25-hydroxyvitamin D is associated with islet function homeostasis in type 2 diabetes patients with abdominal obesity

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Research Article

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Abstract

Aim: Islet α-cells input is essential for insulin secretion from β-cells. The role 25-hydroxyvitamin D (25(OH)D) on islet α-cells function is unclear. We aimed to investigate the association between 25(OH)D and islet function homeostasis in type 2 diabetes (T2D) patients.

Methods: Total 4670 T2D patients were enrolled from seven communities in Shanghai, China. Anthropometric indices, biochemical parameters, serum 25(OH)D, and islet function including C-peptide (C-p) and glucagon were measured.

Results: With increase in 25(OH)D level, FPG, HbA1c, glucagon and C-p were significantly decreased in trend in T2D patients. The population were divided into abdominal obesity and non-abdominal obesity group. 25(OH)D level was associated with HbA1c, glucagon and HOMA-β in non-abdominal obesity group after adjustment. As for abdominal obesity group, there was the significant association between 25(OH)D and HbA1c, glucagon, HOMA-IR, baseline insulin and C-p. The OLS regression and quantile regression further showed that 25(OH)D was associated with glucagon and fasting C-p levels in T2D patients with abdominal obesity. In non-abdominal obesity group, the OLS regression showed that the regression coefficients were not significant in 50th percentile for HOMA-β. The moderate analyses revealed significantly interaction effect of 25(OH)D and glucagon on C-p (P=0.0124), as well as the effect of 25(OH)D and duration of diabetes on glucagon/C-p in abdominal obesity group. According to the range of the duration of diabetes, the conditional indirect effect of 25(OH)D on glucagon/C-p was significantly at 1 SD below the mean (P=0.0002) and the mean of the duration of diabetes (P=0.0007).

Conclusion: 25(OH)D was negatively associated with glucagon and C-p in T2D patients with abdominal obesity. Duration of diabetes influence the effect of 25(OH)D on the ratio of glucagon/C-p, on behalf of islet function homeostasis, in T2D patients with abdominal obesity.

Introduction

Glucagon and insulin respectively secreted from the pancreatic islet α-cells and β-cells are main hormones for regulating glucose homeostasis. And specifically, insulin produced by β-cells lowers blood glucose, and glucagon secreted from α-cells opposes insulin action to maintain euglycemia[1]. However, α-cells and β-cells share a common developmental origin and exhibit overlapping transcriptomes and epigenomes in spite of these distinct functions[2]. Recent studies showed that glucagon served as an insulinotropic hormone rather than counter-regulatory hormone to insulin[3], which suggested that both a- and β-cells dysfunction contribute to the development of type 2 diabetes (T2D).

Nowadays the relationship between 25-hydroxyvitamin D (25(OH)D) level and the risk of T2D has become a hotspot of research. 25(OH)D as an indicator of vitamin D status has likewise been implicated in islet β-cells function. Deficiency of 25(OH)D has been confirmed to affect the synthesis and secretion of insulin in both animal models and human studies[4–4]. In vitro, 25(OH)D induces the biosynthesis of

insulin and insulin secretion in rat pancreatic islet cells[5, 6],[7]. In vivo, subjects recently diagnosed T2D who were supplemented with 25(OH)D had a tendency toward a better insulin secretory response[8], and people with 25(OH)D deficiency might predispose to glucose intolerance[9].

Basically all research about relationship between 25(OH)D and islet function in T2D has focused on the insulin-secreting β-cells. Considering vitamin D-binding proteins (DBP), which could bind and transport vitamin D to vitamin D receptor (VDR) in the nucleus, was highly present in human islet α-cells and also seen in islet β-cells, 25(OH)D may also contribute to glucagon secretion from α-cells. Recently some studies reveal that α-cells lacking DBP secrete less glucagon in response to low glucose concentration despite vitamin D sufficiency[10], and glucagon has a physiologic role to activate β-cells and enhance insulin secretion especially in the fed state[11]. So in response to high glucose level or in type 2 diabetes, how 25(OH)D impact glucagon secretion from islet α-cells as well as the islet function homeostasis between islet α-cells and β-cells should be elucidated, which can provide new strategies for treating glucose metabolism disorders.

Therefore, in this study, we aimed to investigate the relationship between 25(OH)D and islet function homeostasis in type 2 diabetes patients.

Methods

Ethics statement

The study design and procedures were approved by the Ethics Committee of Shanghai Jiaotong University School of Medicine Affiliated Ninth People's Hospital (approval number 2013(86)), and written informed consents were obtained from all participants.

Study design and participants

A cross-sectional study named METAL study (Environmental Pollutant Exposure and Metabolic Diseases in Shanghai, www.chictr.org.cn, ChiCTR1800017573) in August 2018 was conducted to investigate the association between vitamin D and islet function homeostasis in Chinese population. Detailed information in this study has been reported previously[12, 13]. Briefly, we randomly selected participants from registration platform of seven communities healthcare center in Huangpu and Pudong District in Shanghai, China. Total 5827 Chinese citizens who were 23–99 years old and lived in their current area for ≥ 6 months were enrolled.

Participants without type 2 diabetes (n = 1014), or missing laboratory values (n = 143) including glucagon, 25(OH)D and fasting insulin were excluded from our study. Thus a total of 4670 type 2 diabetes patients were involved in the final analyses.

Clinical measurements

The questionnaire about covering demographic characteristics, medical history, lifestyle risk factors and anthropometric data were constructed by the same trained and experienced personnel involved in SPECT-China study (Survey on Prevalence in East China for Metabolic Diseases and Risk Factors)[14]. Information including height, weight, waist circumference (WC) and blood pressure were collected in all participants by following the standard procedure. The detailed protocols had been described in previously published papers[15]. Body mass index (BMI) was calculated as body weight (kg) divided by the square of the body height (m). Current smoking was defined as having smoked at least 100 cigarettes in one's lifetime and currently smoking cigarettes[16].

Biochemical measurements

Blood samples for laboratory assays were obtained, processed and shipped as previously described[15]. Glycated hemoglobin (HbA1c) was measured by high-performance liquid chromatography (HPLC, MQ-2000PT, Medconn, China). Fasting plasma glucose (FPG), triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were performed with a Beckman Coulter AU 680 (Brea, USA). Serum fasting C-peptide (FC-p)was assessed by immunoassay (ARCHITECH i2000SR, Abbott Laboratories, Chicago, IL, USA). Serum 25(OH)D (SIEMENS ADVIA Centaur XP, Siemens, Germany) and fasting insulin (FINS) (Abbott i2000 SR, Chicago, USA) were measured using the chemiluminescence method. Glucagon was by immunoassay (ARCHITECH i2000SR, Beijing North Institute of Biotechnology Co, Ltd). The Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) equation was calculated as HOMA-IR = FPG(mmol/L)× FINS(mU/L)/22.5, and β-cells function (HOMA-β) was calculated as HOMA-β = 20× FINS/(FPG-3.5).

Definition of variables

In accordance with the American Diabetes Association 2014 criteria, diabetes was defined as a previous diagnosis by healthcare professionals, FPG ≥ 7.0mmol/L, 2h post-prandial glucose (PPG) ≥ 11.1mmol/L, or HbA1c ≥ 6.5%. Abdominal obesity was defined as a WC ≥ 90cm for men and a WC ≥ 80cm for women according to WHO recommendations. The definition of hypertension and dyslipidemia were described in previous study [17]. The ratio of glucagon and C-peptide (glucagon/C-p) were used to evaluated islet function hemostasis.

Statistical analysis

The characteristics of the T2D population according to 25(OH)D quartiles were presented as mean ± standard deviation (SD) for continuous variables and percentages (%) for categorical variables. Data with a skewed distribution were log10-transformed. The trend of variable changes according to 25(OH)D quartiles were accessed by linear or logistic regression analysis, providing unadjusted *P* values and *P* values adjusted for age, gender, current smoking, diabetes duration.

The correlation between 25(OH)D and glucose metabolism parameters in T2D or in abdominal obesity group or in non-abdominal obesity group was determined by spearman correlation analysis and partial correlation analysis after adjusting for age, gender, duration of diabetes, current smoking, body mass

index, dyslipidemia, hypertension and use of any antidiabetic agents. Furthermore, We used both ordinary least squares (OLS) and quantile regression analysis to explore the association of 25(OH)D and islets function in T2D with or without abdominal obesity. In the OLS regression model, all independent and dependent variables followed normal distribution after log-transformation. Quantile regression as a robust regression was used to identify the influencing factors at the 50th percentile of the dependent variable distribution. In this study, other quantiles (10th, 25th, 75th, and 95th percentiles) of the dependent variables distribution were also analyzed in quantile regression. In the regression models, participant characteristics, such as age, gender and lipid profiles (TC, TG, HDL, LDL) were included as covariates. The significance level was set at $P < 0.05$, and the collected data were analyzed using STATA 14.0 (Stata Corp, College Station, TX, USA).

Base on the above analysis, we further examined the moderation effects by SPSS PROCESS macro in a bootstrap approach (Bolin 2014). The conditional indirect effect of 25(OH)D on C-peptide by glucagon in type 2 diabetes with abdominal obesity were further analyzed by the model 4 of moderation effects. PROCESS was operated using one independent variables (C-p), one mediator (glucagon), and one dependent variable (25(OH)D).The conditional indirect effect of 25(OH)D on the ratio of glucagon and C-peptide (glucagon/C-p) at different duration of diabetes levels in type 2 diabetes with abdominal obesity were analyzed by the model 1 of moderation effects. PROCESS was operated using one independent variable (25(OH)D), one moderator (duration of diabetes), and one dependent variable (glucagon/C-p). 5000 bootstrap samples in the present study determined the moderating effect of the 95% confidence interval (CI). Gender, age, smoking status, exercising status and use of any antidiabetic agents were adjusted as confounders in the model of moderation analyses.

Data management and statistical analyses were performed using IBM SPSS Statistics (Version 24; SPSS Statistics, IBM Corporation, USA). $P < 0.05$ (two-tailed) was considered statistically significant.

Results

Participant characteristics by quartiles of 25(OH)D

Table 1 presents the characteristics of the T2D population included in our study (n = 4670). The quartile ranges of 25(OH)D were ≤ 30.79, 30.80-39.23, 39.24–48.92, and ≥ 48.92. The participants in the highest 25(OH)D quartile were younger and more likely to be men than those with lower 25(OH)D quartile($P <$ 0.001, Table 1). With increase in 25(OH)D level, BMI, SBP, FPG, HbA1c, glucagon, C-peptide, lipid profiles (TC, LDL, HDL, TG) and HOMA-IR were decreased in trend (all adjusted $P_{\text{for trend}}<0.05$). There were no significant changes in DBP, FINS and HOMA-β among different 25(OH)D levels (Table 1). Moreover, the higher the 25(OH)D quartile, the prevalence of dyslipidemia and abdominal obesity were lower (adjusted $P_{\text{for trend}}<0.05$, Table 1).

Table 1
Comparison of clinical indexes among quartiles of 25(OH)D in type 2 diabetes populations

	25(OH)D					
	Q1(≤ 30.79)	Q2(30.80-39.23)	Q3(39.24–48.92)	Q4(≥ 48.92)	$P_{for\ Trend}$	Adjusted $P_{for\ Trend}$
Participants (male, %)	445(38.2)	502(43)	567(48.4)	650(55.4)	< 0.001	/
Age(y)	68.44 ± 9.10	67.50 ± 8.57	66.73 ± 8.45	66.59 ± 8.76	< 0.001	/
Duration of diabetes (y)	10.01 ± 8.00	9.92 ± 7.76	10.20 ± 8.11	10.27 ± 7.95	0.320	/
Smoke(%)	19.5	18.2	17.6	17.1	0.115	/
BMI (kg/m^2)	24.96 ± 3.74	25.13 ± 3.62	25.09 ± 3.65	24.63 ± 3.36	0.055	0.011
SBP(mmHg)	147.10 ± 20.66	145.40 ± 19.31	144.42 ± 19.20	142.66 ± 19.54	< 0.001	< 0.001
DBP(mmHg)	78.33 ± 11.26	79.00 ± 10.74	79.34 ± 10.43	78.58 ± 10.77	0.443	0.127
FPG(mmol/L)	7.78 ± 2.52	7.92 ± 2.51	7.84 ± 2.42	7.56 ± 2.16	0.017	0.002
HbA1c(%)	7.53 ± 1.48	7.54 ± 1.42	7.52 ± 1.34	7.34 ± 1.25	0.001	< 0.001
TC (mmol/L)	5.39 ± 1.27	5.19 ± 1.21	5.09 ± 1.13	4.77 ± 1.09	< 0.001	< 0.001
LDL-C(mmol/L)	3.32 ± 0.87	3.20 ± 0.85	3.16 ± 0.82	2.95 ± 0.80	< 0.001	< 0.001
HDL-C(mmol/L)	1.23 ± 0.31	1.22 ± 0.29	1.19 ± 0.28	1.18 ± 0.28	< 0.001	0.013
TG (mmol/L)	2.16 ± 2.10	1.92 ± 1.61	1.90 ± 1.44	1.67 ± 1.05	< 0.001	< 0.001
Log(glucagon) (pg/ml)	5.12 ± 0.44	5.10 ± 0.46	4.99 ± 0.48	4.89 ± 0.53	< 0.001	< 0.001
Log(FINS) (pmol/L)	3.99 ± 0.73	4.03 ± 0.73	4.00 ± 0.71	3.94 ± 0.72	0.073	0.088
Log(FC-p) (ng/ml)	0.42 ± 0.51	0.39 ± 0.50	0.38 ± 0.55	0.34 ± 0.56	< 0.001	0.049
Log(HOMA-β)	3.73 ± 0.83	3.73 ± 0.84	3.71 ± 0.82	3.71 ± 0.83	0.947	0.929

	25(OH)D					
Log(HOMA-IR)	0.95 ± 0.81	0.99 ± 0.82	0.96 ± 0.78	0.88 ± 0.79	0.014	0.009
Hypertension(%)	79.6	79.3	78.2	76.9	0.084	0.119
Dyslipidemia(%)	66.4	61.7	62.3	58.5	< 0.001	< 0.001
Abdominal obesity(%)	76.5	74.8	75.7	68.2	< 0.001	0.031

Date are expressed as mean ± SD for continuous variables and as percentages for categorical variables; *P* for trend by regression tests. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; FPG: fasting plasma glucose; HbA1c: glycated hemoglobin; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglyceride; FINS: fasting insulin; FC-p: fasting C-peptide; HOMA-β: homeostasis model assessment of β-cell function; HOMA-IR: homeostasis model assessment of insulin resistance. $P_{for\ trend}$ by regression tests; adjusted *P* values adjusted for age, gender, current smoking, diabetes duration.

Association between 25(OH)D and glucose metabolism parameters

The relationship between 25(OH)D and glucose metabolism-related indexes in T2D population was presented in Table 2. The spearman analysis showed that 25(OH)D concentration was negatively related to HbA1c (r=-0.031, P = 0.031), glucagon (r=-0.17, P < 0.001), FC-p (r=-0.055, P < 0.001), FINS (r=-0.034, P = 0.021), HOMA-IR (r=-0.041, P = 0.005) and remained significant even after further adjustments for age, gender, duration of diabetes, current smoking, BMI, dyslipidemia, hypertension and use of any antidiabetic agents. In addition, there was no significant association between 25(OH)D and FPG or HOMA-β in T2D patients.

Table 2
Correlation between 25(OH)D and glucose metabolism indexes in type 2 diabetes patients

	Spearman Corr.		Partial Corr.	
	r	*P*	*r'*	*P'*
Fasting plasma glucose (FPG)	-0.018	0.226	-0.029	0.094
Glycated hemoglobin (HbA1c)	-0.031	0.031	-0.057	0.001
Glucagon	-0.170	< 0.001	-0.137	< 0.001
Fasting C-peptide (FC-p)	-0.055	< 0.001	-0.048	0.006
Fasting insulin (FINS)	-0.034	0.021	-0.035	0.044
HOMA-β	-0.023	0.106	-0.013	0.447
HOMA-IR	-0.041	0.005	-0.043	0.013

r' and *P'* values were adjusted for age, gender, duration of diabetes, current smoking, body mass index, dyslipidemia, hypertension and use of any antidiabetic agents. HOMA-β: homeostasis model assessment of β-cell function; HOMA-IR: homeostasis model assessment of insulin resistance.

The relationship between 25(OH)D and glucose metabolism parameters in type 2 diabetes with or without abdominal obesity

Table 3 showed the relationship between 25(OH)D and glucose metabolism parameters in T2D population with or without abdominal obesity. In non-abdominal obesity group, 25(OH)D level was negatively associated with FPG (r=-0.112, P = 0.002), HbA1c (r=-0.101, P = 0.004), glucagon (r=-0.146, P< 0.001) and HOMA-β (r=-0.084, P = 0.017) after adjusting for age, gender, duration of diabetes, current smoking, dyslipidemia, hypertension and use of any antidiabetic agents. As for abdominal obesity group, the partial correlation analysis showed the significant association between 25(OH)D and HbA1c (r=-0.078, P< 0.001), glucagon (r=-0.125, P< 0.001), fasting insulin(r=-0.062, P = 0.003), fasting C-p (r=-0.061, P = 0.003) and HOMA-IR (r=-0.058, P = 0.011).

Table 3
The relationship between 25(OH)D and indexes of glucose metabolism in type 2 diabetes patients with or without abdominal obesity

	Type 2 diabetes(n = 340)							
	Non-abdominal obesity				Abdominal obesity			
	Spearman		Partial		Spearman		Partial	
	r	*P*	*r′*	*P′*	*r*	*P*	*r′*	*P′*
FPG	-0.063	0.028	-0.112	0.002	0.002	0.928	-0.040	0.055
HbA1c(%)	-0.066	0.020	-0.101	0.004	-0.013	0.444	-0.078	< 0.001
Log(glucagon)	-0.203	< 0.001	-0.146	< 0.001	-0.150	< 0.001	-0.125	< 0.001
Log(FINS)	0.005	0.853	0.022	0.529	-0.019	0.275	-0.062	0.003
Log(FC-p)	-0.006	0.834	-0.032	0.367	-0.044	0.010	-0.061	0.003
Log(HOMA-β)	0.039	0.173	0.084	0.017	-0.021	0.217	-0.029	0.161
Log(HOMA-IR)	-0.031	0.172	-0.035	0.203	-0.030	0.118	-0.058	0.011

r′ and *P′* values were adjusted for age, gender, duration of diabetes, current smoking, dyslipidemia, hypertension and use of any antidiabetic agents. FPG: fasting plasma glucose; HbA1c: glycated hemoglobin; FINS: fasting insulin; FC-p: fasting C-peptide; HOMA-β: homeostasis model assessment of β-cell function; HOMA-IR: homeostasis model assessment of insulin resistance.

The association between 25(OH)D and islets function in type 2 diabetes with or without abdominal obesity

The ordinary least squares (OLS) and quantile regression analysis were used to explore the association of 25(OH)D with islets function parameters including glucagon, fasting insulin, fasting C-p and HOMA-β, as presented in Table 4. The OLS regression showed that 25(OH)D was associated with glucagon (β=-0.135, P< 0.001) and fasting C-p (β=-0.062, P= 0.002) levels in T2D patients with abdominal obesity, while the quantile regression further showed that 25(OH)D was associated with glucagon and fasting C-p levels in 10th, 25th, 50th, 75th and 95th percentile distribution of 25(OH)D (all P< 0.05). As for T2D patients without abdominal obesity, 25(OH)D was associated with glucagon (β=-0.174, P< 0.001), fasting insulin (β=-0.064, P< 0.001) and HOMA-β (β= 0.058, P< 0.001) after linear regression analysis, and the OLS regression showed that the regression coefficients were significant in 10th, 25th, 50th, 75th and 95th percentiles for glucagon (all P< 0.05), but not in 50th percentile for fasting insulin(β=-0.032, P= 0.209) and HOMA-β (β= 0.040, P= 0.055).

Table 4
The association between 25(OH)D and islets function in type 2 diabetes patients with or without abdominal obesity

Independent Variable		OLS Coefficient	Quantile regression Coefficient				
			10th	25th	50th	75th	90th
Log(Glucagon)	abdominal obesity	-0.135**	-0.074**	-0.091**	-0.119**	-0.103**	-0.090**
	non-abdominal obesity	-0.174**	-0.215**	-0.189**	-0.183**	-0.158**	-0.130**
Log(FINS)	abdominal obesity	0.003	0.006	0.034*	-0.005	-0.009	-0.025
	non-abdominal obesity	-0.064**	-0.045	-0.068*	-0.032	-0.060*	-0.066*
Log(FC-p)	abdominal obesity	-0.062**	-0.046*	-0.045*	-0.025*	-0.034*	-0.045*
	non-abdominal obesity	0.004	0.040	-0.037	-0.012	0.023	0.009
Log(HOMA-β)	abdominal obesity	-0.012	-0.028	-0.038*	0.001	0.019	0.021
	non-abdominal obesity	0.058**	0.018	0.052*	0.040	0.068*	0.069*

OLS = Ordinary Least Square; *: $P < 0.05$; **: $P < 0.01$; Gender, age and lipid indexes were included as covariates in the regression models. FINS: fasting insulin; FC-p: fasting C-peptide; HOMA-β: homeostasis model assessment of β-cell function.

The conditional indirect effect of 25(OH)D on C-peptide by glucagon and on islet function hemostasis at different duration of diabetes levels in type 2 diabetes with abdominal obesity

The conditional indirect effect of 25(OH)D on C-peptide by glucagon in T2D with abdominal obesity were further analyzed by the model of moderation effects. Gender, age, smoking status, exercising status and use of any antidiabetic agents were adjusted as confounders. As shown in Table 5, the moderate analyses revealed significantly interaction effect of 25(OH)D and glucagon on C-p in type 2 diabetes with abdominal obesity ($P = 0.0124$), which implied that the influence of 25(OH)D on C-p levels partly by affecting glucagon levels.

Considering C-peptide and glucagon are the biomarkers of islet β-cells and α-cells function respectively, the ratio of glucagon and C-peptide (glucagon/C-p) were used to evaluate islet function hemostasis. The conditional indirect effect of 25(OH)D on the ratio of glucagon and C-peptide (glucagon/C-p) at different

duration of diabetes levels in type 2 diabetes with abdominal obesity were also analyzed after adjusting confounders (gender, age, smoking status, exercising status and use of any antidiabetic agents). Table 6 showed that there was interaction effect of 25(OH)D and duration of diabetes on glucagon/C-p in type 2 diabetes with abdominal obesity ($P = 0.035$). According to the range of the duration of diabetes, 25(OH)D had significantly conditional indirect effect on glucagon/C-p at 1 SD below the mean ($P = 0.0002$) and the mean of the duration of diabetes ($P = 0.0007$). At 1 SD below the mean and the mean of the duration of diabetes, 25(OH)D was associated with glucagon/C-p (Table 6). These results indicated that type 2 diabetes with abdominal obesity and short duration of diabetes having high 25(OH)D presented lower level of glucagon/C-p than those who had low 25(OH)D.

Table 5
The conditional indirect effect of 25(OH)D on C-peptide by glucagon levels in type 2 diabetes with abdominal obesity

	Interaction effect	Lower 95% CI	Upper 95% CI	*P* for interaction
25(OH)D	-0.0745	-0.1291	-0.0198	**0.0076****
glucagon	0.0372	0.0005	0.0749	**0.0429***
25(OH)D×glucagon	-0.0753	-0.1344	-0.0163	**0.0124****

Bold italic*: $P < 0.05$; Bold italic**: $P < 0.01$. Gender, age, smoking status, exercising status and use of any antidiabetic agents were adjusted as confounders in the model of moderation analyses.

Table 6
The conditional indirect effect of 25(OH)D on the ratio of glucagon and C-peptide (glucagon/C-p) at different duration of diabetes levels in type 2 diabetes with abdominal obesity

	Interaction effect	Lower 95% CI	Upper 95% CI	*P* for interaction
25(OH)D	-0.2308	-0.3546	-0.1070	**0.0003****
Diabetes during	-0.0177	-0.0503	-0.0148	0.2966
1 SD below the mean	-0.2076	-0.3152	-0.1000	**0.0009****
Mean	-0.1317	-0.2078	-0.0556	**0.0017****
1 SD above the mean	-0.0557	-0.1556	0.0441	0.2274
25(OH)D×Diabetes during	0.0096	0.0007	0.0185	**0.0350***

Bold italic*: $P < 0.05$; Bold italic**: $P < 0.01$. Gender, age, smoking status, exercising status and use of any antidiabetic agents were adjusted as confounders in the model of moderation analyses.

Discussion

The impaired insulin secretion resulting from reduced β-cells function is the main pathophysiological character of T2D in Asian individuals. β-cells make up on average only 54% of all endocrine cells, and can

range as low as 28%[18]. It is notable that up to 65% of the human islet cells are α-cells. More and more clinical and experimental evidence have shown that multiple type 2 diabetes risk loci were associated with α-cells dysfunction[2], and glucagon secreted from α-cells can modulate β-cells function[1]. 25(OH)D as an activated vitamin D form is related to the function of pancreatic islet β-cells in patients with T2D. Recently a cross sectional study found an association between increased 25(OH)D level and improvement in modified homeostasis model assessment-β function in Chinese T2D patients[19]. However, this study did not access the relationship between 25(OH)D and α-cells function. And the role of 25(OH)D in patients with T2D on glucagon secretion is still unknown.

C-p (connecting peptide) is secreted from the β-cells of islets of the endocrine pancreas when proinsulin is cleaved into insulin and C-p. The most important indications for C-p levels is a measurement of insulin secretory reserve. The T2D patients with anti-diabetic medication including insulin were included in our study, thereby C-p but not insulin were deemed as indicator of β-cells function. In our study, the participants with higher 25(OH)D level had lower BMI, blood pressure, lipid profiles, glucose level, and lower prevalence of dyslipidemia and abdominal obesity. Both the glucagon and C-p levels decreased with 25(OH)D increasing even though there was no obviously difference in fasting insulin and HOMA-β among 25(OH)D quantiles. Further the spearman and partial correlation revealed significant inverse correlations between 25(OH)D and glucagon, C-p and HbA1c levels. Considering obese individuals require two to three times as much vitamin D as normal weight individuals to obtain physiological levels[20, 21], we further investigate the relationship between 25(OH)D and glucose metabolism parameters in T2D with or without abdominal obesity. The results showed that 25(OH)D was still negatively related to glucagon and HbA1c in both groups, and the improvement of islet β-cells evaluated by HOMA-β was only in non-abdominal obesity group, but C-p and insulin sensitivity evaluated by HOMA-IR only in abdominal obesity group, respectively.

The role of 25(OH)D on glucose metabolism is still controversial. Many previous studies had proved the effect of 25(OH)D on glycemic control, which showed a significant inverse association between serum 25(OH)D and HbA1c levels[22–24], and vitamin D supplementation may significantly reduce serum HbA1c and help to control glycemic response in T2D patients[23]. And some mendelian randomization studies or randomized controlled trials have not found the causal associations between vitamin D and T2D or pre-diabetes[25–28]. However, it was testified that vitamin D status was related to insulin resistance[27, 29–31], and had an indirect effect on regulating glucose-simulated insulin secretion from pancreatic β-cells by regulating cellular calcium signaling or suppressing renin-angiotensin activity or stimulating GLP-1 secretion which can promote β-cells proliferation and suppress glucagon release[32–34]. Corroborating with these findings, in our study, the glucose improvement by 25(OH)D in T2D with non-abdominal obesity was mostly due to decrease of glucagon level and improvement of islet β-cells function. In abdominal obesity group, despite accompanied with basal insulin and C-p lowering, glucose lowering by 25(OH)D was depend on decrease of glucagon and improvement of insulin sensitivity. However, as for T2D with abdominal obesity, if 25(OH)D impact on islet α-cells and β-cells simultaneously, or the influence of 25(OH)D on islet β-cells is partly by affecting α-cells, is still unknown.

Therefore, both OLS regression analysis and quantile regression analysis further showed that 25(OH)D negatively associated with glucagon and fasting C-p levels in T2D patients with abdominal obesity, which mean that 25(OH)D might inhibit insulin and glucagon secretion concurrently. But the previous view positions insulin from β cells and glucagon from α cells as opposites that insulin is mainly responsible for glucose reduction and glucagon drives glucose elevation[35]. Recent advances in the field have revealed a more complex relationship between insulin and glucagon, with data suggesting that α-cells input is essential for β-cells function and glucose homeostasis. The α- to β-cells communication describing α-cells input into β-cells is a key axis for regulating insulin secretion and glucose homeostasis[36]. Then the model of moderation effects showed the significant interaction effect of 25(OH)D and glucagon on C-p in type 2 diabetes with abdominal obesity, which implied that the influence of 25(OH)D on C-p levels was partly by affecting glucagon levels. Therefore, we used the ratio of glucagon and C-p (glucagon/C-p) to evaluate islet function homeostasis. Considering islet function homeostasis changed with duration of diabetes, our results further exhibited that T2D patients with abdominal obesity and short duration of diabetes who had high 25(OH)D level presented lower level of glucagon/C-p than those who had low 25(OH)D. T2D patients with abdominal obesity having shorter duration of diabetes but higher intake of 25(OH)D were more susceptible to affect islet α- to β-cells cross-talk.

It was proved that human β-cells express glucagon and glucagon-like peptide 1 (GLP-1) receptors [37–39]. Vendsen et al. found that complete blockade of glucagon signaling in islet β-cells severely limited insulin secretion, and α-cells may influence β-cells function through indirect intercellular or paracrine interactions[1, 2]. Paracrine glucagon actions are required to maintain normal insulin secretion[38]. In addition, the role of vitamin D binding protein (DBP) highly localized in the liver and pancreatic α-cells in regulating glucagon and insulin secretion was underestimated[40]. Glucagon-dependent insulin secretion was evident only at high glucose levels, indicating that intra-islet glucagon is particularly needed when insulin secretion demand is high. Therefore, in our study, 25(OH)D may negatively influence glucagon secretion from islet α-cells by DBP transporting at pancreatic α-cells in T2D patients. Then decreased glucagon further lower insulin secretion from islet β-cells. However, the extent of glucagon's influence on insulin secretion from β-cells exceed the direct impact of 25(OH)D on β-cells. This can explain the same direction change between 25(OH)D and glucagon and fasting C-p levels, and even the glucagon/C-p in T2D with abdominal obesity and short duration of diabetes. These findings present that 25(OH)D possibly modulate intra islet cross-talk between α- and β-cells.

Our study also has some limitations. Firstly, as a cross-sectional study, it is not appropriate to infer causality of 25(OH)D and islet function homeostasis. Secondly, we did not have data on vitamin D supplementations or sun exposure involving in 25(OH)D metabolism, which might have limited our multivariate approach. Thirdly, postprandial C-p and glucagon levels were not measured in our study, thus if the relationship between 25(OH)D and postprandial islet function homeostasis also engage in HbA1c improvement is unknown. Further prospective studies are needed to confirm these effects.

In conclusion, our present study of a large sample of T2D patients presents 25(OH)D was negatively associated with glucagon and C-peptide in T2D patients with abdominal obesity. Duration of diabetes influence the effect of 25(OH)D on the ratio of glucagon/C-p in T2D patients with abdominal obesity.

Declarations

Ethics approval and consent to participate

The study protocol was in accordance with the Declaration of Helsinki and was approved by the ethics committee of Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine. Written informed consent was obtained from all participants.

Consent for publication

Not applicable.

Availability of data and materials

The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that there are no conflicts of interest or financial interests associated with this manuscript.

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Author contribution

NW and YL conceived and designed research; QL, WZ, BH, YW, HW conducted the research; QL and WZ analyzed the data and wrote the manuscript. The final manuscript was read and approved by all authors.

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