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Association of Plasma Vitamin A Level with Type 2 Diabetes Mellitus: a community aging population-based cross-sectional study

Pengfei Li[a,#], Jingjing Xu[a,#], Yujie Guo[a], Xiaojun Ma[a], Shaobo Zhou[b], Chi Zhang[c], Huiyan Yu[a], Ying Wang[d], Xixiang Wang[a], Linhong Yuan[a,*]

[a] *School of Public Health, Capital Medical University, Beijing, 100069, P.R. China;*

[b] *School of Science, Faculty of Engineering and Science, University of Greenwich, Central Avenue, Chatham ME4 4TB, UK;*

[c] *School of Biological Sciences University of Nebraska Lincoln, NE 68588-0660;*

[d] *The Affiliated Suzhou Science and Technology Town Hospital of Nanjing Medical University, Suzhou, Jiangsu 215153, PR China.*

ABSTRACT: Recent studies indicated that vitamin A (VA) might be involved in the pathology of type 2 diabetes mellitus (T2DM). This cross-sectional study was conducted to explore the association between circulating VA level and T2DM. A total of 1818 subjects aged 50-year-old and above were recruited from the community. Binomial logistic regression and restricted cubic spline (RCS) were applied to analyze the association of plasma VA level with the risk of T2DM. Serum VA and lipid-adjusted VA levels of T2DM patients were significantly higher than that of non-T2DM subjects ($P < 0.05$). The ratios of plasma VA/TC, VA/HDL-c and VA/LDL-c were positively associated with the risk of T2DM in the aging population ($P < 0.05$). Compared with the Q1 level, subjects with Q2 to Q3 levels of plasma VA/TG have decreased risk of T2DM ($OR_{Q2} = 0.68$, $P_{Q2} = 0.021$; $OR_{Q3} = 0.59$, $P_{Q3} < 0.01$). Our results indicated that the imbalance of circulating lipids and VA might affect the relationship between VA and T2DM. The middle and aging subjects with higher ratios of plasma VA/TC, VA/HDL-c, and VA/LDL-c displayed increased risk for T2DM, but the moderate ratio of VA/TG might protect against risk of T2DM.

Keywords: type 2 diabetes mellitus; vitamin A; lipid; nutrition; disease prevention

1. Introduction

Currently, the prevalence of diabetes mellitus (DM) has been increasing dramatically throughout the world, and it has become a global health concern. In 2015, 415 million people were diagnosed with DM, and this number was estimated to 642 million by 2040. Type 2 diabetes mellitus (T2DM) accounted for over 90% of diabetes. Combined with its complications, T2DM contributed numerously to the burden of mortality and disability in the aging population. There was a 15% increased risk of all-cause mortality in subjects with T2DM as compared with non-T2DM population [1,2]. In 2019, the number of diabetes patients in China was about 116.4 million, making China the country with the largest number of diabetes patients in the world. Meanwhile, the number of diabetics continues to rise rapidly. It is predicted that the number of diabetes patients in China will reach 147.2 million in 2045 [3]. Recently, a meta-analysis showed a significant correlation between diabetes and the increased risk of cardiovascular-related diseases, such as coronary heart

# These two authors contribute equally to the work.
***Corresponding author**
ylhmedu@126.com

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disease, myocardial infarction and cerebral infarction [2]. As a result, early screening and timely intervention for diabetes-related risk factors are effective strategies to reduce the prevalence of diabetes and its complications.

Vitamin A is a fat-soluble vitamin with strong antioxidant activity, playing a critical role in cellular antioxidant systems. The defect of antioxidant status has been suggested to be involved in the pathology of T2DM and cardiovascular complications [4]. The protective function of antioxidant vitamin C and vitamin E in diabetes has been extensively reported [5]. However, the application of VA as a therapeutic intervention in T2DM is still in its infancy. Additionally, VA was reported to play an important role in the development of the pancreas [6], reduced hyperglycemia and hyperlipidemia have been observed in VA supplemented obese mice [7]. The deficiency of VA may cause reduced concentration of cytosolic retinol binding protein (CRBP) and cytosolic retinoic acid-binding protein (CRABP) in pancreas, and thus impair the insulin secretion. VA has also been reported to increase glucokinase activity in fetal islets to regulate islet function and insulin release [8,9]. A dietary interventional study found that VA-supplemented STZ-induced diabetic rats showed lower plasma retinol level than the non-diabetic control ones. The drop in plasma retinol level could not be attributed to the dietary VA intake, suggesting the alteration of VA metabolism in these diabetic model animals [10]. All these data highlighted the importance of VA in the disease biology and molecular mechanism involved in T2DM therapy.

Altered oxidation reduction and insulin insensitivity are the typical phenotypes of T2DM. Increasing research evidence demonstrated the regulative impact of VA on insulin release, the augment of insulin signaling, and energy homeostasis. However, the relationship between VA and T2DM is ambiguous. For example, VA supplementation has a regenerative role in the pancreatic tissue damage caused by diabetes [11]. A population-based study showed that T2DM patients have a lower concentration of serum VA than healthy individuals [4]. Vitamin A supplementation in populations with a deficient vitamin A status can delay the onset of T2DM. In contrast, it has also been demonstrated that in certain cases, the abnormal VA metabolism during the onset of diabetes could not be reversed by VA supplementation [12]. A clinical study have established a significant positive correlation of VA and T2DM [13], which was proved by increased serum retinol level in T2DM subjects. Moreover, increased VA intake is positively associated with circulating retinol-binding protein (RBP) level and transport of VA from hepatic stores [14], but the elevated plasma RBP level was found negatively correlated with insulin sensitivity. A study conducted in Canada indicated that T2DM patients (older than 40 years) have similar plasma VA level as the control subjects [15]. Tavridou's study found that subjects with impaired glucose tolerance demonstrated higher serum VA level than those with normal glucose tolerance, implying a role of VA in the development of T2DM diabetes [16]. However, in a Japanese population, the T2DM patients showed lower serum retinol acid (RA) level than the healthy controls [17]. Differences of population, dietary behavior, genetic settings, and disease stages might seemingly contribute to these contradictory results.

Vitamin A deficiency remains a public health problem in the world as well as in China. The decline of the body's digestion and absorptive function predisposed the elderly easily have vitamin A deficiency. As a VA deficiency-prone population, the elderly are also at high risk of diabetes and dyslipidemia. With the rise of the aging population globally, the number of people with diabetes will rise continuously. And the disorder of glucose and lipid metabolism in aging subjects with T2DM might further disturb the metabolism of

lipid-soluble VA. To date, the association of VA with T2DM is not certain, especially, and few studies have explored the relationship between vitamin A and diabetes in the aging population. The present study was to investigate the correlation between plasma vitamin A and T2DM in the elderly, aiming to elucidate the contributions of VA to the development and prevention of T2DM in the middle and aging population. The work will provide a basic scientific dataset for setting the strategy for early dietary intervention and prevention of T2DM in the aging population.

2. Material and Methods

2.1 Study population

A total of 1818 subjects aged 50-year-old and above were recruited from the X community and X community in X city. Diabetes was ascertained according to the guidelines for the prevention and control of T2DM in China (2020 Edition, Chinese Diabetes Society) [18]. The study protocol was approved by the Medical Ethics Committee of X (No.X) *and* conformed to the ethical guidelines of the 1975 Declaration of Helsinki. All the participants provided their written informed consent to participate in this study.

2.2 Demography and dietary survey

A face-to-face interview was conducted to collect demographic, lifestyle, and disease history information of the participants. Anthropometric parameters (height, weight) were measured by nurses in the health center of the community. Body mass index (BMI) was calculated as weight (kilograms) divided by the square of height (meters).

A validated semi-quantitative food frequency questionnaire (FFQ) was used to investigate the daily dietary intake of the subjects. This questionnaire was designed according to the version adopted by the Chinese Nutrition Society. The consumption frequencies (daily and weekly) and the intake amounts of 11 food items were investigated face to face by specifically trained nutritionists and registered nurses. The food-items list included cereal, fruits, vegetables, legume, whole grain, red meat, poultry meat, fish, egg, milk, and cooking oil. Especially, intake of cooking oil was calculated averagely according to the number of family members and the monthly consumed amount of cooking oil.

The dietary balance index was calculated according to the dietary balance index-16 (DBI_16) which was developed according to the Chinese dietary guidelines and Food Guide Pagoda (2016) to reflect the diet quality of the Chinese population [19]. A score of 0 is assigned when the subgroup's food intake met the recommended level. If the subgroup's food intake exceeds or doesn't meet the recommended level, a positive score or a negative score is given, respectively. For lacking intake data of alcoholic beverage, addible sugar, drinking water and salt, we calculated and adjusted three indicators of DBI_16 after calculating each DBI_16 component score. High Bound Score (HBS) is defined as the sum of all positive scores, which indicated an excessive extent of dietary intake. Low Bound Score (LBS) is defined as the absolute value of sum for all negative scores, which indicated the deficient extent of dietary intake. Diet Quality Distance (DQD) is defined as the sum of HBS and LBS, indicating the overall extent of dietary imbalance.

2.3 Plasma parameter measurements

Fasting blood samples were collected from all participants, and plasma was separated for biochemical parameters measurement. Fasting blood glucose (FBG), total cholesterol (TC), and triglyceride (TG) were measured using an ILAB8600 clinical chemistry analyzer (Instrumentation Laboratory, Lexington, WI, USA).

Blood high-density lipoprotein cholesterol (HDL-c) level was measured by a commercially available assay from the Instrumentation Laboratory (Lexington, WI, USA). Blood low-density lipoprotein cholesterol (LDL-c) was calculated according to the Friedewald formula. All samples for each participant were analyzed within a single batch, and the inter-assay coefficients of variation (CV) were less than 5%.

Plasma vitamin A (retinol) level measurement was performed using high-performance liquid chromatography (HPLC) (Waters Chromatograph) according to the method descripted by a previous study [20]. Given the impact of blood lipids on circulating VA concentration, lipids-adjusted plasma VA levels (including the ratios of VA/TC, VA/TG, VA/HDL-c, and VA/LDL-c) were calculated.

2.4 Covariates

We evaluated the modifying effect of demographic characteristics as potential confounding factors, including age, gender (male, female), BMI, drinking alcohol (yes, no), smoking (no, abandon tobacco, current smoking), physical activity (never, 1-3 days/week, 4-6 days/week, every day), the usage of dietary supplement (yes, no), and history of disease, including hyperlipidemia (yes, no), cerebrovascular accident (CVA) (yes, no) and chronic kidney disease (CKD) (yes, no).

2.5 Statistic analysis

All analyses were performed using SPSS 23.0 and R 4.3 software, and all analyses were two-sided. A *P* value less than 0.05 was statistically significant. Continuous and categorical data were represented as mean ± SD or number (n) and percentage (%), respectively. Student's *t* test or chi-square test was applied to compare the differences in demographic characteristics between T2DM and control groups. Mann-Whitney *U* test was used to compare the differences in daily dietary intake and dietary balance index between groups. After adjustment for demographic factors, general linear model (GLM) was applied to analyze the differences of plasma VA and lipid-adjusted VA levels between groups. Pearson correlation was used to explore the relationship between plasma lipids and VA levels. According to the quintiles of plasma VA and lipid-adjusted VA levels, we categorized the participants into Q1 to Q5 groups, respectively (See details in Supplemental Table 1). The prevalence of T2DM was also compared between groups according to the quintile of plasma VA and lipid-adjusted levels by using chi-square test.

Linear regression and binomial logistic regression models were used respectively to analyze the association of plasma VA and lipid-adjusted VA levels with the FBG level and the risk of T2DM. The β coefficient, odds ratio (OR), and corresponding 95% confidence intervals (CIs) were calculated. The variance inflation factor (VIF) was used to determine whether there was multicollinearity in linear and binomial regression analyses. During data analysis, the first quintile was used as reference. In Model 1, covariates including age, gender, BMI, hyperlipidemia, CVA, CKD, smoking, physical activity, drinking alcohol and usage of dietary supplement were adjusted. We further adjusted LBS and HBS in model 2 and DQD in model 3, respectively. The tendency test was conducted with the quintile of plasma VA concentration as an ordered classification variable to observe whether there was a linear trend with the increase of plasma VA concentration. The potential non-linear associations were also explored using restricted cubic spline (RCS) at four knots of 20%, 40%, 60%, and 80% of plasma VA and lipid-adjusted VA levels.

3. Results

3.1 Demographic characteristics

In the current study, subjects who did not accomplish the questionnaire, measurement of anthropometric, dietary investigation, and plasma parameters measurement were excluded. Totally, data from 1818 participants, including 503 T2DM patients and 1315 non-type 2 diabetes (non-T2DM) subjects, were finally used for statistical analysis. As shown in Table 1, the average age of the participants was 66.3 ± 6.3 years. The percentage of male subjects in T2DM group is higher than that in non-T2DM group (38% *vs.* 32.1%, $P < 0.05$). The T2DM subjects have higher BMI than the non-T2DMs ($P < 0.05$). The percentage of subjects with hyperlipidemia (55.7% vs. 38.3%), CVA (10.5% vs. 6.4%) and CKD (9.1% vs. 4.8%) in T2DM group was higher than that in non-T2DM group ($P < 0.05$). In comparison with non-T2DM subjects, the T2DM subjects showed higher FBG and TG levels, but lower TC, HDL-c, and LDL-c levels ($P < 0.05$).

Table 1 Demography of the participants

Variables/Characteristic		T2DM (*n = 503*)	Non-T2DM (*n = 1315*)	Total (*n = 1818*)	*P* value
Age (year)		66.7 ± 6.5	66.2 ± 6.2	66.3 ± 6.3	0.113
Gender (male)		191 (38.0%)	422 (32.1%)	613 (33.7%)	0.018
BMI (kg/m^2)		25.4 ± 3.3	24.9 ± 3.4	25.0 ± 3.4	0.006
Physical activity					
	Never	35 (7.0%)	95 (7.2%)	130 (7.2%)	0.129
	1-3 days/week	47 (9.3%)	138 (10.5%)	185 (10.2%)	
	4-6 days/week	38 (7.6%)	143 (10.9%)	181 (10.0%)	
	Everyday	383 (76.1%)	939 (71.4%)	1322 (72.7%)	
Smoking					
	No	361 (71.8%)	993 (75.5%)	1354 (74.5%)	0.163
	Abandon tobacco	68 (13.5%)	139 (10.6%)	207 (11.4%)	
	Current smoking	74 (14.7%)	183 (13.9%)	257 (14.1%)	
Alcohol drinking (yes)		143 (28.4%)	337 (25.6%)	480 (26.4%)	0.225
Dietary supplement (yes)		132 (26.3%)	301 (22.9%)	433 (23.8%)	0.128
Hyperlipidemia (yes)		280 (55.7%)	504 (38.3%)	784 (43.1%)	< 0.001
CVA (yes)		53 (10.5%)	84 (6.4%)	137 (7.5%)	0.003
CKD (yes)		46 (9.1%)	63 (4.8%)	109 (6.0%)	< 0.001
FBG (mmol/L)		7.57 ± 2.46	5.12 ± 0.56	5.79 ± 1.76	< 0.001
TC (mmol/L)		4.77 ± 1.11	5.08 ± 0.97	4.99 ± 1.02	< 0.001
TG (mmol/L)		1.91 ± 1.58	1.67 ± 1.10	1.73 ± 1.25	0.001
HDL-c (mmol/L)		1.35 ± 0.31	1.46 ± 0.30	1.43 ± 0.31	< 0.001
LDL-c (mmol/L)		2.79 ± 0.96	2.99 ± 0.84	2.93 ± 0.88	< 0.001
VA (μg/ml)		0.55 ± 0.17	0.52 ± 0.16	0.53 ± 0.17	0.006

Data were showed as mean±SD for continuous variables, and n (%) for categorical variables, respectively. BMI, body mass index; FBG, fasting blood-glucose; CVA, cerebrovascular accident; CKD, chronic kidney disease. Student *t* test and chi-square test were used to compare the difference of continuous or categorical variables between T2DM and non-T2DM groups. All statistical analyses were two-sided ($\alpha = 0.05$).

3.2 Daily dietary intake and dietary balance index between groups

As shown in Table 2, the T2DM subjects had lower daily intake of fruits and legume, but higher daily intake of milk and dairy products than non-T2DM subjects ($P < 0.05$). As for dietary pattern, the T2DM subjects showed significant deficiency of daily dietary intake ($P_{LBS} < 0.01$), as well as more imbalance dietary intake ($P_{DQD} < 0.05$) than the non-T2DM subjects. However, there was no statistical difference in dietary over-intake between the two groups ($P_{HBS} > 0.05$).

Table 2 Dietary intake and dietary balance index of T2DM and non-T2DM subjects

Food item (g/d) and DBI		T2DM (*n = 503*)	Non-T2DM (*n = 1315*)	*P* value
Food item				
	Cereal	252.9 ± 103.6	263.7 ±105.6	0.063
	Fruit	131.5 ± 101.6	168.5± 114.2	< 0.001
	Vegetable	315.8 ±144.6	311.1 ± 138.4	0.626
	Legume	31.1 ± 31.0	34.3 ± 31.8	0.019
	Whole grain	39.2 ± 37.0	42.9± 42.8	0.180

	Red meat	31.3 ± 31.2	32.8 ± 32.1	0.381
	Poultry meat	14.5 ± 16.1	15.5 ±18.4	0.615
	Fish	19.5 ± 19.6	21.5± 21.5	0.053
	Egg	36.8 ± 23.5	37.5± 21.0	0.192
	Milk	143.7 ± 113.9	125.2 ± 103.8	0.002
	Cooking oil	31.6 ± 20.2	29.8± 19.2	0.142
DBI				
	LBS	17.4 ± 7.2	16.2 ± 7.1	0.001
	HBS	5.5 ± 5.1	5.8 ± 4.9	0.252
	DQD	22.9 ± 7.4	21.9 ± 7.5	0.016

Data were presented as mean (SD). Mann-Whitney *U* test was used to compare dietary intake between T2DM and control group. DBI: diet balance index; LBS, low bound score; HBS, high bound score; DQD, diet quality distance. All statistical analyses were two-sided (α = 0.05).

3.3 The correlation of plasma VA with lipid level

As shown in Fig. 1, plasma VA level was positively correlated with TG (r = 0.150, P < 0.001), and LDL-c (r = 0.187, P < 0.001) levels in the middle age and aging subjects. There was a significantly negative correlation between plasma VA and HDL-c levels (r = -0.156, P < 0.001).

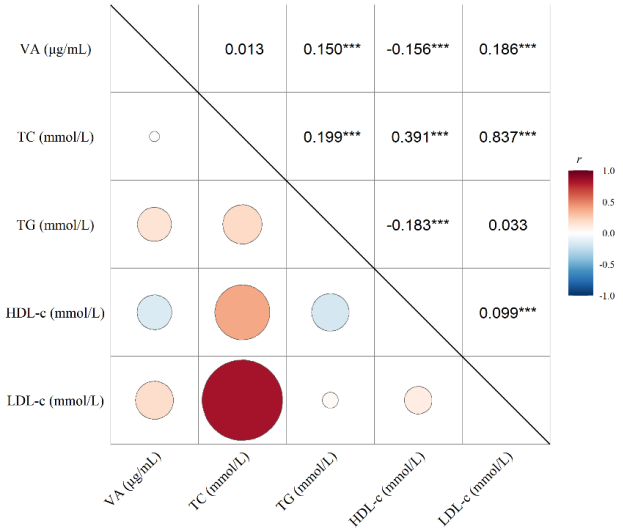
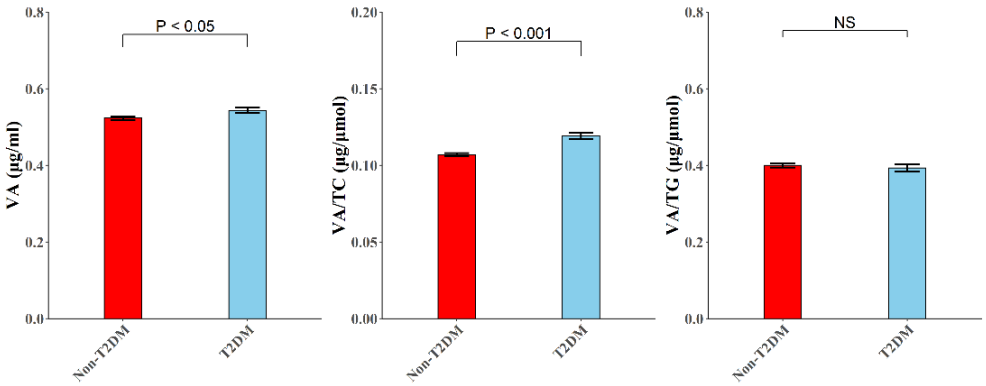


Fig. 1 The correlation of plasma VA and lipid levels. Pearson correlation was used to explore the relationship between blood lipids and VA levels. *P < 0.05; **P < 0.01; ***P < 0.001.

3.4 Differences of plasma VA levels between groups

GLM method was applied to analyze the differences in plasma VA and lipid-adjusted VA levels between groups after adjusting potential confounding factors. Plasma VA concentration of T2DM patients was significantly higher than that of non-T2DM subjects (P < 0.05). After adjusting plasma VA with lipid level, we found that the T2DM subjects continuously showed higher plasma VA/TC, VA/HDL-c, and VA/LDL-c levels than non-T2DM subjects (P < 0.05) (Fig. 2).



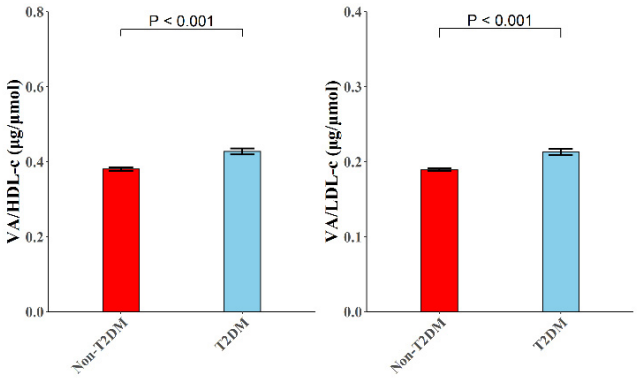


Fig. 2 Plasma VA levels with and without adjustment for circulating lipid levels between T2DM and control groups. Data were expressed as mean±SE. GLM method was performed to compare the difference between groups adjusted by age, gender, BMI, hyperlipidemia, CVA, CKD, smoking, drinking alcohol, physical activity, dietary supplement, DQD. T2DM, type 2 diabetes mellitus. GLM, general linear model. BMI, body mass index. CVA, cerebrovascular accident. CKD, chronic kidney disease. DQD, diet quality distance. All statistical analyses were two-sided (α = 0.05).

3.5 Distribution of T2DM patients according to the quintile of plasma VA level

There is no difference in the percentage of T2DM patients according to the quintile of plasma VA level ($P > 0.05$), we found that subjects with Q1 levels of plasma VA/TC, VA/HDL-c, or VA/LDL-c have the lowest percentage of T2DM patients compared with subjects with Q2 to Q5 levels ($P < 0.05$) (Table 3). We also observed that subjects with Q4 to Q5 level of plasma lipid-adjusted VA showed a higher percentage (more than 30%) of T2DM patients than subjects from other groups ($P < 0.05$). Interestingly, subjects with Q3 level of plasma VA/TG displayed the lowest percentage of T2DM patients in comparison with other groups ($P < 0.05$).

Table 3 Distribution of T2DM patients according to the quintile of plasma VA levels (*n = 1818*)

Parameters	T2DM	Group					χ^2	*P* value
		Q1	Q2	Q3	Q4	Q5		
VA	Yes	90 (24.6%)	95 (26.2%)	98 (26.9%)	108 (29.8%)	112 (30.9%)	4.963	0.291
	No	276 (75.4%)	268 (73.8%)	266 (73.1%)	255 (70.2%)	250 (69.1%)		
VA/TC	Yes	69 (19.0%)	94 (25.8%)	89 (24.5%)	127 (34.8%)	124 (34.3%)	33.332	< 0.001
	No	295 (81.0%)	270 (74.2%)	274 (75.5%)	238 (65.2%)	238 (65.7%)		
VA/TG	Yes	115 (31.7%)	92 (25.1%)	80 (22.2%)	121 (33.2%)	95 (26.2%)	15.481	0.004
	No	248 (68.3%)	275 (74.9%)	281 (77.8%)	244 (66.8%)	267 (73.8%)		
VA/HDL-c	Yes	66 (18.1%)	96 (25.7%)	98 (27.8%)	116 (31.9%)	127 (35.1%)	30.661	< 0.001
	No	299 (81.9%)	278 (74.3%)	255 (72.2%)	248 (68.1%)	235 (64.9%)		
VA/LDL-c	Yes	67 (18.1%)	86 (23.9%)	106 (28.7%)	109 (30.7%)	135 (37.2%)	37.973	< 0.001
	No	304 (81.9%)	274 (76.1%)	263 (71.3%)	246 (69.3%)	228 (62.8%)		

Data were showed as n (%). Chi-square test was performed to compare the difference of T2DM prevalence between groups according to the quintile of plasma VA levels. All statistical analyses were two-sided (α = 0.05).

3.6 Association of plasma VA and FBG level

The data in Table 4 showed that there was no significant multicollinearity between independent variables. Model 1 showed that there was no association between plasma VA, VA/TC levels with FBG concentration ($P > 0.05$, $P_{trend} > 0.05$). After further adjusting HBS, LBS or DQD (model 2 & 3), there was still no significant relationship ($P > 0.05$, $P_{trend} > 0.05$) (Table 4). The results of RCS analysis also showed that there was no statistical association between plasma VA, VA/TC levels with FBG concentration (Fig. 3 and Supplemental Fig. 1).

Table 4 The associations of plasma VA and lipid-adjusted VA levels with FBG concentration (*n = 1818*)

Parameter	N	Model 1			Model 2			Model 3			VIF
		β (95%CI)	*P*	P_{trend}	*β* (95%CI)	*P*	P_{trend}	*β* (95%CI)	*P*	P_{trend}	
VA											
Q1	366	Ref	-		Ref	-		Ref	-		
Q2	363	-0.07 (-0.32, 0.19)	0.593	0.185	-0.07 (-0.32, 0.19)	0.611	0.132	-0.07 (-0.32, 0.19)	0.593	0.186	1.02 – 1.94
Q3	364	0.12 (-0.14, 0.38)	0.367		0.11 (-0.15, 0.37)	0.398		0.12 (-0.14, 0.38)	0.367		
Q4	363	0.01 (-0.25, 0.26)	0.948		-0.01 (-0.27, 0.24)	0.931		0.01 (-0.25, 0.26)	0.949		
Q5	362	-0.23 (-0.49, 0.02)	0.076		-0.25 (-0.51, 0.01)	0.059		-0.23 (-0.49, 0.02)	0.076		
VA/TC											
Q1	364	Ref	-		Ref	-		Ref	-		
Q2	364	0.12 (-0.14, 0.38)	0.353	0.558	0.10 (-0.15, 0.36)	0.433	0.442	0.12 (-0.14, 0.38)	0.353	0.559	1.01 – 1.96
Q3	363	0.11 (-0.15, 0.37)	0.426		0.09 (-0.17, 0.35)	0.514		0.11 (-0.15, 0.37)	0.426		
Q4	365	0.08 (-0.18, 0.34)	0.529		0.06 (-0.20, 0.32)	0.650		0.08 (-0.18, 0.34)	0.529		
Q5	362	-0.07 (-0.33, 0.20)	0.614		-0.09 (-0.36, 0.17)	0.490		-0.07 (-0.33, 0.20)	0.614		
VA/TG											
Q1	363	Ref	-		Ref	-		Ref	-		
Q2	367	-0.34 (-0.60, -0.09)	0.008	< 0.001	-0.33 (-0.58, -0.07)	0.012	< 0.001	-0.34 (-0.60, -0.09)	0.008	< 0.001	1.02 – 1.95
Q3	361	-0.51 (-0.77, -0.25)	< 0.001		-0.49 (-0.75, -0.24)	< 0.001		-0.51 (-0.77, -0.25)	< 0.001		
Q4	365	-0.61 (-0.86, -0.35)	< 0.001		-0.59 (-0.85, -0.34)	< 0.001		-0.61 (-0.86, -0.35)	< 0.001		
Q5	362	-0.77 (-1.02, -0.51)	< 0.001		-0.76 (-1.02, -0.50)	< 0.001		-0.77 (-1.03, -0.51)	< 0.001		
VA/HDL-c											
Q1	365	Ref	-		Ref	-		Ref	-		
Q2	374	0.15 (-0.11, 0.40)	0.255	0.130	0.14 (-0.12, 0.39)	0.300	0.186	0.15 (-0.11, 0.40)	0.255	0.131	1.02 – 1.97
Q3	353	0.35 (0.09, 0.61)	0.009		0.33 (0.07, 0.59)	0.013		0.35 (0.09, 0.61)	0.009		
Q4	364	0.32 (0.06, 0.58)	0.017		0.30 (0.04, 0.56)	0.026		0.32 (0.06, 0.58)	0.017		
Q5	362	0.15 (-0.12, 0.41)	0.271		0.12 (-0.14, 0.39)	0.360		0.15 (-0.12, 0.41)	0.271		
VA/LDL-c											
Q1	371	Ref	-		Ref	-		Ref	-		
Q2	360	0.07 (-0.19, 0.33)	0.596	0.308	0.04 (-0.21, 0.30)	0.738	0.427	0.07 (-0.19, 0.33)	0.598	0.308	1.01 – 1.95
Q3	369	0.07 (-0.19, 0.32)	0.614		0.04 (-0.22, 0.30)	0.770		0.07 (-0.19, 0.32)	0.615		
Q4	355	0.05 (-0.21, 0.31)	0.697		0.03 (-0.24, 0.29)	0.851		0.05 (-0.21, 0.31)	0.696		
Q5	363	0.16 (-0.10, 0.42)	0.226		0.13 (-0.13, 0.39)	0.336		0.16 (-0.10, 0.42)	0.226		

Model 1 was adjusted by age, gender, BMI, CVA, CKD, smoking, physical activity, drinking alcohol, dietary supplement (for VA, model 1 was also adjusted by hyperlipidemia); Model 2 = Model 1 + LBS+HBS; Model 3 = Model 1 + DQD. BMI, body mass index. CVA, cerebrovascular accident. CKD, chronic kidney disease. LBS, low bound score. HBS, high bound score. DQD, diet quality distance.

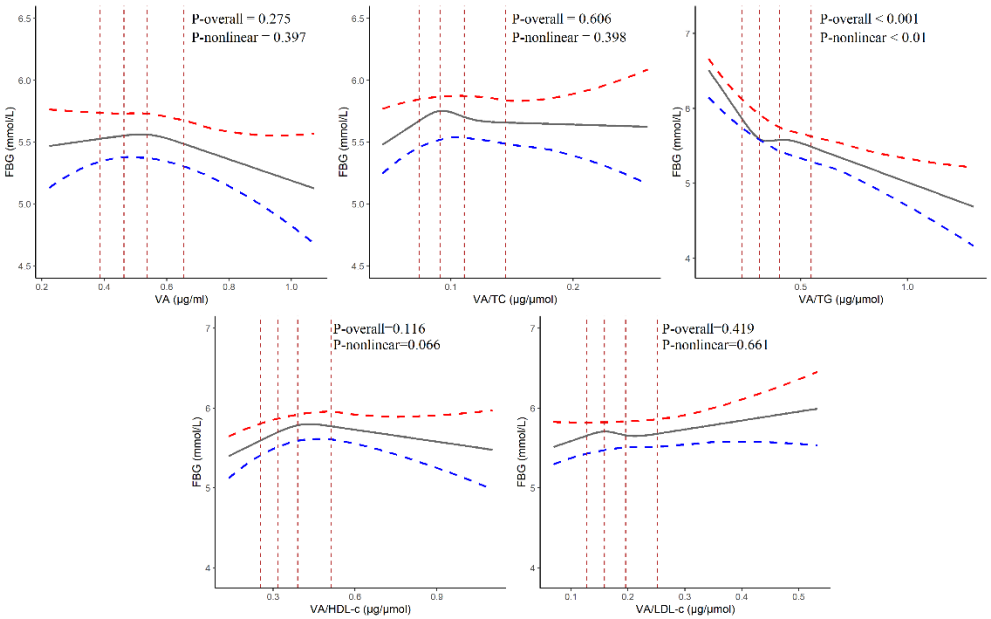


Fig. 3 Restricted cubic spline for association between plasma VA and lipid-adjusted VA levels with FBG concentration. The linear regression was fitted by restricted cubic spline to analyze the overall and nonlinear associations with four knots at 20%, 40%, 60%, and 80% cut-off points. Model 1 was adjusted by age, gender, BMI, CVA, CKD, smoking, physical activity, drinking alcohol, dietary supplement (for VA, model 1 was also adjusted by hyperlipidemia). FBG, fast blood glucose. BMI, body mass index. CVA, cerebrovascular accident. CKD, chronic kidney disease.

After adjusting for demographic and dietary variables, a negative association of plasma VA/TG level with FBG concentration was observed consistently in three models ($P < 0.05$). The trend test further demonstrated the negative linear association ($P_{trend} < 0.001$) (Table 4). Although a nonlinear trend showed statistical significance ($P_{nonlinear} < 0.01$), results of RCS analysis also indicated that, at the quintile nodes, plasma VA/TG level and FBG concentration displayed a significant negative linear trend ($P_{overall} < 0.001$) (Fig. 3 and Supplemental Fig. 1).

A non-linear association of VA/HDL-c with FBG was observed in three models, and the positive relationship between VA/HDL-c with FBG became statistically significant from Q3 and Q4 groups ($P_{Q3} = 0.009$; $P_{Q4} < 0.05$). The results of the trend test also showed a nonlinear trend ($P_{trend} > 0.05$) (Table 4). The results of RCS analysis also showed a nonlinear relationship between plasma VA/HDL-c and FBG levels, but the nonlinear trend was not statistically significant ($P_{overall} > 0.05$; $P_{nonlinear} > 0.05$) (Fig. 4 and Supplementary Fig. 2).

We did not find any significant association between plasma VA/LDL-c level and FBG concentration ($P > 0.05$) (Table 4, Fig. 3 and Supplemental Fig. 2).

3.7 3.7 Association of plasma VA levels with the risk of T2DM

The data in Table 5 showed that there was no significant multicollinearity between independent variables. There was no significant relationship between plasma VA concentration and the risk of T2DM ($P > 0.05$, $P_{trend} > 0.05$) (Table 5), and the results of RCS analyses showed a slight linear increase in the risk of T2DM, but the relationship was not statistically significant ($P_{overall} > 0.05$; $P_{nonlinear} > 0.05$) (Fig. 4 and Supplemental Fig. 3).

Table 5 The associations of plasma VA, lipid-adjusted VA levels with risk of T2DM (*n = 1818*)

Parameter	N	Model 1			Model 2			Model 3			VIF
		OR (95%CI)	*P*	P_{trend}	OR (95%CI)	*P*	P_{trend}	OR (95%CI)	*P*	P_{trend}	
VA											
Q1	366	1.00 (Ref)	-		1.00 (Ref)	-		1.00 (Ref)	-		
Q2	363	1.04 (0.74, 1.47)	0.826	0.067	1.05 (0.74, 1.48)	0.785	0.112	1.04 (0.74, 1.47)	0.816	0.068	1.02 – 1.95
Q3	364	1.04 (0.74, 1.47)	0.820		1.03 (0.73, 1.45)	0.873		1.04 (0.74, 1.47)	0.829		
Q4	363	1.22 (0.87, 1.72)	0.245		1.19 (0.85, 1.67)	0.317		1.23 (0.88, 1.73)	0.228		
Q5	362	1.31 (0.93, 1.84)	0.118		1.28 (0.91, 1.80)	0.160		1.31 (0.93, 1.84)	0.123		
VA/TC											
Q1	364	1.00 (Ref)	-		1.00 (Ref)	-		1.00 (Ref)	-		
Q2	364	1.44 (1.01, 2.06)	0.046	< 0.001	1.40 (0.98, 2.01)	0.066	< 0.001	1.44 (1.01, 2.07)	0.044	< 0.001	1.01 – 1.93
Q3	363	1.28 (0.89, 1.84)	0.187		1.24 (0.86, 1.79)	0.249		1.28 (0.89, 1.84)	0.189		
Q4	365	2.19 (1.55, 3.11)	< 0.001		2.11 (1.49, 3.01)	< 0.001		2.19 (1.55, 3.11)	< 0.001		
Q5	362	2.12 (1.49, 3.02)	< 0.001		2.03 (1.43, 2.91)	< 0.001		2.11 (1.49, 3.02)	< 0.001		
VA/TG											
Q1	363	1.00 (Ref)	-		1.00 (Ref)	-		1.00 (Ref)	-		
Q2	367	0.68 (0.49, 0.94)	0.021	0.701	0.70 (0.50, 0.98)	0.036	0.809	0.68 (0.49, 0.94)	0.021	0.750	1.02 – 1.92
Q3	361	0.59 (0.42, 0.83)	0.002		0.61 (0.43, 0.85)	0.004		0.59 (0.42, 0.83)	0.002		
Q4	365	1.04 (0.75, 1.43)	0.821		1.08 (0.78, 1.49)	0.639		1.05 (0.76, 1.44)	0.783		
Q5	362	0.76 (0.54, 1.05)	0.101		0.77 (0.55, 1.08)	0.133		0.76 (0.55, 1.07)	0.113		
VA/HDL-c											
Q1	365	1.00 (Ref)	-		1.00 (Ref)	-		1.00 (Ref)	-		
Q2	374	1.52 (1.06, 2.18)	0.023	< 0.001	1.49 (1.04, 2.15)	0.031	< 0.001	1.52 (1.06, 2.19)	0.022	< 0.001	1.02 – 1.94
Q3	353	1.70 (1.19, 2.45)	0.004		1.67 (1.16, 2.40)	0.006		1.70 (1.19, 2.45)	0.004		
Q4	364	2.01 (1.41, 2.88)	< 0.001		1.94 (1.36, 2.79)	< 0.001		2.00 (1.40, 2.87)	< 0.001		
Q5	362	2.39 (1.68, 3.43)	< 0.001		2.31 (1.62, 3.32)	< 0.001		2.40 (1.68, 3.44)	< 0.001		
VA/LDL-c											
Q1	371	1.00 (Ref)	-		1.00 (Ref)	-		1.00 (Ref)	-		
Q2	360	1.46 (1.02, 2.11)	0.042	< 0.001	1.43 (0.99, 2.07)	0.058	< 0.001	1.49 (1.03, 2.15)	0.034	< 0.001	1.01 – 1.93
Q3	369	1.81 (1.28, 2.60)	0.001		1.75 (1.23, 2.51)	0.002		1.83 (1.29, 2.62)	0.001		
Q4	355	2.01 (1.41, 2.88)	< 0.001		1.93 (1.35, 2.77)	< 0.001		2.01 (1.41, 2.88)	< 0.001		
Q5	363	2.56 (1.81, 3.65)	< 0.001		2.44 (1.72, 3.48)	< 0.001		2.56 (1.81, 3.65)	< 0.001		

Model 1 was adjusted by age, gender, BMI, CVA, CKD, smoking, physical activity, drinking alcohol, dietary supplement (for VA, model 1 was also adjusted by hyperlipidemia); Model 2 = Model 1 + LBS+HBS; Model 3 = Model 1 + DQD. BMI, body mass index. CVA, cerebrovascular accident. CKD, chronic kidney disease. LBS, low bound score. HBS, high bound score. DQD, diet quality distance.

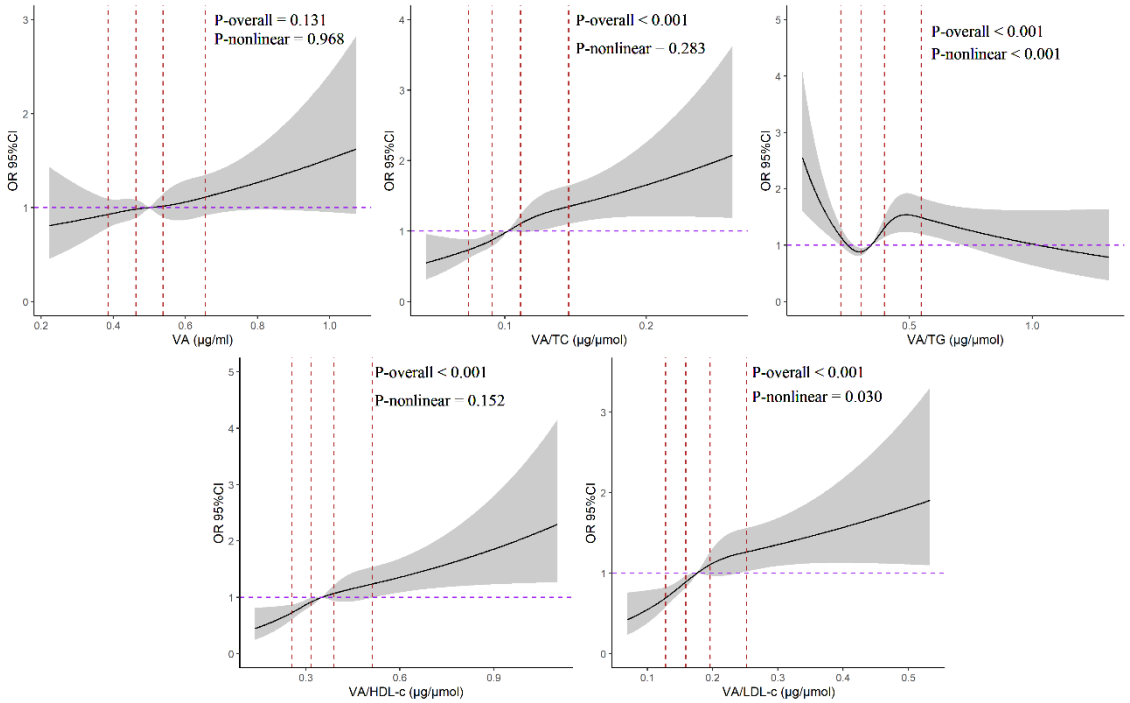


Fig. 4 Restricted cubic spline for association between plasma VA and lipid-adjusted VA levels with risk of T2DM. The logistic regression was fitted by restricted cubic spline to analyze the overall and nonlinear associations with four knots at 20%, 40%, 60%, and 80% cut-off points. Model 1 was adjusted by age, gender, BMI, CVA, CKD, smoking, physical activity, drinking alcohol, dietary supplement (for VA, model 1 was also adjusted by hyperlipidemia). T2DM, type 2 diabetes mellitus. BMI, body mass index. CVA, cerebrovascular accident. CKD, chronic kidney disease.

As shown in Table 5, the higher plasma VA/TC level increased the risk of T2DM in the aging population, and in comparison with the Q1 level, statistical significance at Q2, Q4 and Q5 levels was observed ($P < 0.05$). The trend test further confirmed the positive impact of plasma VA/TC level on the risk of T2DM (OR_{Q2} = 1.44, P_{Q2} = 0.046; OR_{Q4} = 2.19, $P_{Q4} < 0.001$; OR_{Q5} = 2.12, $P_{Q5} < 0.001$; $P_{trend} < 0.001$). RCS analysis also demonstrated a linear positive association between plasma VA/TC level and the risk of T2DM ($P_{overall} < 0.001$) (Fig. 4 and Supplemental Fig. 3). Even after further adjustment for HBS, LBS or DQD, the statistical relationship remained unchanged.

In model 1, we detected a non-linear relationship between plasma VA/TG level with the risk of T2DM. Compared with the Q1 level, subjects with Q2 to Q3 level of plasma VA/TG level have decreased risk of T2DM (OR_{Q2} = 0.68, P_{Q2} = 0.021; OR_{Q3} = 0.59, P_{Q3} = 0.002). After adjusting for the dietary balance index in model 2 and model 3, the negative relationship continuously existed in Q2 and Q3 levels ($P < 0.05$). The RCS analysis results also showed that the risk of T2DM in subjects with Q2 and Q3 levels of plasma VA/TG was lower than that at the Q1 level, and a statistically significant nonlinear trend was also observed ($P_{nonlinear} < 0.001$) (Table 5, Fig. 4 and Supplemental Fig. 3).

Significant positive associations of plasma VA/HDL-c and VA/LDL-c levels with the risk of T2DM were found in the current study. Following the increase of plasma VA/HDL-c and VA/LDL-c levels from Q2 to Q5 levels, the risk of T2DM increased gradually, and in comparison with the Q1 group, the differences reached statistical significance ($P < 0.05$). The results of the trend test also demonstrated the positive linear relationships between plasma VA/HDL-c and VA/LDL-c levels with the risk of T2DM in aging subjects ($P_{trend} < 0.001$), even further adjusting dietary balance index in model 2 and model 3, the relationship remained

unchanged (Table 5). Results of RCS analysis also showed a linear increased risk of T2DM with the increase of plasma VA/HDL-c and VA/LDL-c levels in three models ($P_{overall} < 0.001$) (Fig. 4 and Supplemental Fig. 4).

4. Discussion

Diabetes is a metabolic disease characterized by increased blood glucose and insulin levels. High blood glucose level will lead to chronic damage of various tissues, as well as disturbed lipid metabolism. It was reported that diabetic patients with dyslipidemia mainly showed elevated TG, decreased HDL-C, and elevated or normal LDL-C levels [21]. Consistently, in the current study, we found that T2DM subjects displayed higher plasma TG and lower HDL-c levels than the non-T2DM ones, and the T2DM subjects have higher percentage of subjects with hyperlipidemia, CVA, and CKD than the non-T2DM, further demonstrating a lipid metabolism disorder in aging subjects with T2DM.

According to the recommendation of WHO, blood retinol at the concentration of 0.35 - 0.70 μmol/L, 0.70 - 1.05 μmol/L, and ≥ 1.05 μmol/L was a VA deficiency, marginal VA deficiency, and VA sufficient, respectively [22]. Wang's study reported that the serum VA level of the Chinese urban elderly was 1.92 (1.50 – 2.45) μmol/L [23]. Another study conducted in Chongqing city of China also found that the average serum VA level in subjects aged over 50 years was 1.27 μmol/L [24]. Consistently, our study found the average concentration of the participants was 0.53 ± 0.17 μg/ml (1.86 ± 0.60 μmol/L) (Table 1), demonstrating that the middle age and aging subjects were in an optimal VA nutritional status.

Li's study found that individuals receive 70-90% of their retinoid from provitamin A carotenoids in developing countries, and up to 75% of daily total dietary retinoid comes from preformed retinoid in industrialized countries [25]. A study conducted in the United States found that South Asians living in the United States with T2DM actually have less daily VA intake than the controls [26]. The strong correlation between dietary VA intake and a higher risk of metabolic syndrome was also demonstrated in Iranian women [27]. The study conducted in the Alberta region of Canada, however, found that T2DM patients (older than 40 years) had similar daily VA intake and plasma VA level as the control subjects [15]. These results suggested the potential impact of dietary VA intake on circulating VA level in subjects with T2DM. In the current study, our data further showed that the dietary pattern of the T2DM and the non-T2DM subjects was significantly different, which was demonstrated by lower daily fruit and legume, but higher milk intake in T2DM subjects (Table 2). Besides, the significant differences in LBS and DQD between groups further indicated the discrepancy of dietary patterns between the T2DMs and the non-T2DMs. Given the essential role of dietary VA intake in affecting an individuals' VA nutritional status, we, therefore, took dietary balance indexes as potential covariates during analyzing the association between plasma VA level and T2DM.

Researchers reported a significant increase in retinol levels in the liver of patients with diabetes. Data from clinical studies and animal experiments also showed that diabetes caused abnormal VA metabolism *in vivo* [28], indicating an imbalance in VA level in diabetic patients with disrupted homeostasis of blood sugar, lipids, and insulin. In the current study, T2DM subjects have higher plasma VA level than the non-T2DM subjects (Table 1). After adjusting potential confounding factors, the differences in plasma VA concentrations between groups were continuously maintained (Fig. 2). These results were in line with the data derived from the studies conducted in Japanese and French populations, which reported that T2DM patients showed significantly higher circulating retinol level than the control ones [13,29]. Besides, subjects with impaired glucose tolerance demonstrated a higher serum VA level than those with normal glucose tolerance [16]. In

contrast, similar blood retinol level was found in T2DM patients (28-74 years) and control subjects in Saudi Arabia [30]. Recently, Jyothi's case-control study found a significantly positive correlation of serum retinol with TC and LDL-c in subjects with T2DM, and reported that serum retinol could be a predictor of cardiovascular risk in subjects with T2DM [31]. Although the relationship between the blood VA level and T2DM remained unclear, the published documents suggested a potential association of VA nutritional status with T2DM.

A study found that retinoic acid supplement increased circulating TG level in rats [32]. In a population-based randomized controlled trial, Farhangi and coworkers found that the serum TG level of women who took VA supplements was higher than that of control subjects [33]. Cartmel also reported that retinol supplement caused a significant increase in serum triacylglycerol in a population at moderate risk of skin cancer [34]. In our study, we found a significantly positive correlation of plasma VA and TG level (Fig. 1), suggesting that plasma VA positively affects circulating TG level. After stratifying the subjects according to the quintile of plasma VA level, we failed to detect the significant association of plasma VA with FBG, as well as plasma VA with the risk of T2DM. Interestingly, after adjusting plasma VA level with TG, we detected a significant negative association of plasma VA/TG with FBG level (Table 4, Fig. 3 and Supplemental Fig. 1). It was reported that the diabetes with dyslipidemia exhibited elevated circulating TG level, and the T2DM patients with longer disease duration displayed much higher blood TG level [35,36]. Existed hypertriglyceridemia in the diabetics could be exacerbated due to loss of glycemic control, which was demonstrated by elevated FBG and serum TG levels [37,38]. A study conducted in the Chinese elderly also found that T2DM and prediabetic subjects had elevated serum TG level than the non-T2DM and non-prediabetes subjects [39]. In our study, we consistently observed a significant increase in plasma TG and VA levels in T2DM patients (Table 1 and Fig. 2), suggesting that, for middle age and aging subjects with high plasma TG level, deficient VA intake or lower plasma VA level might increase their susceptibility to have higher FBG level and sufficient VA intake should be suggested to subjects with hypertriglyceridemia for maintaining blood glucose level.

Association analysis of plasma VA/TG and risk of T2DM indicated that subjects with Q2 (the corresponding average VA and TG levels are 0.478 ± 0.141 μg/ml and 1.808 ± 0.543 mmol/L, respectively) and Q3 (the corresponding average VA and TG levels are 0.513 ± 0.158 μg/ml and 1.477 ± 0.455 mmol/L, respectively) levels of plasma VA/TG showed a significantly decreased risk of T2DM (Table 5 and Supplemental Table 2). The non-linear association proved by the trend test and RCS analysis further indicated that optimal plasma VA/TG is beneficial to attenuate the risk of T2DM. According to our data, we suggested a reference of circulating VA/TG ratio ranging from 0.224 μg/μmol to 0.400 μg/μmol for middle age and aging subjects to decrease the risk for T2DM (Supplemental Table 1).

The relationship between plasma cholesterol with VA status remains unclear. Data from experimental animals suggested that VA promoted cholesterol clearance in mice fed with a high-fat diet [40]. However, in Cartmel's randomized controlled trial study, the authors reported a significant increase in serum TC and LDL-c levels, but a decrease in HDL-c after retinyl palmitate supplement for 3.8 years [34]. The study of the elderly in Chongqing city in China showed that serum VA level significantly affected serum LDL-c level, but had no effect on HDL-c and TC levels, indicating that LDL-c may be the main target of VA-mediated regulation of blood cholesterol [24]. In the present study, a strong correlation between plasma cholesterol and

VA levels was also detected, which was demonstrated by significantly positive correlation of plasma VA with LDL-c levels, but negative correlation with HDL-c level (Fig. 1). We also found that the T2DM subjects had higher plasma VA level, but lower TC, HDL-c, and LDL-c levels than the non-T2DM subjects. Additionally, we also found a dramatically increased prevalence of T2DM in subjects with plasma VA/TC (≥ 0.112 μg/μmol), VA/HDL-c (≥ 0.391 μg/μmol), and VA/LDL-c (≥ 0.197 μg/μmol) at and above Q4 level (Table 3 and Supplemental Table 1). Consistently, the dramatically increased *OR* value following the increase of plasma VA/TC, VA/HDL-c and VA/LDL-c from Q2 to Q5 levels further demonstrated a significantly positive associations of VA/TC, VA/HDL-c, and VA/LDL-c with the risk of T2DM. The results of the trend test and RCS analysis also displayed a positive linear relationship between plasma VA/TC, VA/HDL-c, and VA/LDL-c levels with the risk for T2DM. These results indicated the abnormal glucose and lipid metabolisms in T2DM might modify the relationships between TC, LDL-c with VA, and subjects with the increased level of plasma cholesterol-adjusted VA might be at higher risk for T2DM. Although our data indicated that high plasma VA to cholesterol ratio might indicated a significantly increased T2DM risk, we could not conclude that high plasma VA and low cholesterol is a risk factor for T2DM in the middle-age and elderly. The increase of blood VA to cholesterol ratio may be due to different circumstances, for example, a significant increase in plasma VA accompanied by the unchanged or decreased plasma TC level, and a significant increase in plasma VA level accompanied with a marginal increase of TC level ect. Therefore, we speculated that the disequilibrated relationship between circulating VA and cholesterol under the abnormal glycolipid metabolism status in T2DM patients might partly explain the negative relation between VA to cholesterol ratio and the risk of T2DM.

The positive correlation of cholesterol-adjusted VA with T2DM might be explained by the potential regulating effect of VA on circulating cholesterol levels. Feng's study found that, in comparison with the subjects with normal circulating LDL-c (2.34 - 3.38 mmol/L), the risk of diabetes increased more than one fold in the subjects with very low circulating LDL-c (< 1.04 mmol/L, *OR* = 2.31), and significant association of very low LDL-c with diabetes was found in normal and obesity individuals [41]. Moreover, plasma LDL is mainly derived from VLDL particles, hence VA supplement-mediated LDL-c elevation might be due to an enhanced hepatic VLDL production [42], and the down-regulation of hepatic apolipoprotein A-I gene expression might be attributable to VA-mediated decrease in serum HDL-c [43,44]. Some studies also reported that supplement of synthetic retinoids caused an increase in blood TC and LDL-c levels and a concomitant decrease in HDL-c level [45,46], indicating a physiological adjustment of cholesterol metabolism in response to VA supplement. Similarly, we observed plasma VA positively correlated with TC and LDL-c, but negatively correlated with HDL-c level in the middle and aging subjects (Fig. 1).

Unexpectedly, we found that the T2DM subjects showed significantly lower plasma TC, HDL-c, and LDL-c levels, but higher plasma VA level than the non-T2DM subjects (Table 1). Reduced HDL-c and elevated LDL-c levels were demonstrated as the typical diabetic dyslipidemia in T2DM patients [47,48]. Low TC and LDL-c concentration in T2DM group in the current study might be explained by the use of LDL-c lowering medicine, such as statin, ezetimibe and evolocumab [47,48]. Interestingly, RA was reported to inhibit high fat diet-induced obesity through the activation of nuclear RA receptors (RARs) and peroxisome proliferator–activated receptor β/δ (PPARβ/δ) [49], which is potential signaling pathways to reduce plasma TC and LDL-c levels in T2DM patients. Moverover, the increased transfer of TG into LDL in exchange for

LDL cholesteryl ester at the present of cholesteryl ester transfer protein (CETP) was reported during insulin resistance, which might lead to a smaller and denser lipid-depleted LDL particle [48]. Therefore, the unparalleled change of plasma VA with TC and LDL-c in T2DM subjects might indicate a retarded adaptive adjustment of cholesterol metabolism to increase of circulating VA level in T2DM patients with dyslipidemia. Additionally, more studies are needed to interpret the unexpected reductions of TC and LDL-c levels in T2DM individuals. Following the increase of plasma VA/LDL-c ratio from Q1 to Q5 level, the average plasma LDL-c level decreased dramatically from 3.624 ± 0.816 mmol/L to 2.124 ± 0.628 mmol/L (Supplemental Table 2). Although a higher plasma LDL-c level than the recommended normal range (2.34 - 3.38 mmol/L), the subjects in Q1 group of VA/LDL-c showed the lowest T2DM risk when compared with subjects from Q2 to Q5 group (the average plasma LDL-c level ranged from 3.212 ± 0.707 mmol/L to 2.124 ± 0.628 mmol/L) (Supplemental Table 2), suggesting that the ratio of plasma VA to LDL-c is probably a more reliable index than LDL-c to predict the risk for T2DM in aging subjects.

Additionally, we also detected a significant positive association of VA/HDL-c with FBG level, and the statistical significance was detected in Q3 and Q4 level of VA/HDL-c (the ratio ranged from 0.318 μg/μmol to 0.513 μg/μmol) (Supplemental Table 1). The trend test and RCS analysis also displayed a nonlinear relationship of VA/HDL-c with FBG level, indicating that plasma VA/HDL-c from 0.318 to 0.513 μg/μmol (Supplemental Table 1) could predict an increase of plasma FBG level in middle and aging subjects. The significant negative correlation of plasma HDL-c with VA further demonstrated that the imbalance between plasma VA and HDL-c might predispose the subjects to have elevated FBG level and increased risk for T2DM, indicating that VA and HDL-c might be potential regulators of blood glucose level. Totally, our data implied that the imbalance between plasma VA and cholesterol levels in the development of T2DM, and plasma VA/TC, VA/TG, VA/HDL-c and VA/LDL-c were potential indices for predicting the risk of T2DM in the middle and aging subjects.

There are some limitations to our study. Firstly, a cross-sectional study failed to explain the causal relationship between VA nutritional status and T2DM, a large-scale population-based cohort study is needed to reveal the mechanism of VA affecting the pathology of diabetes. Secondly, the relationship between plasma VA levels and T2DM might depend on the studied population, individual's food choices, eating behavior, genetic background, and disease stage. Our study was conducted in a Chinese population, due to lifestyle, dietary patterns, and racial differences, extrapolation of our conclusion to other races should be cautious. Thirdly, the plasma lipid level might reduce in T2DM patients because of abnormal lipid mobilization [50], which might cause an imbalance of plasma VA and cholesterol ratio, terminally modifying the relation between plasma VA and the risk of T2DM. Failure to detect lipid mobilization disorders in T2DM patients precludes us from ruling out the possibility of abnormal lipid mobilization on the relation between plasma VA level and the risk of T2DM in the middle-age and elderly. The innovation of this tstudy is reflected in the following aspects: 1) We explored the dose-responsive relationship between plasma VA level and risk of T2DM in the elderly with the adjustement of dietary pattern (the Chinese dietary balance indexes); 2) Given the significant relation between plasma VA and lipids [47,48,51,52], the potential modifying impact of circulating lipids on the relationship between plasma VA level and the risk of T2DM was further explored. The significance of the study is that we provide more reliable data and scientific references than previous

studies for establishing the relationship between VA nutritional status and the risk of T2DM in middle-age and elderly population.

5. Conclusions

Totally, our data indicated the modifying effect of circulating lipid on the relationship between VA and T2DM. That might partly explain the discrepant conclusions in different studies regarding the association between VA nutritional status and the risk of T2DM. We also found that ratios of VA/TC, VA/TG, VA/HDL-c, and VA/LDL-c are potential predictors of the risk of T2DM in middle age and aging subjects. Subjects with higher ratios of plasma VA/TC (0.112 - 0.341 μg/μmol), VA/HDL-c (0.391 - 1.404 μg/μmol), and VA/LDL-c (0.197 - 1.203 μg/μmol) might have an increased risk for T2DM, but the moderate ratio of VA/TG (0.224 - 0.400 μg/μmol) might protect against risk of T2DM.

Acknowledgment

We thank all study participants for their participation.

Ethics approval and consent to participate

This study protocol was reviewed and approved by [the Medical Ethics Committee of X], approval number [No.X] *and* conducted ethically in accordance with the World Medical Association Declaration of Helsinki. All the participants provided their written informed consent to participate in this study.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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