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Vitamin D deficiency in diabetes exacerbates longitudinal risk for atherosclerotic cardiovascular disease in Lanzhou, China

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ABSTRACT

Background and Objectives: Vitamin D deficiency has been considered a risk factor for atherosclerotic cardiovascular disease (ASCVD). The aim of this study was to investigate the correlation between serum 25(OH)D concentration and the risk of ASCVD in Chinese, especially in Type 2 diabetes mellitus (T2DM) patients. **Methods and Study Design:** Based on the "REACTION" study conducted in 2011, some 9,014 Lanzhou residents aged 40-75 years were followed from 2014 to 2016. A total of 7,061 with complete data were analyzed. Baseline population was classified into four groups based on 25(OH)D quartiles. Cox proportional hazard models were used to estimate relations between 25(OH)D concentration and ASCVD. **Results:** The prevalence of vitamin D deficiency [25(OH)D <20 ng/mL] was 75.1%. Followed-up for 3.3 years, those with the lowest of 25(OH)D concentration had higher rates of ASCVD (HR: 1.748, 95% CI: 1.149-2.660, p<0.01). A 10 ng/mL increase in baseline serum 25(OH)D was accompanied by a 24 % decrease in ASCVD risk (HR: 0.760, 95% CI: 0.590-0.980, p<0.05). For 25(OH)D and ASCVD risk with glycaemic status, low 25(OH)D plus T2DM was highly associated with ASCVD (HR: 2.296, 95% CI: 1.246-4.232, p<0.01). With diabetes, ASCVD risk decreased by 36% when serum 25(OH)D increased by 10 ng/mL (HR: 0.644, 95% CI: 0.440-0.941, p<0.05). **Conclusions:** Serum 25(OH)D is independently and inversely associated with the risk of ASCVD in Lanzhou Chinese, especially those with T2DM. Maintaining sufficient levels of vitamin D adequacy may be an effective measure in ASCVD prevention.

Key Words: type 2 diabetes mellitus, atherosclerotic cardiovascular disease, vitamin D deficiency, middle aged and elderly individuals, longitudinal study

INTRODUCTION

Atherosclerotic cardiovascular disease (ASCVD) is the leading cause of mortality in the world, which endangers public health and aggravates the socio-economic burden.[1] Type 2 diabetes mellitus (T2DM) is a major hazard for ASCVD.[2] Patients with T2DM have a 2-4 times higher risk of ASCVD than do those without.[3] Blood glucose normalization is the usual focus for the prevention and management of macroangiopathy which involves attention to personal behaviours relevant also to blood lipid and blood pressure control. Regular vascular ultrasound screening in diabetes can facilitate management,[4] and direct it to more comprehensive dietary management.[5]

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Vitamin D plays a vital role in bone metabolism as a fat-soluble vitamin.[6] However, its deficiency is a global health issue[7,8] for reasons beyond bone health with linkage to several chronic diseases[9] including those involving vascular health, immune function and cellular differentiation or neoplasia.[10] How these manifest in North East Asia requires greater understanding and attention.[11] Dietary pattern, insufficient sunlight exposure, severe liver and kidney dysfunction, gastrointestinal malabsorption and metabolic disorders may contribute to vitamin D deficiency.[7] Cardiovascular system health may be affected by vitamin D status through vascular endothelial cell function and arterial immunology, and by inhibition of coronary artery calcification.[12,13] Consequently, vitamin D deficiency has been considered a candidate for ASCVD in several settings.[14-16]

Lanzhou, the capital of Gansu province, is located on the northwestern inland China. The average duration of sunshine in Lanzhou is 2446 h per year. Although sunshine is relatively plentiful, there is a high rate of vitamin D deficiency.[17] Moreover, cardiovascular disease is now common disease in China, notably in Gansu Province. Large-scale prospective studies to explore any correlation between vitamin D and ASCVD are lacking in Northwest China, and putative mechanisms in question. Thus, we have investigated the relationship between vitamin D concentration and ASCVD risk in Lanzhou, Gansu Province.

MATERIALS AND METHODS

Study population

The "REACTION" study, known as The Risk Evaluation of Cancers in Chinese Diabetic Individuals: A Longitudinal Study, is a large, nationwide, prospective study involving 259,657 adults aged 40 years and older in 25 communities across mainland China from 2011 to 2012 in order to investigate the association of diabetes and cancer.18 The present report is to do with a sub-set of the REACTION study, randomly selected from three communities in the urban Lanzhou using stratified, multistage probability population sampling. Only persons who had been living in their current residence for at least five years were eligible to participate. The study recruited some 9,014 individuals aged 40 to 75 years randomly from the original 2011-2012 cohort. Participants with coronary heart disease (CHD), ischemic stroke or peripheral arterial disease at baseline were excluded. At follow up from 2014 to 2016, there was an eligible 7,061 participants. The follow-up end points were the new onset of ASCVD events, death, loss to follow-up and study termination (December 31, 2016, Figure 1). The ASCVD events and deaths were confirmed by verifying the time and place of cardiovascular events or deaths, physical examination results and hospital certificates or other

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supportive documents. The study was approved by the Ethics Committee of Shanghai Jiao Tong University [Approval No. 2011(14) and 2014(52)]. All study participants provided written informed consent.

Clinical and laboratory measurements

The personal interview was conducted by trained health workers using a standardized questionnaire. The information included necessary personal details (name, gender, age, residential region, survey date), personal behaviour (drinking and smoking), medical history (hypertension, diabetes, hyperlipidemia, stroke, coronary heart disease, peripheral vascular disease, and tumour), operation history, physical activity level, and use of medications. Physical examination provided measurement of height, weight and blood pressure. Body mass index (BMI) was calculated as body weight in kilograms divided by body height squared in metres (kg/m^2). Blood pressure was consecutively measured at the non-dominant arm three times.

Blood samples were obtained from all participants after overnight fasting (at least 8~10 h) or 2-h in 75-g oral glucose tolerance test (OGTT). Fasting (FPG) and 2h plasma glucose (2h-PG) were measured, and glycated haemoglobin (HbA1c) was measured by high performance liquid chromatography using the VARIANT II Hemoglobin Testing System at a laboratory in the institute of endocrinology, the first hospital of Lanzhou University. Serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured by the Shanghai Institute of Endocrine and Metabolic Diseases using an autoanalyser (Abbott Laboratories). Serum 25(OH)D concentration was determined by enzyme immunoassay (EIA; IDS Ltd, Boldon, UK).

Clinical and laboratory definitions

By World Health Organization criteria in 1999,19 the participants were categorized as follows: normal glucose tolerance [NGT: FPG <6.1 mmol/L and 2h-PG <7.8 mmol/L], impaired glucose regulation [IGR:6.1 mmol/L ≤ FPG < 7.0 mmol/L or 7.8 mmol/L ≤ 2hPG < 11.1 mmol/L] and diabetes mellitus [DM: diagnosed diabetes and FPG ≥ 7.0 mmol/L, or 2h-PG ≥ 11.1 mmol/L). Vitamin D status was defined as “deficiency” [25(OH)D < 20 ng/mL], “insufficiency” [20 ng/mL ≤ 25(OH)D < 30 ng/mL and “sufficiency” [25(OH)D ≥30 ng/mL].[7] Hypertension was defined as a sitting blood pressure of at least 140 mmHg systolic or 90 mmHg diastolic or anti-hypertensive drug use. Smoking was defined as the current smoking

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of cigarettes or tobacco leaves with one branch or 1 g or more of tobacco leaves per day; alcohol consumption was defined as drinking at least once a week for more than one year. ASCVD events were defined by the WHO diagnostic criteria for MONICA including coronary heart disease events (stable angina pectoris, unstable angina pectoris and acute myocardial infarction and sudden cardiac death, chronic coronary heart disease death) and fatal and nonfatal ischemic cerebral apoplexy,[20] and diagnoses established by questionnaire survey, telephone follow-up, medical history, medication history and from photos of medical records. Glucolipid metabolic indexes refer to blood glucose and blood lipids, including glucose metabolic indicators (FPG, 2h-PG, HbA1c) and lipid metabolic indicators (LDL-C, HDL-C, TC, TG).

Statistical analysis

All analyses were performed using SPSS software version 23.0. The participants were divided into four groups according to quartiles of serum 25(OH)D concentration. Normally distributed continuous variables were expressed as the mean ± standard deviation (SD), and non-normally distributed variables were presented as medians (interquartile ranges). One-way ANOVA with LSD analysis was used to evaluate differences in quantitative data or Chi-square test with Bonferroni correction for categorical variables. Kruskal-Wallis H test was used for multiple comparisons of non-normally distributed data. Partial correlation analysis was used to explore the correlation between serum 25(OH)D concentration and the glucolipid metabolic indexes (including FPG, 2h-PG, HbA1c, LDL-C, HDL-C, TC and TG) concentrations. The cumulative incidence of ASCVD in different serum 25(OH)D groups and different glycaemia groups was calculated and tested by Log-Rank. The linear-by-linear association test and Chi-square test value for trends were used to explore the trend in ASCVD incidence by 25(OH)D level in different glycaemia groups. The hazard ratios (HR) and 95% confidence intervals (CI) were calculated by multivariate Cox regression analyses. A *p*-value <0.05 was considered statistically significant.

RESULTS

Baseline characteristics of participants

A total of 7,061 participants was enrolled in the study, including 2012 males (28.5%) and 5049 females (71.5%). The mean age was 57.6 years (57.6±8.4 years) and the median concentration of serum 25(OH)D was 15.91 ng/mL. The prevalence of vitamin D deficiency [25(OH)D <20 ng/mL] was 75.1%, and merely 2.0% were vitamin D sufficient [25(OH) ≥30

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ng/mL]. Table 1 shows the baseline clinical and biochemical parameters by quartile for 25(OH)D. Overall, participants in the lower quartile of 25(OH)D were more likely to be older, female, to have lower smoking and drinking rates and have a higher prevalence of hypertension. The BMI, FPG, and 2h-PG values decreased with an increase in 25(OH)D (all $p<0.05$). However, no statistically significant differences were detected between the 25(OH)D quartile groups for HbA1c, HDL-C, LDL-C, TC or TG ($p>0.05$). From the first to the fourth quartile, the prevalence of IGR showed a significant downward trend ($p<0.05$), but not for diabetes prevalence ($p>0.05$).

Correlation between serum 25(OH)D concentration and glucolipid metabolic index

Correlations between 25(OH)D concentration and glucolipid metabolic index were evident in our study. Partial correlation analysis showed that serum 25(OH)D concentration was negatively correlated with FPG, 2h-PG, HbA1c and TG when adjusted for gender, age, smoking, drinking, BMI, hypertension, and diabetes; the partial correlation coefficients were -0.044, -0.053, -0.028, -0.040, respectively (all $p<0.05$). Serum 25(OH)D was positively correlated with HDL-C and LDL-C; the partial correlation coefficients were 0.049 and 0.035, respectively ($p<0.05$), but no correlations were evident between serum 25(OH)D and TC (Table 2).

Incidence of ASCVD among 25(OH)D quartiles

After a median follow-up of 3.3 years, a total of 216 participants (3.1%) experienced ASCVD events; the ASCVD cumulative incidences by 25(OH)D quartile were 4.1%, 3.0%, 3.1%, and 2.0%, respectively. There was a linear trend for decreasing ASCVD events as serum 25(OH)D increased, statistically significant using the Log-Rank test ($\chi^2=11.676$, $p=0.009$, Figure 2).

Hazard ratios for ASCVD events in participants with different 25(OH)D concentrations

Table 3 shows the hazard ratios for ASCVD incidence by 25(OH)D quartile compared with the referent group (the fourth quartile). When unadjusted, the risk of ASCVD in the first quartile group increased compared with the last quartile (HR: 1.972, 95% CI: 1.322-2.942, $p<0.01$); with adjustment for confounding variables, significance remained (HR: 1.748, 95% CI: 1.149-2.660, $p<0.01$). However, the ASCVD risk in the second and third quartiles was not significantly different from that in the fourth quartile. Using serum 25(OH)D as a continuous

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variable, Cox regression analyses showed an inverse association of the 25(OH)D concentration (per 10 ng/mL increase) with the risk of ASCVD. This correlation remained significant after adjustments for age, gender, education, physical activity, smoking, drinking, BMI, diabetes, hypertension, FPG, 2h-PG, HbA1c, HDL-C, LDL-C, TC and TG (HR: 0.760,95% CI: 0.590-0.980, *p*=0.035). We assessed the interaction of vitamin D concentrations with various clinical ASCVD variables; the risk of ASCVD decreased markedly with increased vitamin D concentration in diabetic patients (HR: 0.551, 95%CI: 0.468-0.649, *p* for interaction <0.001), and a similar phenomenon was found in those 60 years or older (Figure 3).

Incidence of ASCVD among populations of different glucose metabolism status within 25(OH)D quartiles

By glycaemic status, 7,061 individuals were classified as NGT (n=3,272), IGR (n=2,001) or T2DM (n=1,788). The 3.3-year cumulative incidence of ASCVD in the three groups was 1.7%, 2.7%, and 5.9%, respectively with no statistically significant difference in ASCVD incidence between the 25(OH)D quartiles in either the NGT and IGR populations. Interestingly, for the T2DM population, the incidences of ASCVD were 8.8%, 5.1%, 6.0%, and 3.6% from the first quartile of serum 25(OH)D to fourth, respectively. Overall, the incidence of ASCVD showed a downward trend with an increase of 25(OH)D level in T2DM patients (*p*=0.003, Figure 4).

Hazard ratios for ASCVD events in T2DM patients with different 25(OH)D concentrations

Compared with the fourth quartile of vitamin D in T2DM patients, the first quartile group had a higher risk of ASCVD. After adjustment for all confounding factors, the trend was still significant, and the hazard ratio of ASCVD events (95% CI) was 2.296 (1.246-4.232) in the lowest quartile compared with the highest (*p*=0.008). For a 10 ng/mL increase in baseline serum 25(OH)D concentration, the risk of ASCVD decreased by about 40% in T2DM patients without adjustment for relevant risk factors (HR: 0.597, 95% CI: 0.415-0.857, *p*=0.005). This significance remained after further adjustment for confounders. Thus, the risk of ASCVD was reduced by 35% for each 10 ng/ml elevation of 25(OH)D in the presence of diabetes (HR: 0.644, 95% CI: 0.440-0.941, *p*=0.023, Table 4).

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DISCUSSION

Vitamin D deficiency is common worldwide.8 A population-based study in metropolitan West Germany measured vitamin D concentrations in 4,149 participants aged 45 to 75 years. It found the median concentration of vitamin D to be 19.8 ng/mL and the prevalence of deficiency (<20 ng/mL) to be 50.6%. Women had a lower vitamin D than men.[21] Our study found the median serum 25(OH)D level of middle-aged and elderly people in urban Lanzhou urban to be 15.91 ng/mL, lower than that found among German residents. The Lanzhou prevalence of vitamin D deficiency was 75.1%, and also more common in women than men (79.6% vs 63.8%), and just 2.0% with vitamin D sufficiency. Our findings are supported by two large-scale surveys in Shanghai and Beijing, which showed that as high as 70% to 90% of the participants had vitamin D deficiency.[22,23] On the whole, the prevalence of vitamin D deficiency is relatively high in China, although there are slight differences by region. Therefore, screening for dietary pattern, use of supplements, sunlight exposure and serum vitamin D may be both public health and clinical nutrition considerations.

Vitamin D deficiency may be associated with chronic disease, causally or consequentially, and this includes ASCVD.[24-25] A dose-response meta-analysis of observational studies suggests that an inverse correlation exists between serum 25(OH)D and cardiovascular events including mortality. CVD mortality is increased by 57%, in people with 25(OH)D <25 ng/mL.[15] For a 10 ng/mL increase in 25(OH)D concentration, there is a decrease of 10% in total cardiovascular events and 12% in cardiovascular mortality.[15] The incidence of ASCVD in our study underwent a marked decline from the lowest of 25(OH)D to the highest after 3.3 years of follow-up. Additionally, for each 10 ng/mL increase in serum 25(OH)D from baseline, ASCVD risk was reduced by 24%, which suggested that 25(OH)D-deficiency is significantly associated with an increased incidence of ASCVD events in the Lanzhou urban population, a finding supported by Chen et al.[26] However, our study further assessed ASCVD incidence by glycaemic status and baseline vitamin D. We found that the 3.3-year cumulative incidence rate of ASCVD in T2DM patients was statistically higher than in those with NGT or IGR. Diabetes is a major risk factor for ASCVD, and our study adds insight into how this might in part be exacerbated. We did not find vitamin D to be related to ASCVD incidence in either the IGR and NGT populations, which would be consistent with such an exacerbation if ‘dose’-related. Those with the lowest 25(OH) D had a 2.3fold increased risk of ASCVD compared with those in the highest category for the T2DM population; and for each 10 ng/ml increase of 25(OH)D, ASCVD risk was reduced by 36%, which indicated that very low serum 25(OH)D presents a danger of ASCVD events for those in theT2DM population. A

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prospective Iranian cohort study also showed that the incidence of CHD in patients with T2DM decreased as serum 25(OH)D increased after followed for a median of 8.5 years.[27] Our follow-up time was shorter, but consistent and indicative of the potential merits of early intervention.

The mechanisms which might explain an association between vitamin D and ASCVD are unknown, but vitamin D may play a cardiovascular protective role by reducing inflammatory factors, inhibiting oxidative stress, preventing the proliferation of vascular smooth muscle cells, or down-regulating the renin-angiotensin-aldosterone system (RAAS).[12,13,28,29] Vitamin D is an immunomodulator, which specifically binds to the vitamin D receptor (VDR) on T lymphocytes to inhibit the proliferation of Th1 cells, reduces the secretion of pro-inflammatory factors such as IL-2 and IFN-γ, so potentially limiting vascular endothelial injury.[12] The VDR also exists in vascular smooth muscle cells (VSMCs). *In vitro*, $1,25(OH)_2D_3$ can induce VDR binding directly to the promoter of vascular endothelial growth factor (VEGF) in VSMCs, increase VEGF synthesis and release, and improve endothelial function. In addition, the active form of vitamin D can inhibit the proliferation of VSMCs by preventing the activation of cyclin-dependent kinase 2 to reduce the impact of cholesterol and lipids.[13] In addition, vitamin D can improve and protect vascular endothelial function by inducing endothelial cells to produce nitric oxide which can protect blood vessels and inhibit oxidative stress.[28] Vitamin D can also negatively regulate the RAAS to interfere with the transcription of the renin gene and reduce the production of renin, thus maintaining blood pressure and protecting cardiovascular function.[29]

Hypertension, hyperglycaemia and dyslipidemia are known to be the major risks for ASCVD.[30-32] In the present study, we found that, with the increased 25(OH)D, hypertension prevalence showed a downward trend, and serum 25(OH)D was negatively correlated with glucolipid metabolism (TG, FPG, 2h-PG and HbA1c), and positively with HDL-C and LDL-C after covariate adjustments. A meta-analysis to back up our findings, demonstrated that vitamin D treatment was linked to a decrease in blood pressure, total TG, TC, LDL and an increase in HDL, such that adequate vitamin D status could decrease the risk for ASCVD.[33]

Advantages in this study include the investigation methods, quality control measures, and large sample size. Furthermore, the ASCVD outcome was robust. Limitations are, firstly, the short follow-up time since it might be expected that the impact of vitamin D on ASCVD would take time. However, the relatively short time to altered incidence augurs well for the merit of early intervention with vitamin D to limit ASCVD, especially in diabetes Secondly,

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whether sunlight exposure in its own right (for which vitamin D may be a surrogate) or vitamin D supplements can alter ASCVD risk has not been ascertained.

In conclusion, a high prevalence of hypovitaminosis D in the Lanzhou region of China is associated with a high incidence of ASCVD in the middle-aged and elderly population, especially in those with T2DM. Additionally, severe deficiency of vitamin D is independently connected with an increased risk of ASCVD. Vitamin D deficiency can now rate as a risk factor for ASCVD in certain locations and populations. Early attention to vitamin D nutritional status and sufficiency may be an added measure for ASCVD prevention and management.

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CONFLICT OF INTEREST AND FUNDING DISCLOSURE

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Table 1. Baseline characteristics of the study participants stratified by 25(OH)D quartiles

Characteristics	Total	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Cut points (ng/mL)	1.35-65.69	≤11.56	11.57-15.91	15.92-19.98	≥19.99
No. of participants	7061	1767	1764	1765	1765
Male vs. female	2012/5049	307/1460	447/1317‡	528/1237‡§	730/1035‡§¶
Age (years)	57.6±8.4	58.9±8.8	57.5±8.6‡	57.2±8.0‡	56.9±8.1‡
Hypertension (%)	2525 (35.8)	702 (39.7)	614 (34.8)‡	608 (34.4) ‡	601 (34.1)‡
Smoking (%)	1151 (16.3)	218 (12.3)	276 (15.6)‡	285 (16.1) ‡	372 (21.1)‡§¶
Drinking (%)	2008 (28.4)	389 (22.0)	484 (27.4)‡	532 (30.1) ‡	603 (34.2)‡§
Education (%)					
High (>9 years)	3928 (55.6)	859 (21.9)	1005 (25.6)‡	1008 (25.7) ‡	1056 (26.9)‡
Low (≤9 years)	3133 (44.4)	908 (29.0)	759 (24.2)‡	757 (24.2) ‡	709 (22.6)‡
Physical activity(%)					
Low	5153 (73.0)	1340 (26.0)	1303 (25.3)	1299 (25.2)	1211 (23.5)‡§¶
Moderate	1712 (24.2)	402 (23.5)	418 (24.4)	415 (24.2)	477 (27.9)‡
Heavy	196 (2.8)	25 (12.8)	43 (21.9)	51 (26.0) ‡	77 (39.3)‡§
Glucose metabolism status					
NGT (%)	3272 (46.3)	757 (42.8)	818 (46.4)	827 (46.9)	870 (49.3)‡
IGR (%)	2001 (28.3)	558 (31.6)	499 (28.3)	491 (27.8)	453 (25.7)‡
T2DM (%)	1788 (25.3)	452 (25.6)	447 (25.3)	447 (25.3)	442 (25.0)
BMI (kg/m^2)	24.17±3.30	24.38±3.60	24.21±3.42	24.06±3.10‡	24.01±3.04‡
FPG (mmol/L)	6.00±1.71	6.09±1.84	6.03±1.76	5.95±1.57‡	5.94±1.65‡
2h-PG (mmol/L) †	7.60 (6.10, 10.00)	7.90 (6.28, 10.50)	7.60 (6.13, 9.90)	7.50 (6.04, 9.86) ‡	7.41 (6.00, 9.70)‡
HbA1c (%)	6.16±1.03	6.18±1.08	6.18±1.06	6.16±1.02	6.10±0.97
HDL-C (mmol/L)	1.23±0.30	1.21±0.31	1.23±0.31	1.23±0.29	1.23±0.30
LDL-C (mmol/L)	2.56±0.77	2.55±0.82	2.56±0.76	2.59±0.77	2.56±0.75
TC (mmol/L)	4.57±1.05	4.57±1.12	4.58±1.07	4.58±1.04	4.53±0.97
TG (mmol/L) †	1.50 (1.07, 2.14)	1.53 (1.09, 2.19)	1.51 (1.06, 2.15)	1.52 (1.05, 2.14)	1.45 (1.06, 2.07)
25 (OH)D (ng/mL) †	15.91 (11.56, 19.98)	9.86 (8.66, 10.64)	13.81 (12.76, 14.85)‡	17.89 (16.84, 18.93)‡§	22.70 (21.19, 24.86)‡§¶

NGT: normal glucose tolerance; IGR: impaired glucose regulation; T2DM: type 2 diabetes mellitus; BMI: body mass index; FPG: fasting plasma glucose; 2h-PG: 2h postprandial plasma glucose; HbA1c: glycated haemoglobin; HDL-C: high density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TC: total cholesterol; TG: triglyceride; 25(OH)D: 25-hydroxyvitamin D.
†Non-normally distributed variables were presented as medians (interquartile ranges); ‡p<0.05 versus Quartile 1; §p<0.05 versus Quartile 2; ¶p<0.05 versus Quartile 3.

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Table 2. Correlations between serum 25(OH)D concentration and glucolipid metabolic index

Index	Unadjusted		Model 1†		Model 2‡	
	Partial correlation coefficient	*p* value	Partial correlation coefficient	*p* value	Partial correlation coefficient	*p* value
FPG (mmol/L)	-0.035	0.003	-0.048	<0.001	-0.044	<0.001
2h-PG (mmol/L)	-0.056	<0.001	-0.053	<0.001	-0.053	<0.001
HbA1c (%)	-0.038	0.001	-0.040	0.001	-0.028	0.019
HDL-C (mmol/L)	0.013	0.274	0.061	<0.001	0.049	<0.001
LDL-C (mmol/L)	0.004	0.743	0.031	0.009	0.035	0.004
TC (mmol/L)	-0.022	0.069	0.018	0.126	0.022	0.060
TG (mmol/L)	-0.047	<0.001	-0.055	<0.001	-0.040	0.001

FPG: fasting plasma glucose; 2h-PG: 2h postprandial plasma glucose; HbA1c: glycated haemoglobin; HDL-C: high density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TC: total cholesterol; TG: triglyceride; 25(OH)D: 25-hydroxyvitamin D.
†Model 1: adjusted for age, gender.
‡Model 2: adjusted for age, gender, education, physical activity, smoking, drinking, body mass index, and history of hypertension and diabetes.

Table 3. Hazard ratios for ASCVD in participants at different 25(OH)D concentrations

Model	Quartile 1	Quartile 2	Quartile 3	Quartile 4	Per 10 ng/mL increase
Incidence rate	4.1 (72/1767)	3.0 (53/1764)	3.1 (55/1765)	2.0 (36/1765)	-
Unadjusted	1.972 (1.322-2.942) *p*=0.001	1.430 (0.937-2.184) *p*=0.098	1.522 (1.000-2.317) *p*=0.050	Ref.	0.696 (0.545-0.890) *p*=0.004
Model 1†	1.902 (1.258-2.877) *p*=0.002	1.458 (0.951-2.235) *p*=0.084	1.561 (1.024-2.381) *p*=0.039	Ref.	0.711 (0.554-0.913) *p*=0.008
Model 2‡	1.762 (1.161-2.673) *p*=0.008	1.392 (0.907-2.136) *p*=0.130	1.510 (0.989-2.305) *p*=0.056	Ref.	0.754 (0.586-0.971) *p*=0.028
Model 3§	1.748 (1.149-2.660) *p*=0.009	1.381 (0.898-2.122) *p*=0.142	1.514 (0.990-2.315) *p*=0.056	Ref.	0.760 (0.590-0.980) *p*=0.035

†Model 1: adjusted for age, gender.
‡Model 2: adjusted for age, gender, education, physical activity, smoking, drinking, diabetes, hypertension, and body mass index.
§Model 3: additionally adjusted for fasting plasma glucose, 2h postprandial plasma glucose, glycated haemoglobin, high density lipoprotein cholesterol, low-density lipoprotein cholesterol, total cholesterol, triglyceride.

Table 4. Hazard ratios for ASCVD in T2DM patients at different 25(OH)D concentrations

Model	Quartile 1	Quartile 2	Quartile 3	Quartile 4	Per 10 ng/mL increase
Incidence rate	8.8(40/452)	5.1(23/447)	6.0(27/447)	3.6(16/442)	-
Unadjusted	2.481(1.389-4.430) *p*=0.002	1.373(0.725-2.599) *p*=0.330	1.666(0.897-3.091) *p*=0.106	Ref.	0.597(0.415-0.857) *p*=0.005
Model 1	2.614(1.434-4.765) *p*=0.002	1.455(0.765-2.768) *p*=0.253	1.759(0.945-3.275) *p*=0.075	Ref.	0.578(0.397-0.843) *p*=0.004
Model 2	2.330(1.274-4.263) *p*=0.006	1.373(0.721-2.615) *p*=0.334	1.749(0.938-3.260) *p*=0.079	Ref.	0.633(0.434-0.921) *p*=0.017
Model 3	2.296(1.246-4.232) *p*=0.008	1.338(0.700-2.565) *p*=0.377	1.730(0.924-3.239) *p*=0.087	Ref.	0.644(0.440-0.941) *p*=0.023

†Model 1: adjusted for age, gender.
‡Model 2: adjusted for age, gender, education, physical activity, smoking, drinking, hypertension, and body mass index.
§Model 3: additionally adjusted for fasting plasma glucose, 2h postprandial plasma glucose, glycated haemoglobin, high density lipoprotein cholesterol, low-density lipoprotein cholesterol, total cholesterol, triglyceride.

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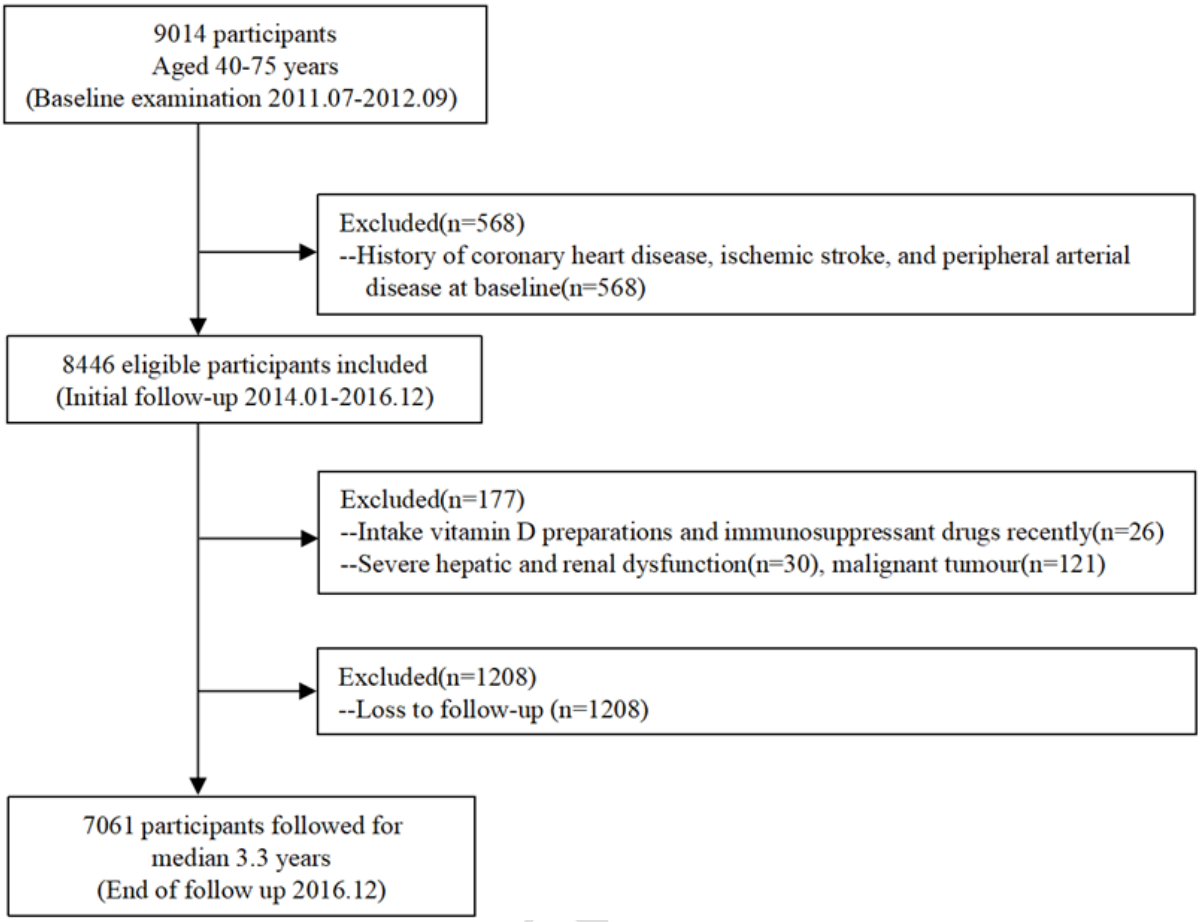

Figure 1. Flow chart for participant selection.

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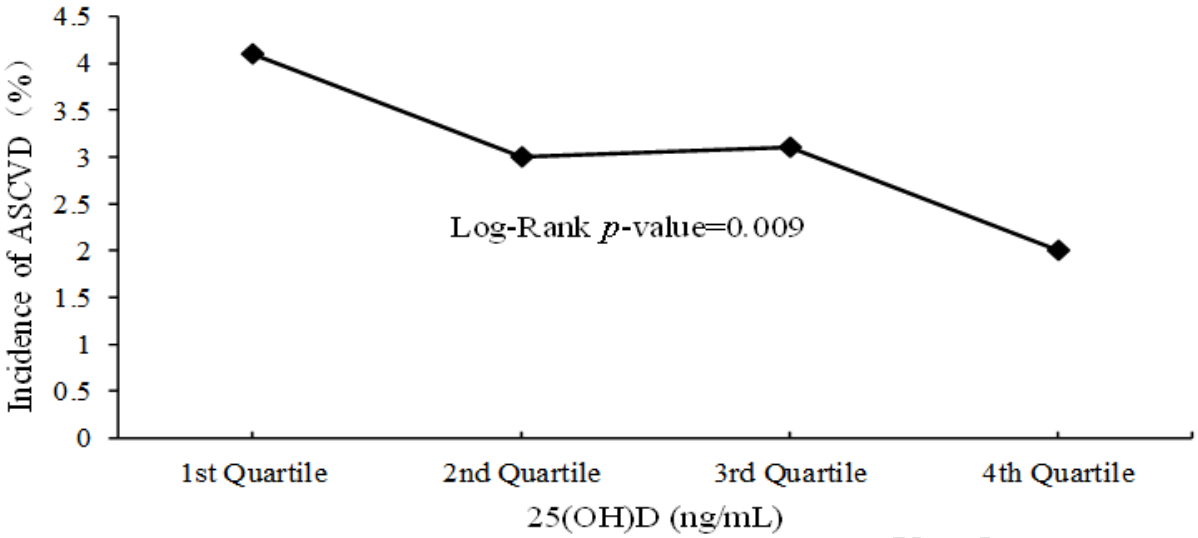

Figure 2. Incidence of ASCVD by 25(OH)D quartile.

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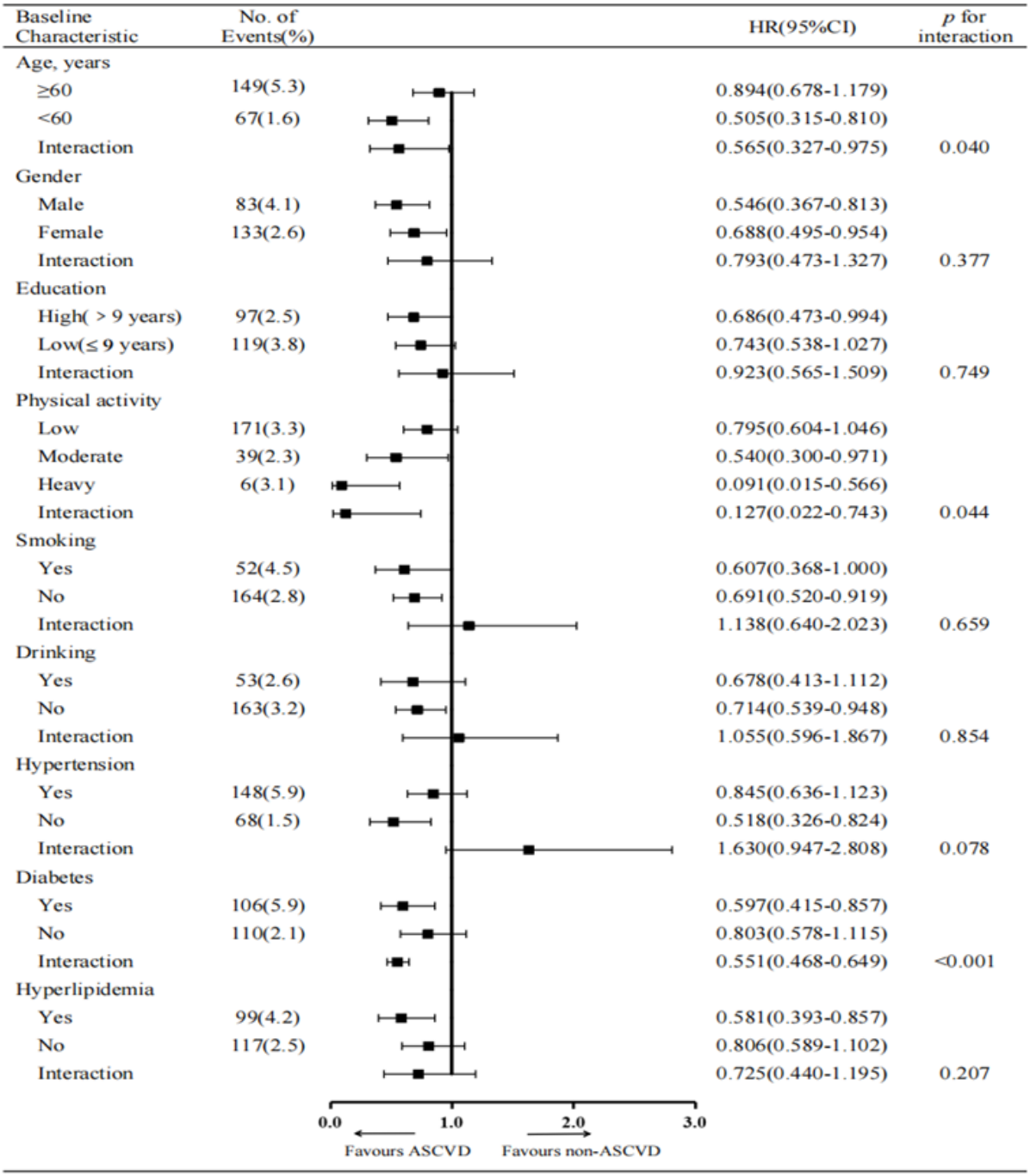

Figure 3. Subgroup analysis of ASCVD with 10 ng/mL per increase of 25(OH)D.

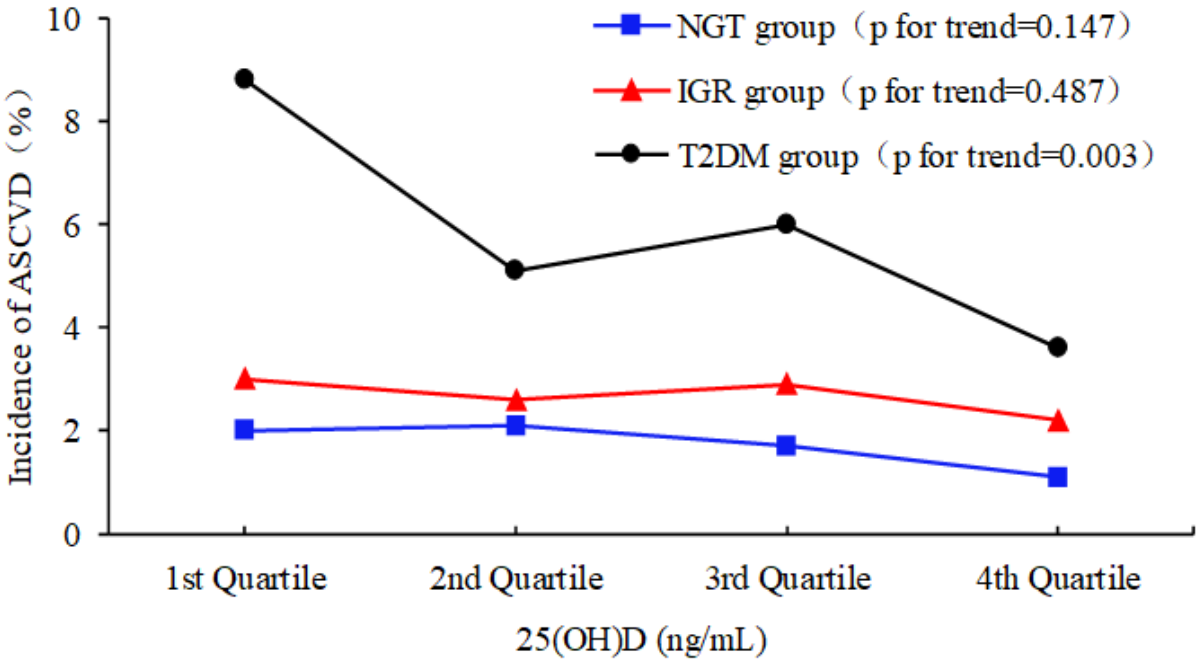

Figure 4. Incidence of ASCVD among populations of different glucose metabolism status within 25(OH)D quartiles.

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