

Comparison of 25-hydroxy vitamin D3, adiponectin and apolipoprotein A5 levels in subjects with different glucose tolerance and their correlation with blood lipid.

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Abstract

This study aims to compare 25-hydroxy vitamin D3 (25(OH)D3), adiponectin (ADPN) and apolipoprotein A5 (apoA5) levels among subjects with different glucose tolerance and to explore their correlation with lipid metabolism. Subjects were divided into 98 cases of type 2 diabetes mellitus (T2DM group), 87 cases of impaired glucose regulation (IGR group) and 100 cases of normal glucose tolerance (NGT group). 25(OH)D3, adiponectin (ADPN), apolipoprotein A5 (apoA5), hemoglobin A1c (HbA1c) and blood lipid were estimated and analyzed. It was found that the levels of 25(OH)D3, ADPN, apoA5 and high-density lipoprotein cholesterol (HDL-C) in T2DM and IGR groups were lower than that of NGT group, while HbA1c, total cholesterol (TC), triacylglycerol (TG) and LDL-C levels were higher than that of NGT group ($P < 0.05$). Levels of 25(OH)D3, ADPN, apoA5 and HDL-C in T2DM group were lower than that of IGR group. However, HbA1c, TC, TG and LDL-C levels were higher than that of IGR group ($P < 0.05$). Therefore, low plasma levels of 25(OH)D3, ADPN and apoA5 may possibly be the risk factors for impaired glucose tolerance, thus the regulation of 25(OH)D3, ADPN, and apoA5 concentrations could be important in the prevention of dyslipidemia and T2DM.

Keywords: Impaired glucose regulation; 25-hydroxy vitamin D3; Adiponectin; Apolipoprotein A5; Blood lipid

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Introduction

Lipotoxicity is one of the important factors for decreased number of pancreatic β -cells and insulin resistance (IR) from normal glucose tolerance (NGT) to impaired glucose tolerance (IGT) and type 2 diabetes mellitus (T2DM), thus diabetes is also known as glycolipid disease that emphasizes the importance of lipotoxicity in the development of T2DM [1]. It has been shown that plasma 25-hydroxy vitamin D 3(25(OH)D3) level can predict hyperglycemia and insulin resistance (IR) [2]. 25(OH)D3 has been extensively focused in the diabetes development process; the researches about the Since correlation among 25(OH)D3, adiponectin (ADPN), apolipoprotein A5 (apoA5) and blood lipid has not yet been reported, we aimed to explore whether there is any correlation among 25(OH)D3, ADPN, apoA5 and blood lipid among people with NGT, IGR,

or T2DM. Which might provide important guidance in the prevention and treatment of IGT, T2DM and dyslipidemia.

Materials and Methods

Subjects

Between Jan. 2011 and Nov. 2011, we recruited 306 outpatients from Chongqing Medical University, of which 108 patients (53 males and 55 females) with newly diagnosed T2DM aged (55.6 ± 9.7) years, 97 patients with IGR who were subdivided into 48 IGT patients (24 males and 24 females) aged (53.7 ± 9.2) years and 49 IFG patients (23 males and 26 females) aged (54.2 ± 9.1) years and 101 subjects with NGT (52 males and 49 females) aged (53.5 ± 9.2) years. Patients were subjected to oral glucose tolerance test (OGTT). This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics

Committee of Zunyi Medical College. Written informed consent was obtained from all participants.

Inclusion and exclusion criteria

Inclusion criteria for T2DM: i) newly diagnosed T2DM according to 1999 World Health Organization (WHO) diagnostic criteria; ii) normal hepatorenal function without severe organic disease or acute diabetic complications; iii) no pregnancy or lactation; iv) no previous diabetes treatment including diet and exercise therapy. Inclusion criteria for IGR: patients consistent with 2010 ADA diagnostic criteria, including people with NGT confirmed by OGTT (fasting plasma glucose (FPG) <5.6 mmol/l and 2hPG <7.8 mmol/l); IGT (FPG <5.6 mmol/l and 7.8 mmol/l ≤2hPG <11.1 mmol/l); IFG (5.6mmol/l ≤FPG <7.0 mmol/l and 2hPG <7.8 mmol/l). No medications on the day of test.

Exclusion criteria

Heart, liver or kidney disease or endocrine disease; administered the drugs that may influence the glucose or lipid metabolism within one month; history of any inflammation two weeks before the examination.

Detection methods

Access to food and water were restricted 12 h and 8 h, respectively before experiment. Fasting venous blood sample was drawn to measure blood lipid and glucose. OGTT was performed in the morning by orally taking glucose solution (75 g of anhydrous dextrose in 300 ml of water) within 5 min while fasting.

Electrochemiluminescence was used to detect 25(OH) D3 (Kit was from Roche Diagnostics GmbH, NY, USA); plasma ADPN was determined with ELISA Kit from US R&D with sensitivity of 100 pg/ml and measurement range of 100 pg/ml-6 ng/ml; plasma apoA5 was measured with ELISA Kit from US Phoenix Pharmaceuticals, Inc., with sensitivity of 0.85 pg/ml and measurement range of 0.85 pg/ml-61.5 ng/ml, and the inter- and intra-batch variations were ±5.2% (7.4 ng/ml) and ±15.0% (6.7 ng/ml). High performance liquid chromatography (HPLC) was employed to detect HbA1c (the kit was purchased from Beckman coulter Co., Ltd., USA); high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured with direct method; chemiluminescence was performed to determine fasting insulin (FINS) level; total cholesterol (TC) and triacylglycerol (TG) were detected with enzyme (Kit was from Roche Diagnostics GmbH, NY, USA); FPG was measured with oxidase assay; insulin resistance index (IRI) = FPG × FINS/22.5.

Statistical analysis

Data was analyzed with SPSS16.0 and presented as ($\bar{x} \pm s$). Comparison between groups utilized variance analysis and *t*-test. Multiple factors were analyzed using

stepwise multiple regressions. $P < 0.05$ was considered statistically significant.

Results

General data - HbA1c and blood lipid

There were no statistical differences in age, gender, body mass index (BMI), waist-to-hip ratio (WHR), systolic and diastolic blood pressure (SBP and DBP) among three groups ($P > 0.05$) so far tested. The differences in 25(OH)D3, ADPN, apoA5, HbA1c, TC, TG, LDL-C, HDL-C, free fatty acid (FFA), 2 h OGTT FFA (2hFFA), FINS, FPG, 2hPG, homeostatic model assessment of β -cell function and IR (HOMA-B and HOMA-IR) were significant ($P < 0.05$). In T2DM and IGR groups, levels of 25(OH)D3, ADPN, apoA5, HDL-C and HOMA-B were lower than that of NGT group, while HbA1c, TC, TG, LDL-C, FFA, 2hFFA, FINS, FPG, 2hPG and HOMA-IR levels were higher than that of NGT group ($P < 0.05$). Levels of 25(OH)D3, ADPN, apoA5, HDL-C and HOMA-B in T2DM group were found to be lower than that of IGR group; HbA1c, TC, TG, LDL-C, FFA, 2hFFA, FINS, FPG, 2hPG and HOMA-IR levels were higher than that of IGR group ($P < 0.05$, Table 1).

Multiple regression analysis

Pearson correlation analysis indicated that 25(OH)D3 did not correlate with gender, age and blood pressure ($P > 0.05$), positively correlated with serum levels of ADPN, apoA5, HOMA-B and HDL-C (correlation coefficient r was 0.432, 0.465, 0.447 and 0.317, $P < 0.05$) and negatively correlated with HbA1c, HOMA-IR, BMI, WHR, TC, TG, LDL-C, FFA, 2hFFA (correlation coefficient r was -0.345, -0.368, -0.427, -0.437, -0.365, -0.412, -0.423, -0.587 and -0.441, $P < 0.05$). Stepwise multiple regression analysis, with 25(OH)D3 as dependent variable and ADPN, apoA5, HbA1c, HOMA-IR, BMI, WHR, TC, TG, LDL-C, HOMA-B, HDL-C, FFA, 2hFFA as independent variables, showed the independent influencing factors of 25(OH)D3 were ADPN, apoA5, HbA1c, TG, WHR and HOMA-IR ($\beta = 0.534, 0.621, -0.472, -0.526, -0.465, -0.375$; $P < 0.05$). After correction of insulin sensitivity, 25(OH)D3 still negatively correlated with HbA1c, HOMA-IR, BMI, WHR, TC, TG, LDL-C, FFA, 2hFFA (correlation coefficient r was -0.323, -0.356, -0.421, -0.438, -0.381, -0.375, -0.382, -0.481, -0.363, $P < 0.05$) and positively correlated with serum levels of ADPN, apoA5, HOMA-B, HDL-C (correlation coefficient r was 0.385, 0.317, 0.432, 0.301, $P < 0.05$). Stepwise multiple regression analysis displayed the independent influencing factors of 25(OH)D3 were TG, HbA1c, ADPN, apoA5, WHR, HOMA-IR ($\beta = -0.531, -0.352, 0.576, 0.457, -0.334, -0.268$; $P < 0.05$). The multiple regression equation was as follows: $Y_{25(OH)D3} = 71.462 - 25.231X_{TG} - 32.421X_{HbA1c} + 33.543X_{ADPN} + 35.524X_{apoA5} - 36.472X_{WHR} - 38.467X_{HOMA-IR}$ (Table 2).

Table 1. Comparison of general clinical data and other index among three groups ($\bar{x} \pm s$).

Item	T2DM group (n = 108)	IGR group (n = 97)	NGT group (n = 101)
Age (year)	55.6±9.7	53.7±9.3	53.5±9.2
Male (F/M)	55/53	50/47	49/52
BMI (kg/m ²)	23.13±2.19	22.98±2.12	22.31±2.23
W-H-R	0.85±0.05	0.84±0.04	0.82±0.05
Systolic BP (mmHg)	123.24±16.18	121.32±14.51	118.78±13.58
Diastolic BP (mmHg)	83.22±8.73	81.58±8.56	79.65±9.32
25(OH)D3 (ng/ml)	19.8±10.7* [#]	26.3±11.5*	34.7±10.5
ADPN (μg/ml)	3.28±1.21* [#]	8.32±2.19*	15.18±8.25
apoA5 (μg/ml)	6.75±1.58* [#]	25.78±10.35*	37.48±12.23
HbA1c (%)	8.19±2.32* [#]	6.24±1.10*	4.23±0.61
TC (mmol/l)	6.54±1.23* [#]	5.32±1.21*	4.34±0.58
TG (mmol/l)	4.12±1.32* [#]	2.45±1.63*	1.32±0.51
LDL-C (mmol/l)	4.27±0.64* [#]	3.51±0.71*	2.34±0.58
HDL-C (mmol/l)	0.86±0.24* [#]	1.12±0.31*	1.63±0.49
FFA (mmol/l)	0.88±0.21* [#]	0.67±0.09*	0.56±0.08
2hFFA (mmol/l)	0.56±0.16* [#]	0.34±0.05*	0.23±0.07
FINS (mU/l)	23.49±12.46* [#]	15.76±12.14*	7.57±3.34
FPG (mmol/l)	9.79±2.52* [#]	6.37±0.28*	5.26±0.24
2hPG (mmol/l)	14.53±3.47* [#]	9.78±1.21*	5.45±0.71
HOMA-B	44.23±16.42* [#]	92.22±57.13*	102.47±57.17
HOMA-IR	4.87±2.36* [#]	2.18±1.42*	1.61±0.72

Note: * $P < 0.05$, compared with NGT group; [#] $P < 0.05$, compared with IGR group.

Table 2: Correlation coefficient (r value) and partial correlation coefficient (r' value) of 25(OH)D3 and other indicators before and after correcting insulin sensitivity.

Correlation coefficient	ADPN	apoA5	HDL-C	TG	TC	LDL-C	HOMA-IR	HbA1c	BMI	WHR	FFA	2hFFA	HOMA-B
r _{25(OH)D3}	0.432*	0.465*	0.317*	-0.412*	-0.365*	-0.423 [△]	-0.368*	-0.345*	-0.427*	-0.437*	-0.587*	-0.441*	0.471*
r' _{25(OH)D3}	0.385*	0.317*	0.301*	-0.375*	-0.381	-0.382 [△]	-0.356 [△]	-0.323*	-0.421*	-0.438*	-0.481*	-0.363*	0.432*
P	0.032	0.032	0.026	0.029	0.042	0.003	0.004	0.037	0.027	0.031	0.034	0.026	0.036

In multiple linear stepwise regression analysis, values included for analysis were 25(OH)D3, (apoA5), ADPN, WHR, BMI, FPG, FINS, 2hPG, HOMA-IR, FFA, 2hFFA, TG, TC, LDL-C and HDL-C. * $P < 0.05$, compared with NGT group; [△] $P < 0.01$, compared with IGR group.

Discussion

WHO has predicted that there will be 46 million Chinese diabetic patients in 2025, ranking the second in the world. Chinese Diabetes Society believes that attention to IGR risk group is required to prevent this trend. Many studies have demonstrated that blood concentration of 25(OH)D3 is correlated with T2DM morbidity [3-5] and people with vitamin D deficiency are more susceptible to metabolic syndrome and diabetes [6-8]. In 2001, lipotoxicity was reported as primary pathophysiologic factor for diabetes and milestone in research on pathogenesis of diabetes [9]. Dyslipidemia is important in T2DM development as it damages the function of mitochondria in muscle and disturbs the role of insulin in glucose metabolism, resulting in glucose metabolism disorder [10, 11]. In NGT-IGR-

T2DM development, glucotoxicity and lipotoxicity are reciprocal causation and contribute to the occurrence and development of diabetes. However, the relationship among levels of 25(OH)D3, ADPN, apoA5 and blood lipid is rarely reported.

Norman et al [12] have discovered that the pancreatic islets from rats with vitamin D deficiency display decreased insulin synthesis and secretion stimulated by glucose and arginine. Research has shown a significant lower level of vitamin D in patients with T2DM than that of healthy controls [13] and a close correlation between reduction of plasma 25(OH)D3 level, IR, obesity and T2DM [14-17]. The above results were consistent with the results of this study. ADPN mainly regulates glucose and lipid metabolism, increases fatty acid oxidation and

Insulin sensitivity and correlates with obesity, IR and diabetes [18], and protects pancreatic β -cells. Recent research [19, 20] has revealed that plasma apoA5 level negatively correlates with TG, BMI, HOMA-IR and low plasma level of apoA5 is involved in IR-related hypertriglyceridemia. Vaessen et al [21] have reported that overexpression of apoA5 decreases plasma TG level, apoA5 deficiency, increase in TG level in human and murine and apoA5 negatively correlates with TG level. In our study, plasma levels of 25(OH)D3, ADPN and apoA5 gradually decreased and HbA1c, TC, TG, LDL-C levels were gradually increased in NGT-IGR-T2DM development; 25(OH)D3 concentration was positively correlated with serum levels of ADPN, apoA5, HOMA-B, HDL-C and negatively correlated with HbA1c, HOMA-IR, BMI, WHR, TC, TG and LDL-C; the independent influencing factors of 25(OH)D3 were ADPN, apoA5, HbA1c, TG, WHR and HOMA-IR. Levels of HbA1c, TC, TG and LDL-C in T2DM group were significantly higher than that of IGR and NGT groups, while 25(OH)D3, ADPN, apoA5 and HDL-C levels were distinctly lower than that of IGR and NGT groups. Therefore, we speculate that 25(OH)D3, ADPN and apoA5 could be early indicators for dyslipidemia at increasing risk for diabetes; decrease in plasma levels of 25(OH)D3, ADPN and apoA5 probably act as high risk factors for IGT.

In conclusion, IGT severity closely correlates with the levels of 25(OH)D3, ADPN, apoA5, HbA1c and dyslipidemia. Reduced plasma level of 25(OH)D3 might cause decrease in ADPN, apoA5 levels and dyslipidemia. Conversely, dyslipidemia decreases plasma 25(OH)D3, ADPN and apoA5 levels and increases HbA1c level to form a vicious circle, thus 25(OH)D3, ADPN, apoA5 levels, glucose and lipid metabolism disorders are reciprocal causation and collectively contribute to the occurrence and development of IGR, T2DM and dyslipidemia. Therefore, maintenance of 25(OH)D3, ADPN and apoA5 levels at control values could essentially prevent IGR and T2DM development. However, further studies are required to explore the underlying mechanism.

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