SERUM 25-HYDROXYVITAMIN D AND INCIDENCE OF DIABETES IN *ELDERLY PEOPLE*: THE PRO.V.A STUDY

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Context: increasing research has shown that low levels of serum 25-hydroxyvitamin (25OHD) predict the onset of diabetes, but no research is available on this issue in *elderly* people.

Objective: to examine whether low serum levels of 25OHD are associated with a higher risk of incident type 2 diabetes over a lengthy follow-up in a representative group of *elderly* people.

Design: a population-based cohort study as part of the Progetto Veneto Anziani (Pro.V.A) over a follow-up of 4.4 years

Setting: general community.

Participants: 2227 participants (1728 with follow-up visits and 499 died during the follow-up) over 65 years of age without diabetes at the baseline, among 2352 initially included.

Main outcome measure: incident diabetes.

Results: there were no baseline differences in known factors for the onset of diabetes (BMI, waist circumference, total cholesterol, renal function and HbA1c levels) between the groups with different serum 25OHD levels (≤ 25, 25–50, 50–75 and ≥ 75 nmol/L). Over a 4.4-year follow-up, 291 individuals developed diabetes, with an incidence of 28 events per 1000 person-years. No significant difference in the incidence of diabetes emerged between the baseline 25OHD groups. Cox's regression analysis, adjusted for potential confounders, revealed no relationship between low vitamin D levels and incident diabetes during the follow-up (HR: 1.05, 95%CI: 0.76–1.45, p=0.77; 1.44, 0.95–1.98, p=0.12 and 1.37, 0.87–2.16, p=0.17 for those with 25OHD ≤ 25, 25–50, and 50–75, respectively)

Conclusion: baseline serum concentrations of 25OHD were unassociated with the incidence of diabetes in community-dwelling *elderly* people over a follow-up of 4.4 years.

Diabetes is the fourth leading cause of death in most industrialized countries and takes an extraordinary toll on public health systems. Nowadays, more than 40% of the diagnosed cases of diabetes involve people over 65 years old, and recent studies suggest that there will be a huge increase in this disease among elderly people in years to come. It has been estimated that the number of cases among 65- to 74-year-old women will rise by 252%, for

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Abbreviations:

instance, and for men over 75 the figures will increase by 537% over the next 20 years (1–3).

Vitamin D deficiency is common in community-dwelling ***elderly*** people: in Europe, 36% of elderly men and 47% of elderly women have a severe deficiency (ie, serum 25OHD levels below 30 nmol/L), and the percentages may be as high as 60% in the Mediterranean countries of Europe (4–6).

Low vitamin D levels have been considered a potential risk factor for the onset of diabetes. A complex link has been suggested between diabetes and vitamin D, and serum 25-hydroxyvitamin (25OHD) in particular. Metabolites of vitamin D significantly influence glucose metabolism by improving insulin secretion and insulin sensitivity (7, 8).

A potential role for poor vitamin D status in increasing the risk of type 2 diabetes is supported by several cross-sectional studies largely (though not always consistently) showing that low serum 25OHD levels are associated with impaired glucose tolerance and diabetes (9–17). Longitudinal studies in the general population have found a substantial, statistically significant association between low serum 25OHD levels and the onset of diabetes (18–27), as confirmed by two recent meta-analyses (28–29). The studies that involved an adequate proportion of elderly people found no strong association between serum 25OHD levels and prevalent or incident diabetes, however (17, 30–32). The reasons for the different findings between older and younger people are not known, but probably relate to a physiological decline in vitamin D receptor expression in the peripheral tissues with aging (33), and to differences in the role of low vitamin D status in older and younger people. Given the above-mentioned findings, we hypothesized that there is no direct relationship between low serum 25OHD levels and incident diabetes in ***elderly*** people.

The aim of the present study was thus to examine whether low serum 25OHD levels were associated with any increased risk of incident type 2 diabetes in a representative group of ***elderly*** men and women over a lengthy follow-up (4.4 years).

Materials and Methods

Data source and subjects

The data for this analysis came from the *Progetto Veneto Anziani* (Pro.V.A.), an observational cohort study on the Italian population aged ≥ 65 years. Our study population included 3099 age- and sex-stratified Caucasian participants (1854 women and 1245 men) randomly selected between 1995 and 1997 using a multistage stratified method. Sampling procedures and data collection methods have been described elsewhere (34). Trained physicians and nurses examined participants at various clinics. The present study concerns the information collected on the incidence of diabetes over a mean 4.4 years (± 1.2 SD) of follow-up.

Among the 3099 subjects considered, 211 were excluded because their serum 25OHD levels had not been tested at the time of their enrollment; another 536 were excluded because they were already diabetic at the baseline; and 125 participants were lost to follow-up. The final sample thus consisted of 2227 participants (1728 who completed the follow-up and 499 who died) (Figure 1). Those lost to follow-up had baseline characteristics in terms of the proportion of females, age, anthropometric parameters and fasting plasma glucose (FPG), glycosylated hemoglobin (HbA1c), and 25OHD levels comparable with those of participants included in the study.

The ethical committees of Padua University and the Veneto Region's Local Health Units (USSL) n. 15 and n. 18 approved our study protocol, and participants gave their written informed consent to the study.

Clinical data

Participants were examined at the city hospitals by trained physicians and nurses at both the baseline and the follow-up visit. Information was collected on their formal education, monthly income, physical activity, smoking, and number and type of drugs being used by means of a face-to-face interview. Educational level was categorized as up to as opposed to more than five years of schooling (ie, primary school attendance in Italy). Monthly income (as an indicator of socio-economic condition) was classified as below or above 500 €. Smoking habits were dichotomized as "never/former smoker" (if a subject had given up smoking at least a year earlier) vs. "current smoker". Regular physical activity was defined as ≥ 4 hours/wk in the previous month of at least moderate physical activity (brisk walking, cycling, gardening, dancing, or physical exercising). Body weight and height were measured by trained physicians and the body mass index (BMI) (BMI, kg/m^2) was calculated; waist circumference at umbilical level was also recorded. Obesity was defined as a BMI over 30 kg/m^2, underweight as a BMI below 20 kg/m^2 (a widely accepted cut-off for underweight in older people), and abdominal obesity as a waist circumference over 102 cm in men

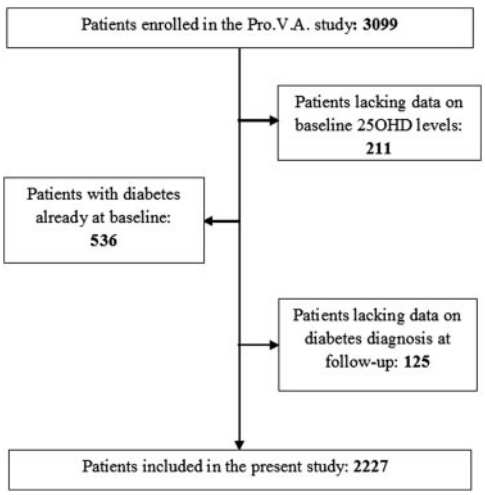

Figure 1. Flow chart of how the study population was selected: the PRO.V.A. study

and over 88 in women (35–36). Blood pressure was recorded as the mean of three readings obtained while the subject was supine.

Any diseases were assessed by board-certified physicians, who examined all the clinical details collected for each participant in the study, including their clinical history, symptoms (self-reported by means of standardized questionnaires), medical and hospital records, blood tests, and a physical examination. A history of major disease was recorded for any of the following: cardiovascular diseases (CVD: congestive heart failure (CHF), angina and myocardial infarction (MI), stroke, and peripheral artery disease (PAD)), chronic pulmonary diseases (COPD), cancer, and hypertension. Cognitive function was assessed by administering the 30-item Mini-Mental State Examination (MMSI) (MMSE).

Definition of diabetes

Incident and prevalent diabetes were defined as FPG $\geq$ 7.0 nmol/L (37). Subjects were also considered diabetic if any of the following applied: $HbA_1c \geq 6.5\%$ (=48 mmol/mol), use of glucose-lowering drugs, or a 2h postload glucose $\geq$ 11.1 nmol/L. Participants' medical information was checked by board-certified physicians at the follow-up visit and confirmed with the aid of a standardized questionnaire (34). Details of FPG were unavailable for 18 participants at the follow-up visit. The hospital records were reviewed for all participants, and death certificates too for those who had died, using codes 250 to 250.9 (International Classification of Diseases – ninth Revision– Clinical Modifications- 2002).

Laboratory data

Venous blood samples were obtained after an overnight fast for biochemical tests, which were performed at the city hospital's central laboratory using standard, quality-controlled procedures. The season of blood collection was categorized as winter, spring, summer or autumn.

Fasting plasma glucose (FPG) was measured with hexokinase glucose-6-phosphate dehydrogenase (Dimension Vista System, Siemens). Glycosylated hemoglobin (HbA1c) was measured using high-performance liquid chromatography (HPLC). Serum 25OHD (25-hydroxyvitamin D) and PTH levels were tested at the Padova University laboratory. Serum 25OHD levels were measured by radioimmunoassay (RIA) (RIA kit; DiaSorin); the intra-assay and interassay coefficients of variation for 25OHD were 8.1% and 10.2%, respectively. Serum intact PTH levels were measured using a two-site immunoradiometric assay (IRMA) kit (N-tact PTHSP; DiaSorin): the intra-assay and interassay coefficients of variation for PTH were 3.0% and 5.5%, respectively. Serum creatinine levels were measured using Jaffe rate reactions for the Roche/Hitachi autoanalyzer (Roche Diagnostic GmbH, Mannheim, Germany) calibrated with the uncompensated method, while the estimated glomerular filtration rate (eGFR) was calculated using the MDRD formula. Total cholesterol, triglycerides and HDL (high-density lipoprotein (HDL)) cholesterol were calculated using the enzymatic method. LDL (low-density lipoprotein (LDL)) cholesterol was calculated with the Friedewald equation, unless triglycerides were higher than 400 mg/dl. The LDL values were thus unavailable for 27 participants. Except for 25OHD and PTH, all laboratory measures were obtained at both the baseline and the follow-up visit.

Statistical analyses

Participants' characteristics were summarized using means ($\pm$ standard deviations) for continuous variables, and counts and percentages for categorical variables. Means and proportions were compared between study participants according to their 25OHD baseline values: $\leq$ 25 nmol/L, 25–50 nmol/L, 50–75 nmol/L and $\geq$ 75 nmol/L (38).

For continuous variables, normal distributions were tested using the Shapiro-Wilk test. Age- and gender-adjusted p values for trends were calculated, checking the differences between the means of the covariates by 25OHD group using analysis of variance (ANOVA) and Bonferroni's correction. Differences in categorical variables were examined using the *chi*-square test.

Cox's proportional hazard models were used to assess associations between 25OHD groups and incident type 2 diabetes. Known factors associated with 25OHD levels and/or diabetes were considered for inclusion in the analysis. To explore whether a variable should be included as a predictor in the final survival model, the log-rank test of equality across strata was performed for all the categorical variables and Cox's univariate proportional hazards regression for all the continuous variables considering the onset of diabetes as the outcome.

The predictors included in the final model were all the variables reaching a $P < .20$ in the univariate analyses ie, baseline vitamin D groups, age, gender, waist circumference, hypertension, formal education, monthly income, smoking habits, serum levels of FPG, Hb1Ac and total cholesterol, HDL, triglycerides, and eGFR. When the collinearity among the cholesterol parameters was assessed, serum HDL and triglycerides were found closely correlated with total cholesterol. Of these three variables, only total cholesterol was retained because it indicates the participants' lipid profile better than any other single parameter.

Hazard ratios (HRs) and 95% confidence intervals (CIs) were used to compare rates of diabetes across different 25OHD groups, and they were also obtained for all covariates found significantly associated with the outcome. All analyses were performed using the SPSS 21.0 for Windows (SPSS Inc., Chicago, Illinois). All statistical tests were two-tailed and statistical significance was assumed for a p-value < 0.05

Results

Baseline characteristics

The sample consisted of 2227 community-dwelling elderly subjects without diabetes. They were a mean 76.1 $\pm$ 7.8 years old [range: 65–103]; 59% were females; and the sample's mean BMI was 27.17 $\pm$ 4.43 kg/m^2. The mean serum 25OHD level was 80.12 $\pm$ 54.66 nmol/L, while the FPG and HbA1c levels were 5.34 $\pm$ 0.65 nmol/L and 5.05 $\pm$ 0.50% (=31.68 $\pm$ 5.5 mmol/mol), respectively. The prevalence of participants with 25OHD levels below 25 nmol/L was 11.4%, while in 32.6% they were less than 50 nmol/L, and in 55.2% they were less than 75 nmol/L.

Participants in the lowest 25OHD group ($\leq$25 nmol/L) were significantly older and there were more females than among those in the higher 25OHD groups (p for trend $<$ 0.0001, for both variables). After adjusting for age and

gender, participants with 25OHD levels below 25 nmol/L had a longer school attendance (p for trend = 0.001), a more frequent history of cardiovascular diseases, and were more often cognitively impaired (p for trend < 0.0001 for both) and less active (p for trend < 0.0001). No differences emerged between the 25OHD groups in terms of their seasonal blood collection (P = .15) (Table 1).

Concerning the potential risk factors for diabetes at the baseline, there were no differences between the 25OHD groups in terms of BMI (p for trend = 0.27) or waist circumference (p for trend = 0.08). Participants in the lowest 25OHD group had significantly more obesity and abdominal obesity, however, balanced by a higher presence of underweight condition (p for trend = 0.02, 0.01 and 0.007, respectively). As for the laboratory tests, participants with 25OHD levels below 25 nmol/L had significantly lower FPG levels than those with higher 25OHD values (p for trend = 0.002), while no significant differences emerged for HbA1c (Table 1). No differences came to light for the cholesterol parameters, uric acid or renal function.

Follow-up data

Over a period of 4.4 years, 291 subjects became diabetic (173 F and 118 M), with an incidence of 28 events per 1000 person-years. The incidence of diabetes was highest in the group with 25OHD levels in the range of 25–50 nmol/L (Table 2), and participants who became diabetic had similar baseline 25OHD values to those who did not (82.77 ± 60.83 vs. 83.92 ± 53.75 nmol/L, P = .75). Ultimately, no significant difference emerged in the cumulative incidence of diabetes between the 25OHD groups during the course of the follow-up (p for trend = 0.29) (Figure 2).

Using Cox's regression analysis, 25OHD levels ≤ 25, 25–50 and 50–75 nmol/L were not associated with a higher probability of developing diabetes during the follow-up (Table 2). Modeling 25OHD level as a continuous variable revealed no significant association between 25OHD and incident diabetes. In the final model, what did predict the onset of diabetes during the follow-up was the baseline FPG (HR: 1.32; 95% CI: 1.09–1.61; P = .005).

The results of all our statistical analyses remained much the same after stratifying by gender or abdominal obesity (Figure 3), or using gender-specific quartiles to classify 25OHD status, or quartiles of PTH serum levels instead of 25OHD groups (details not shown).

Discussion

Our large population-based prospective study found no evidence of any significant association between circulating 25OHD levels and incident diabetes over a 4.4-year follow-up.

About one in three participants in our study had 25OHD levels below 50 nmol/L, but more than half of them had 25OHD levels over 75 nmol/L, although the sample's use of vitamin D supplementation was extremely low (1.2% of the whole sample at the baseline and 2.6% at the follow-up); these low figures are substantially similar to those of other studies conducted around the same time as our study (39). The relatively high vitamin D levels in our population could be because our 25OHD groups included both males and females, and because participants came from a rural area of the Veneto, where gardening is still widespread.

We also found that BMI and waist circumference did not differ across 25OHD groups. This is hardly surprising in elderly people because any poor vitamin D status at this age is usually due not only to a greater fat mass (as in middle-aged people), but also to malnutrition and frailty, conditions exclusive to older people and typically associated with low BMI values (40).

Cross-sectional studies have substantially demonstrated that low vitamin D status is associated with a higher prevalence of diabetes, but such studies have always focused on middle-aged or younger people (9–16). The only cross-sectional study on people over 65 indicated that low 25OHD levels led to an increased risk of developing diabetes, but no association was found between 25OHD and markers of glucose metabolism or insulin sensitivity in cases recently diagnosed with diabetes (17). Another cross-sectional study exploring the relationship between low 25OHD and hyperglycemia in older people showed that other confounders (particularly BMI and alcohol intake) drastically reduced this association (32).

Several longitudinal observational studies have reported a significant association between low circulating levels of 25OHD and a higher incidence of type 2 diabetes (18–26), findings also confirmed by two recent meta-analyses (28, 29). Conversely, our present findings are consistent with two longitudinal studies involving a sizable number of ***elderly*** people, in which: Pilz et al found no association between 25OHD and incident diabetes or poor glycemic control in a group of participants with a mean age of 68 years; and Robinson et al reported similar results in a cohort of 5140 postmenopausal women (8, 31). Our results are also in partial agreement with another study reporting a significant association between low 25OHD levels and diabetes only in ***elderly*** women (30).

There may be several reasons why old people lack the association between low vitamin D levels and diabetes seen in the middle-aged. For a start, low 25OHD levels are only an independent mortality factor in the elderly. We

Table 1. Participants' characteristics by serum 25-hydroxyvitamin D (25OHD) cutoffs of 25, 50 and 75 nmol/liter: the PRO.V.A. study. Numbers are mean values (and standard deviations) or percentages (%), as appropriate.

	25-hydroxyvitamin D (25OHD) groups				
	≤25 nmol/liter (*n* = 255)	**25–50 nmol/liter (*n* = 470)**	**50–75 nmol/liter (*n* = 502)**	**≥75 nmol/liter (*n* = 1000)**	***p* value***
Age (years)	81.48 (7.99)	77.73 (8.06)	76.17 (7.48)	73.81 (6.95)	<0.0001†
Female sex (%)	76.6	75.7	68.1	41.2	<0.0001†
BMI (kg/m^2)	26.69 (5.36)	27.39 (4.84)	27.46 (4.34)	27.03 (4.05)	0.24
Obesity (%)	24.9	25.8	24.6	20.0	0.02
Underweight (%)	7.7	3.2	2.9	3.1	0.01
Waist circumference (cm)	94.77 (12.78)	95.37 (11.96)	95.38 (11.08)	95.38 (10.13)	0.18
Abdominal obesity (%)	31.1	28.3	28.0	22.6	0.007
Systolic BP (mmHg)	155.17 (24.38)	153.14 (21.94)	152.66 (21.10)	151.27 (20.65)	0.72
Diastolic BP (mmHg)	82.06 (12.43)	83.16 (11.62)	82.56 (11.04)	83.00 (10.89)	0.22
Education ≥ 5 ys (%)	15.2	15.0	12.4	14.2	0.001
Monthly income ≤ 500 (%)	60.2	67.0	65.7	57.3	0.16
Current smokers (%)	6.3	6.8	8.4	11.8	0.40
Physical activity ≥ 4 h/week (%)	8.2	16.8	22.9	31.3	<0.0001
Use of cortisone (%)	3.5	3.7	1.9	3.0	0.64
Vitamin D supplementation (%)	2.4	1.3	1.2	0.8	0.45
Medical conditions					
Cognitive impairment (%)	29.4	10.9	8.6	2.8	<0.0001
CVD (%)	32.5	22.6	14.9	18.9	<0.0001
COPD (%)	10.2	10.9	6.6	10.5	0.14
Cancer (%)	7.8	9.1	7.6	6.9	0.21
Hypertension (%)	76.8	77.0	71.3	69.4	0.06
Biohumoral tests					
FPG (mmol/liter)	5.18 (0.73)	5.27 (0.67)	5.33 (0.64)	5.41 (0.60)	0.002
HbA1c (%)	5.10 (0.52)	5.10 (0.51)	5.11 (0.47)	4.99 (0.51)	0.10
HbA1c (mmol/mol)	*32.13 (5.64)*	*32.25 (5.50)*	*32.32 (5.25)*	*30.98 (5.50)*	
25OHD (nmol/liter)	16.35 (5.56)	38.82 (6.97)	62.19 (7.20)	124.54 (50.5)	<0.0001
PTH (ng/liter)	61.32 (46.99)	46.83 (25.99)	42.11 (20.80)	36.31 (23.18)	<0.0001
eGFR (ml/min)	66.58 (18.64)	65.64 (19.34)	68.20 (18.27)	72.34 (17.26)	0.09
Total cholesterol (mg/dl)	227.29 (47.82)	233.78 (43.43)	235.56 (43.77)	230.93 (41.27)	0.38
HDL (mg/dl)	56.89 (15.96)	58.10 (15.29)	57.50 (15.46)	58.26 (15.79)	0.16
Triglycerides (mg/dl)	136.97 (67.99)	136.19 (66.46)	130.08 (60.63)	126.58 (67.86)	0.15
LDL (mg/dl)	144.90 (41.22)	149.00 (36.99)	152.30 (39.61)	147.32 (36.21)	0.12
Uric acid (mg/dl)	5.29 (1.55)	5.17 (1.49)	5.14 (1.43)	5.30 (1.40)	0.67
Blood collection					0.15
Winter (%)	22.1	27.0	21.4	30.0	
Spring (%)	37.6	27.0	23.3	20.6	
Summer (%)	15.9	19.3	21.0	17.0	
Autumn (%)	22.9	26.0	33.8	30.7	

Notes: obesity was defined as BMI≥30, underweight as BMI<20 and abdominal obesity as a waist circumference ≥102 cm in males and ≥88 in females. *Unless otherwise specified, *p values* are adjusted for age and gender using a general linear model or

logistic regression, as appropriate. † Not adjusted for age or gender.

Abbreviations: BMI: body mass index; BP: blood pressure; CVD: cardiovascular diseases; COPD: chronic obstructive pulmonary disease; FPG: fasting plasma glucose; HbA1c: glycosylated hemoglobin; 25OHD: serum 25-hydroxyvitamin D; PTH: parathormone; eGFR: estimated glomerular filtration rate; HDL: high-density lipoproteins; LDL: low-density lipoproteins.

Table 2. Association between serum 25-hydroxyvitamin D concentration and incident diabetes: the PRO.V.A. study.

	No. of events	*No. of participants*	*Incidence rate* *	*Unadjusted hazard ratio (95%CI)*	*p –value*	*Adjusted hazard ratio*† *(95%CI)*	*p –value*
25OHD categories							
≥ 75 nmol/liter	131	1000	28 (26–30)	1 [reference]		1 [reference]	
50–75 nmol/liter	60	502	27 (22–32)	0.99 (0.73–1.35)	0.98	1.05 (0.76–1.45)	0.77
25–50 nmol/liter	70	470	33 (30–36)	1.28 (0.96–1.71)	0.10	1.44 (0.95–1.98)	0.12
≤25 nmol/liter	30	255	28 (24–32)	1.24 (0.82–1.90)	0.31	1.37 (0.87–2.16)	0.17
25OHD as continuous							
For change of 1 nmol/liter	-	-	-	1.00 (0.998–1.002)	0.80	0.999 (0.997–1.002)	0.50
+2 SD **from the mean**	17	99	28 (27–29)	1 [reference]		1 [reference]	
+1 SD **from the mean**	110	830	29 (28–30)	0.81 (0.49–1.36)	0.43	0.89 (0.52–1.53)	0.89
- 1 SD **from the mean**	164	1298	40 (36–40)	0.75 (0.44–1.26)	0.27	0.79 (0.47–1.35)	0.39

Unless otherwise specified, data are presented as relative risk and 95% confidence interval.

Notes:

* Incidence rates are per 1000 person-years. Data are expressed as means with 95% confidence intervals.

† Adjusted for: age, gender, **and baseline** waist circumference, hypertension, formal education, monthly income, smoking habits, and serum levels of: FPG, Hb1Ac, total cholesterol, eGFR.

found that the people who had died in our sample not only had significantly lower 25OHD levels than the other participants, but also significantly more conditions associated with a higher mortality, and cardiovascular diseases in particular. It may be that people with low 25OHD levels die before they develop diabetes (41). Secondly, 25OHD levels are known to differ between older and younger people, so while younger people's baseline 25OHD levels substantially increase over a period of time, the same cannot be said of old people (42–44). Finally, we need to consider the possible influence of vitamin D receptor on these different findings: this receptor's expression is known to decline with age in the tissues that determine insulin sensitivity (33), meaning that much higher levels of 25OHD might be needed in later life to achieve the same protective effect as in younger people. Indirect support for these hypotheses comes from several studies correlating 25OHD levels with cardiovascular diseases: unlike the situation in young people, these studies were unable to find any significant association between poor vitamin D status and cardiovascular diseases in ***elderly*** people (27).

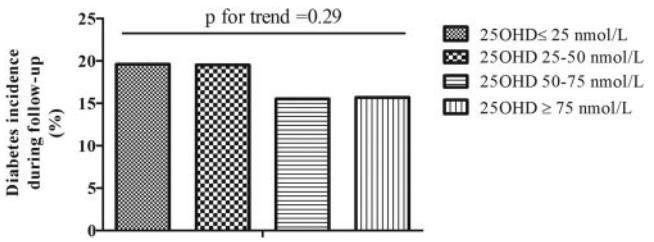

Figure 2. Incidence of diabetes (as a percentage) at the follow-up, by baseline serum 25-hydroxyvitamin D (25OHD) level, using 25, 50 and 75 nmol/L as cutoffs: the PRO.V.A. study.

Our study has both strengths and limitations. The main limitation lies in the number of participants lost to follow-up (although this was much the same as in other longitudinal studies on ***elderly*** people). Another limitation concerns the shortage of other laboratory tests useful for diagnosing diabetes at the follow-up visit, which may have led to an underestimation of the new diagnoses of diabetes. Finally, no information was available on any prior vitamin D supplementation, family history of diabetes, or 25OHD levels at the follow-up visit. Vitamin D supplementation

was found negligible in our sample (1.2% at the baseline and 2.6% at the follow-up), so we assume that any use of vitamin D in previous periods was probably likewise extremely low, and not enough to affect participants' baseline 25OHD values. Moreover, since vitamin D supplementation is one of the most important factors affecting serum 25OHD levels in elderly people (4), we assume that participants' baseline 25OHD levels were scarcely affected by this factor. A family history of diabetes is an important factor influencing the risk of developing diabetes, but this information was not obtained from our participants, nor was it reported in other studies exploring the association between 25OHD and diabetes (18–27). The same applies to the 25OHD levels at the follow-up: serum 25OHD was only measured at the baseline, as in other studies exploring the same association (8, 20, 24), so it may not reflect long-term vitamin D status. On the other hand, serum 25OHD levels drop extremely slowly (by about 0.3 mmol/L/y) (44), as recently confirmed in a sample of elderly individuals living in the same geographical area as the participants involved in this study (43). The vitamin supplementation rate was also very low in our sample, at both the baseline and the follow-up visits, so our results are unlikely to have been substantially influenced by our not having measured 25OHD levels at the follow-up. On the other hand, board-certified experts on metabolic diseases used standardized questionnaires to confirm the information collected on the cases of diabetes diagnosed during the follow-up. Using this approach, we found an incidence of diabetes during the follow-up that was similar to the figures reported in other studies (3). The main strengths of our work lie in that it was the first such study to focus exclusively on the elderly, and in the longitudinal design and long-term follow-up of this sample of ***elderly*** people. Another strength concerns the use of 25OHD, which is the best indicator of total body stores of vitamin D. An additional strength of our work relates to the large number of potential confounders that we analyzed.

In conclusion, baseline serum concentrations of 25OHD were not associated with the incidence of diabetes over a 4.4-year follow-up in our sample of elderly people. Since the prevalence of diabetes is rising in people over 65 years of age, and the condition is associated not only with chronic complications typical of the middle-aged, but also with a higher risk of diseases specifically of old age (such as sarcopenia, disability and frailty), we need to clarify the real role of vitamin D in the onset of diabetes.

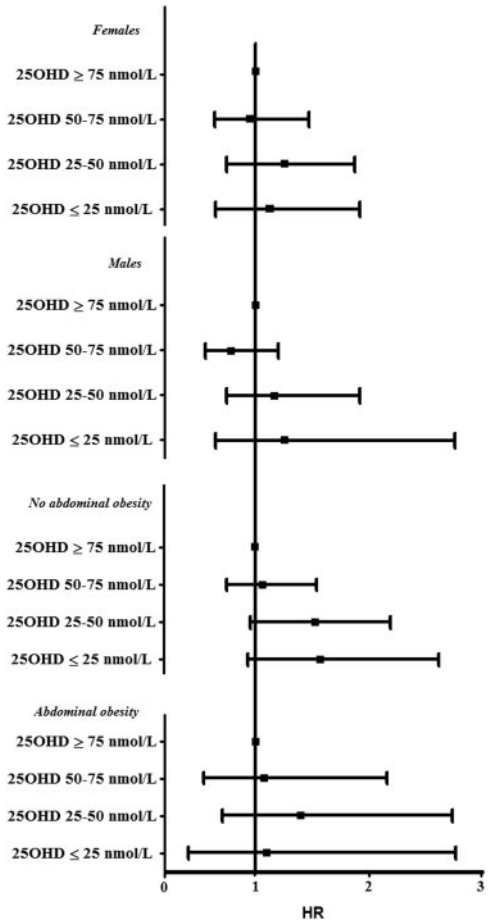

Figure 3. Risk of diabetes (adjusted for the same covariates as in Table 2) by gender and abdominal obesity: the PRO.V.A. study.

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References

1. **Punjabi NM.** Do sleep disorders and associated treatments impact glucose metabolism? *Drugs*. 2009;69 Suppl.2:13–27.
2. **Mokdad AH, Ford ES, Bowman BA, Nelson DE, Engelgau MM,**

Vinicor F, Marks JS. Diabetes trends in the U.S.: 1990–1998. *Diabetes Care*. 2000;23:1278–1283.
3. **McBean AM, Li S, Gilbertson DT, Collins AJ.** Differences in diabetes prevalence, incidence, and mortality among the elderly of four racial/ethnic groups: whites, blacks, hispanics, and asians. *Diabetes Care*. 2004;27:2317–2324.
4. **Mosekilde L.** Vitamin D and the elderly. *Clin Endocrinol (Oxf)*. 2005;62:265–281.
5. **Timpini A, Pini L, Tantucci C, Cossi S, Grassi V.** Vitamin D and health status in elderly. *Intern Emerg Med*. 2011;6:11–21.
6. **Toffanello ED, Perissinotto E, Sergi G, Zambon S, Musacchio E, Maggi S, Coin A, Sartori L, Corti MC, Baggio G, Crepaldi G, Manzato E.** Vitamin D and physical performance in elderly subjects: the Pro.V.A study. *PLoS One*. 2012;7:e34950.
7. **Gonzalez-Molero I, Rojo-Martinez G, Morcillo S, Gutierrez-Repiso C, Rubio-Martin E, Almaraz MC, Olveira G, Soriguer F.** Vitamin D and incidence of diabetes: a prospective cohort study. *Clin Nutr*. 2012;31:571–573.
8. **Pilz S, van den Hurk K, Nijpels G, Stehouwer CD, Van't Riet E, Kienreich K, Tomaschitz A, Dekker JM.** Vitamin D status, incident diabetes and prospective changes in glucose metabolism in older subjects: the Hoorn study. *Nutr Metab Cardiovasc Dis*. 2012;22: 883–889.
9. **Isaia G, Giorgino R, Adami S.** High prevalence of hypovitaminosis D in female type 2 diabetic population. *Diabetes Care*. 2001;24: 1496.
10. **Need AG, O'Loughlin PD, Horowitz M, Nordin BE.** Relationship between fasting serum glucose, age, body mass index and serum 25 hydroxyvitamin D in postmenopausal women. *Clin Endocrinol (Oxf)*. 2005;62:738–741.
11. **Scragg R, Sowers M, Bell C.** Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. *Diabetes Care*. 2004;27:2813–2818.
12. **Chailurkit LO, Aekplakorn W, Ongphiphadhanakul B.** The association between vitamin D status and type 2 diabetes in a Thai population, a cross-sectional study. *Clin Endocrinol (Oxf)*. 2012;77: 658–664.
13. **Hurskainen AR, Virtanen JK, Tuomainen TP, Nurmi T, Voutilainen S.** Association of serum 25-hydroxyvitamin D with type 2 diabetes and markers of insulin resistance in a general older population in Finland. *Diabetes Metab Res Rev*. 2012;28:418–423.
14. **Brock KE, Huang WY, Fraser DR, Ke L, Tseng M, Mason RS, Stolzenberg-Solomon RZ, Freedman DM, Ahn J, Peters U, McCarty C, Hollis BW, Ziegler RG, Purdue MP, Graubard BI.** Diabetes prevalence is associated with serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D in US middle-aged Caucasian men and women: a cross-sectional analysis within the Prostate, *Lung, Colorectal and Ovarian Cancer Screening Trial*. *Br J Nutr*. 2011;106:339–344.
15. **Ford ES, Ajani UA, McGuire LC, Liu S.** Concentrations of serum vitamin D and the metabolic syndrome among U.S. adults. *Diabetes Care*. 2005;28:1228–1230.
16. **Hypponen E, Boucher BJ, Berry DJ, Power C.** 25-hydroxyvitamin D, IGF-1, and metabolic syndrome at 45 years of age: a cross-sectional study in the 1958 British Birth Cohort. *Diabetes*. 2008;57: 298–305.
17. **Dalgard C, Petersen MS, Weihe P, Grandjean P.** Vitamin D status in relation to glucose metabolism and type 2 diabetes in septuagenarians. *Diabetes Care*. 2011;34:1284–1288.
18. **Forouhi NG, Luan J, Cooper A, Boucher BJ, Wareham NJ.** Baseline serum 25-hydroxy vitamin d is predictive of future glycemic status and insulin resistance: the Medical Research Council Ely Prospective Study 1990–2000. *Diabetes*. 2008;57:2619–2625.
19. **Anderson JL, May HT, Horne BD, Bair TL, Hall NL, Carlquist JF, Lappe DL, Muhlestein JB.** Relation of vitamin D deficiency to cardiovascular risk factors, disease status, and incident events in a general healthcare population. *Am J Cardiol*. 2010;106:963–968.
20. **Gagnon C, Lu ZX, Magliano DJ, Dunstan DW, Shaw JE, Zimmet PZ, Sikaris K, Grantham N, Ebeling PR, Daly RM.** Serum 25-hydroxyvitamin D, calcium intake, and risk of type 2 diabetes after 5 years: results from a national, population-based prospective study (the Australian Diabetes, *Obesity and Lifestyle study)*. *Diabetes Care*. 2011;34:1133–1138.
21. **Grimnes G, Emaus N, Joakimsen RM, Figenschau Y, Jenssen T, Njolstad I, Schirmer H, Jorde R.** Baseline serum 25-hydroxyvitamin D concentrations in the Tromso Study 1994–95 and risk of developing type 2 diabetes mellitus during 11 years of follow-up. *Diabet Med*. 2010;27:1107–1115.
22. **Knekt P, Laaksonen M, Mattila C, Harkanen T, Marniemi J, Heliovaara M, Rissanen H, Montonen J, Reunanen A.** Serum vitamin D and subsequent occurrence of type 2 diabetes. *Epidemiology*. 2008;19:666–671.
23. **Pittas AG, Sun Q, Manson JE, Dawson-Hughes B, Hu FB.** Plasma 25-hydroxyvitamin D concentration and risk of incident type 2 diabetes in women. *Diabetes Care*. 2010;33:2021–2023.
24. **Thorand B, Zierer A, Huth C, Linseisen J, Meisinger C, Roden M, Peters A, Koenig W, Herder C.** Effect of serum 25-hydroxyvitamin D on risk for type 2 diabetes may be partially mediated by subclinical inflammation: results from the MONICA/KORA Augsburg study. *Diabetes Care*. 2011;34:2320–2322.
25. **Husemoen LL, Thuesen BH, Fenger M, Jorgensen T, Glumer C, Svensson J, Ovesen L, Witte DR, Linneberg A.** Serum 25(OH)D and type 2 diabetes association in a general population: a prospective study. *Diabetes Care*. 2012;35:1695–1700.
26. **Afzal S, Bojesen SE, Nordestgaard BG.** Low 25-hydroxyvitamin D and risk of type 2 diabetes: a prospective cohort study and meta-analysis. *Clin Chem*. 2013;59:381–391.
27. **Bolland MJ, Bacon CJ, Horne AM, Mason BH, Ames RW, Wang TK, Grey AB, Gamble GD, Reid IR.** Vitamin D insufficiency and health outcomes over 5 y in older women. *Am J Clin Nutr*. 2010; 91:82–89.
28. **Mitri J, Muraru MD, Pittas AG.** Vitamin D and type 2 diabetes: a systematic review. *Eur J Clin Nutr*. 2011;65:1005–1015.
29. **Song Y, Wang L, Pittas AG, Del Gobbo LC, Zhang C, Manson JE, Hu FB.** Blood 25-hydroxy vitamin D levels and incident type 2 diabetes: a meta-analysis of prospective studies. *Diabetes Care*. 2013;36:1422–1428.
30. **Schottker B, Herder C, Rothenbacher D, Perna L, Muller H, Brenner H.** Serum 25-hydroxyvitamin D levels and incident diabetes mellitus type 2: a competing risk analysis in a large population-based cohort of older adults. *Eur J Epidemiol*. 2013;28:267–275.
31. **Robinson JG, Manson JE, Larson J, Liu S, Song Y, Howard BV, Phillips L, Shikany JM, Allison M, Curb JD, Johnson KC, Watts N.** Lack of association between 25(OH)D levels and incident type 2 diabetes in older women. *Diabetes Care*. 2011;34:628–634.
32. **Hirani V.** Relationship between vitamin D and hyperglycemia in older people from a nationally representative population survey. *J Am Geriatr Soc*. 2011;59:1786–1792.
33. **Alvarez JA, Ashraf.** A Role of vitamin d in insulin secretion and insulin sensitivity for glucose homeostasis. *Int J Endocrinol*. 2010; 2010:351385.
34. **Corti MC, Guralnik JM, Sartori L, Baggio G, Manzato E, Pezzotti P, Barbato G, Zambon S, Ferrucci L, Minervini S, Musacchio E, Crepaldi G.** The effect of cardiovascular and osteoarticular diseases on disability in older Italian men and women: rationale, design, and sample characteristics of the Progetto Veneto Anziani (PRO.V.A.) study. *J Am Geriatr Soc*. 2002;50:1535–1540.
35. **Veronese N, De Rui M, Toffanello ED, De Ronch I, Perissinotto E, Bolzetta F, D'Avanzo B, Cardin F, Coin A, Manzato E, Sergi G.** Body mass index as a predictor of all-cause mortality in nursing home residents during a 5-year follow-up. *J Am Med Dir Assoc*. 2013; 14:53–57.
36. **Expert Panel on the Identification, Evaluation, and Treatment of Overweight in Adults.** Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: executive summary. *Am J Clin Nutr*. 1998;68:899–917.

37. **American Diabetes Association.** Standards of medical care in diabetes–2013. *Diabetes Care*. 2013;36 Suppl 1:S11–66.
38. **Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM.** Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2011;96:1911–1930.
39. **Kim HJ, Giovannucci E, Rosner B, Willett WC, Cho E.** Longitudinal and Secular Trends in Dietary Supplement Use: Nurses' Health Study and Health Professionals Follow-Up Study, 1986–2006. *J Acad Nutr Diet*. 2013;114:436–443.
40. **Hubbard RE, Lang IA, Llewellyn DJ, Rockwood K.** Frailty, body mass index, and abdominal obesity in older people. *J Gerontol A Biol Sci Med Sci*. 2010;65:377–381.
41. **Bjelakovic G, Gluud LL, Nikolova D, Whitfield K, Wetterslev J, Simonetti RG, Bjelakovic M, Gluud C.** Vitamin D supplementation for prevention of mortality in adults. *Cochrane Database Syst Rev*. 2011;Cd007470.
42. **Baker MR, Peacock M, Nordin BE.** The decline in vitamin D status with age. *Age Ageing*. 1980;9:249–252.
43. **Mosele M, Coin A, Manzato E, Sarti S, Berton L, Bolzetta F, Imoscopi A, Rinaldi G, Perissinotto E, Sergi G.** Association between serum 25-hydroxyvitamin d levels, bone geometry, and bone mineral density in healthy older adults. *J Gerontol A Biol Sci Med Sci*. 2013;68:992–998.
44. **Jorde R, Sneve M, Hutchinson M, Emaus N, Figenschau Y, Grimnes G.** Tracking of serum 25-hydroxyvitamin D levels during 14 years in a population-based study and during 12 months in an intervention study. *Am J Epidemiol*. 2010;171:903–908.