–19	56	5.6	59	5.9
20–22	167	16.7	161	16.1
23–24	125	12.5	128	12.8
25–29	221	22.1	221	22.1
30–34	135	13.5	136	13.6
35+	296	29.6	295	29.5

[a] $\chi^2=38.6$, $p<0.0001$; [b] $\chi^2=0.23$ $p=0.99$

Table 2 Prediagnostic serum 25(OH)D concentrations in the study population of 1,000 insulin-requiring diabetes cases and 1,000 matched controls, active-duty US military service members, 2002–2011

Race	Serum 25(OH)D (nmol/l)		p value
	Cases	Controls	
White	62.2 (31.8)	72.5 (33.0)	≤0.0001
Black	41.8 (26.0)	46.5 (24.8)	≤0.01
Other	60.8 (28.5)	69.2 (33.2)	≤0.01
All races	62.2 (31.8)	72.5 (33.0)	≤0.0001

Results are presented as mean (SD)

diabetes than those with a higher concentration. This is consistent with two previous case–control studies of serum 25(OH)D [8, 9], but not a third [10]. An inverse association between serum 25(OH)D and risk of insulin-requiring diabetes was present in the same direction and was statistically significant in all race groups.

There was a substantial inverse association comparing the lowest quintile with the third quintile (Fig. 1). Based on the present study, it may be that no further reduction in risk would be expected once a serum 25(OH)D concentration of >60 nmol/l has been attained.

Limitations of this study include the possibility that some of the individuals with insulin-requiring diabetes may have had a disorder that some might regard as complicated or severe forms of type 2 diabetes [13]. Cases in the present study were included solely because they were dependent on insulin.

Only one serum sample was used, to control costs of the study. The findings could have been different if the study had analysed the earliest samples collected from each individual, rather than the most recent. However, the association might have been missed if the effect of vitamin D deficiency is short lived. Since insulin-requiring diabetes occurs even in young children [1], the time interval between exposure and diagnosis is probably rather short.

There may be an association between higher BMI and higher risk of type 1 diabetes [14, 15], but obesity is only a modest risk factor for type 1 diabetes, at least in children and adolescents [15]. BMI values of the individuals in this study were not provided in the available database. Therefore, caution should be exercised when interpreting the results, since the higher risk of insulin-requiring diabetes observed in individuals with low serum 25(OH)D could be partly due to their having a higher BMI. On the other hand, all subjects in this study were active-duty military personnel who were required to meet annual standards for body weight. The required BMI in the military for males is ≤26 kg/m^2 and for females it is ≤28 kg/m^2. These maxima are well below the usual criterion of ≥30 kg/m^2 for obesity.

The results of this study should be generalised to the US population only with caution. Patients were not examined

specifically for the purposes of this study. This could have contributed to misdiagnosis.

This study used prediagnostic serum for analysis of the 25(OH)D concentration. This is an advantage over preceding studies that analysed serum collected after diagnosis of diabetes. A further advantage of this study is its large sample size, greater than that of all known previous studies of this association combined.

Higher serum 25(OH)D concentration provides the substrate for biosynthesis of 1,25(OH)$_2$D, which inhibits occurrence of insulin-requiring diabetes in animal models [16]. Vitamin D deficiency is associated with major effects on the innate immune system [17]. This could potentially influence the risk of diabetes by reducing risk of infection of islet cells. However, vitamin-D-deficient and VDR-KO mice do not have known major abnormalities of the immune system, except for minor effects on dendritic cell markers [17]. Individuals in this study who had serum levels of 25 (OH)D greater than 100 nmol/l had a 70% lower risk of developing insulin-dependent diabetes than those with levels below 43 nmol/l. While the results are intriguing, caution should be exercised when supplementing with very large doses of vitamin D [18].

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