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Changes of adipocytokine expression after diabetic rats received sitagliptin and the molecular mechanism

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ABSTRACT

Objective: To study the effect of sitagliptin on adipocytokines expression in diabetic rats and its molecular mechanism.
Methods: Male SD rats were chosen and randomly divided into NC group, T2DM group, SP group and SP + LY group. NC group received conventional breeding, T2DM group, SP group and SP + LY group received intraperitoneal injection of streptozotocin after 12 weeks of high-fat diet to establish diabetes animal model, SP group received sitagliptin intervention and SP + LY group received sitagliptin combined with PI3K inhibitor LY294002 intervention. Six weeks after the intervention, serum was collected to determine the levels of biochemical indexes and adipocytokines, and visceral adipose tissue was collected to determine expression levels of adipocytokines.
Results: Serum TC, TG, LDL-C, FBG, FINS, Leptin and Chemerin levels as well as HOMA-IR of T2DM group were higher than those of NC group, and HDL-C, Adiponectin and Omentin-1 levels were significantly lower than those of NC group; serum TC, TG, LDL-C, FBG, FINS, Leptin and Chemerin levels as well as HOMA-IR of SP group were lower than those of T2DM group, and HDL-C, Adiponectin and Omentin-1 levels were significantly higher than those of T2DM group; Leptin and Chemerin levels in serum and visceral adipose tissue of SP + LY group were higher than those of SP group while Adiponectin and Omentin-1 levels were significantly lower than those of SP group.
Conclusion: Sitagliptin can regulate the expression of adipocytokines in adipose tissue of diabetic rats through PI3K-AKT pathway.

1. Introduction

Type 2 diabetes mellitus (T2DM) is an endocrine disease characterized by insulin resistance and relatively insufficient insulin secretion, is with rising morbidity, and causes adverse effect on both life health and quality of life of the patients. Adipocytokines are a type of cytokines synthesized and secreted by adipocytes, include Adiponectin, Leptin, (Omentin-1), Chemerin, *etc.*, and have regulating effect on inflammation, insulin sensitivity, endothelial function, *etc* [1,2]. There are abnormal glucolipid metabolism and abnormal abdominal visceral adipose tissue accumulation in T2DM patients, and the adipocytokines synthesized by visceral adipose tissue will change, resulting in a variety of diabetic complications [3–5]. Sitagliptin is an oral hypoglycemic drug used in treatment of T2DM patients in recent years, belongs to the dipeptidyl peptidase-4 (DPP-4) inhibitor, and can inhibit glucagon-like peptide (GLP-1) degradation, regulate insulin sensitivity and lower blood glucose [6,7]. At present, the regulating effect of sitagliptin on adipocytokines in patients with T2DM is unclear. In the following study, the diabetic rat models were established and the changes of adipocytokines after sitagliptin treatment were analyzed.

2. Materials and methods

2.1. Experimental materials

Experimental animals were 50 male SD rats with 6–8 weeks of age and body mass of 160–220 g, they were purchased from

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the experimental animal center of Zhejiang University, and license No. was SCXK 2008-0016, the animal study was approved by the hospital ethics committee; streptozotocin and PI3K inhibitor LY294002 were from Sigma Company, enzyme-linked immunosorbent assay kits were from Elabscience Company, and RNA extraction kits, the first strand of cDNA synthesis kits and fluorescence quantitative PCR kits were from Beijing Tiangen Biochemical Company. OneTouch Ultra for Johnson & Johnson Company, microplate reader was from the Bio-rad Company, and fully automatic biochemical analyzer was from Switzerland Roche Company.

2.2. Experimental methods

2.2.1. Animal grouping and model establishing methods

Experimental animals were randomly divided into NC group, T2DM group, SP group and SP + LY group. NC group (n = 8) received conventional breeding, the remaining 42 were used for T2DM model establishment, and the method was as follows: they received high-fat diet for 12 weeks, and then were given intraperitoneal injection of 35 mg/kg streptozotocin, streptozotocin was temporarily configured with pH = 4.5 0.1 mmol/L sodium citrate buffer before injection, blood was collected via caudal vein 7 days after injection, rats with fasting glucose > 11.1 mmol/L were considered as successfully established, 5 were dead, 7 were with substandard fasting glucose, and only 30 were successfully established and randomly divided into T2DM group, SP group and SP + LY group, 10 in each group.

2.2.2. Intervention methods

T2DM group received intragastric administration of hydroxymethyl amylase, 2 mL/time, 1 time/day, and then continued to receive normal diet; SP group received intragastric administration of sitagliptin, 10 mg/(kg·d), the sitagliptin was dissolved in 2 ml hydroxymethyl amylase, and after intervention, they continued to receive normal diet; SP + LY group received intragastric administration of sitagliptin with the same method as that of SP group and received subcutaneous injection of PI3K inhibitor LY294002, 50 μg/(kg·d), LY294002 was dissolved in 1 mL saline, and after intervention, they continued to receive normal diet.

2.2.3. Sample collecting methods

Six weeks after intervention, four groups of rats were fasting for 12 h and then was sacrificed by cervical dislocation, blood samples were collected, let stand for 15 min at room temperature and then centrifuged in the centrifugal machine for 10 min at 3000 r/min, and serum was separated and saved at −80 °C; abdominal cavity of rats was opened after execution, abdominal visceral adipose tissue was collected, frozen with liquid nitrogen and then saved at −80 °C.

2.2.4. Index detecting methods

Serum specimen were collected, automatic biochemical analyzer was used to determine fasting blood glucose (FBG), fasting blood insulin (FINS), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) content, and the steady-state model was used to calculate the insulin resistance index (HOMA-IR) = FINS × FBG/22.5. Enzyme-linked immunosorbent assay kits were used to determine Adiponectin, Leptin, Omentin-1 and Chemerin levels. Abdominal visceral adipose tissue was collected, RNA extraction kits and the first strand of cDNA synthesis kits were used to separate and obtain cDNA samples, then fluorescent quantitative PCR amplification was performed, and the mRNA levels of Adiponectin, Leptin, Omentin-1 and Chemerin were calculated.

2.2.5. Statistical methods

SPSS20.0 software was used to input and analyze data, measurement data was by variance analysis, pair-wise comparison was by LSD-t test and $P < 0.05$ indicated statistical significance in differences.

3. Results

3.1. Serum biochemical indexes of all groups

Serum biochemical indexes TC, TG, LDL-C, HDL-C, FBG and FINS as well as HOMA-IR of NC group, T2DM group, SP group and SP + LY group were significant different ($P < 0.05$), and pair-wise comparison and analysis showed that: serum TC, TG, LDL-C, FBG and FINS levels as well as HOMA-IR of T2DM group were higher than those of NC group, and HDL-C was significantly lower than that of NC group; serum TC, TG, LDL-C, FBG and FINS levels as well as HOMA-IR of SP group were lower than those of T2DM group, and HDL-C was significantly higher than that of T2DM group; serum TC, TG, LDL-C, FBG and FINS levels as well as HOMA-IR of SP + LY group were higher than those of SP group, and HDL-C was significantly lower than that of SP group (Table 1).

3.2. Effect of sitagliptin intervention on serum levels of adipocytokines in T2DM rats

Serum adipocytokines Adiponectin, Leptin, Omentin-1 and Chemerin levels of NC group, T2DM group and SP group were

Table 1
Serum biochemical indexes of all groups.

Group	n	TC (mmol/L)	TG (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)	FBG (mmol/L)	FINS (IU/mL)	HOMA-IR
NC group	8	1.74 ± 0.25	0.72 ± 0.10	0.97 ± 0.12	0.82 ± 0.10	4.89 ± 0.74	6.14 ± 0.89	1.62 ± 0.22
T2DM group	10	2.95 ± 0.32△	1.32 ± 0.18△	1.93 ± 0.25△	0.45 ± 0.07△	13.20 ± 1.85△	17.58 ± 2.51△	8.48 ± 1.03△
SP group	10	2.04 ± 0.31▲	0.89 ± 0.11▲	1.21 ± 0.18▲	0.74 ± 0.09▲	5.85 ± 0.71▲	8.39 ± 1.05▲	3.38 ± 0.44▲
SP + LY group	10	2.77 ± 0.35*	1.25 ± 0.22*	1.82 ± 0.26*	0.56 ± 0.07*	12.14 ± 1.88*	14.59 ± 2.35*	7.14 ± 0.91*

△$P < 0.05$ compared with NC group; ▲$P < 0.05$ compared with T2DM group; *$P < 0.05$ compared with SP group.

significantly different ($P < 0.05$), and pair-wise comparison and analysis showed that serum Adiponectin and Omentin-1 levels of T2DM group were significantly lower than those of NC group while Leptin and Chemerin levels were significantly higher than those of NC group; serum Adiponectin and Omentin-1 levels of SP group were significantly higher than those of T2DM group while Leptin and Chemerin levels were significantly lower than those of T2DM group (Table 2).

Table 2
Effect of sitagliptin intervention on serum levels of adipocytokines in T2DM rats.

Group	Sample size	Adiponectin (μg/mL)	Leptin (μg/mL)	Omentin-1 (μg/mL)	Chemerin (pg/mL)
NC group	8	11.48 ± 1.67	4.22 ± 0.92	22.42 ± 4.85	451.24 ± 67.13
T2DM group	10	6.42 ± 8.10△	14.12 ± 1.86△	8.92 ± 1.06△	792.32 ± 92.42△
SP group	10	10.14 ± 1.52▲	6.79 ± 0.91▲	17.85 ± 2.86▲	527.85 ± 71.45▲

△$P < 0.05$ compared with NC group, there were differences; ▲$P < 0.05$ compared with T2DM group.

3.3. Effect of sitagliptin intervention on the expression of adipocytokines in visceral adipose tissue of T2DM rats

Adipocytokines Adiponectin, Leptin, Omentin-1 and Chemerin levels in visceral adipose tissue of NC group, T2DM group and SP group were significantly different ($P < 0.05$), and pair-wise comparison and analysis was as follows: Adiponectin and Omentin-1 levels in visceral adipose tissue of T2DM group were significantly lower than those of NC group while Leptin and Chemerin levels were significantly higher than those of NC group; Adiponectin and Omentin-1 levels in visceral adipose tissue of SP group were significantly higher than those of T2DM group while Leptin and Chemerin levels were significantly lower than those of T2DM group (Table 3).

Table 3
Effect of sitagliptin intervention on the mRNA levels of adipocytokines in visceral adipose tissue of T2DM rats.

Group	*n*	Adiponectin	Leptin	Omentin-1	Chemerin
NC group	8	1.00 ± 0.15	1.00 ± 0.17	1.00 ± 0.13	1.00 ± 0.14
T2DM group	10	0.38 ± 0.07△	2.42 ± 0.37△	0.32 ± 0.06△	2.71 ± 0.33△
SP group	10	0.84 ± 0.11▲	1.32 ± 0.19▲	0.89 ± 0.12▲	1.52 ± 0.22▲

△$P < 0.05$ compared with NC group, there were differences; ▲$P < 0.05$ compared with T2DM group.

3.4. Effect of PI3K inhibitor combined with sitagliptin on the generation of adipocytokines

Serum Leptin and Chemerin levels in serum and visceral adipose tissue of SP + LY group were higher than those of SP group while Adiponectin and Omentin-1 levels were significantly lower than those of SP group (Table 4).

4. Discussion

Insulin resistance and relatively insufficient secretion are the most prominent pathological features of T2DM, and increasing insulin sensitivity and promoting insulin secretion or supplementing exogenous insulin are important in the clinical treatment of T2DM. GLP-1 is a polypeptide hormone newly discovered in recent years, and it can lower the blood glucose by promoting insulin secretion, restraining glucagon secretion, slowing gastric emptying and other ways [8,9]. Under physiological conditions, GLP-1 can be rapidly degraded by DPP-4 and lose biological activity. Sitagliptin is a highly selective inhibitor of DPP-4 that can increase the GLP-1 levels in the body and regulate blood glucose metabolism [10,11]. In order to specify the value of sitagliptin for treatment of diabetes, high-fat diet combined with intraperitoneal injection of streptozotocin was adopted in the study to establish the diabetic animal models, and the analysis of blood glucose metabolism indexes showed that serum FBG and FINS levels as well as HOMA-IR of T2DM group were higher than those of NC group. Thus it confirmed that the diabetic models were successfully established, and there were hyperglycemia, hyperinsulinism and insulin resistance in model rats. After sitagliptin treatment, serum FBG and FINS levels as well as HOMA-IR of SP group were lower than those of T2DM group. This meant that sitagliptin treatment could improve the insulin resistance and reduce blood glucose levels in diabetic rats.

In progression of diabetes, abnormal glucose metabolism and hyperinsulinemia will affect lipid metabolism, cause elevated blood lipid levels and promote fat deposition in abdominal visceral tissue [12–14]. Elevated blood lipid level is the risk factor causing atherosclerosis, and is also one of the reasons for the increased risk of diabetic macro-vascular complications. In the study, the analysis of blood lipid metabolism confirmed that serum TC, TG and LDL-C levels of T2DM group were higher than those of NC group and HDL-C level was lower than that of NC group; after sitagliptin intervention, serum TC, TG and LDL-C levels of SP group were lower than those of T2DM

Table 4
Effect of PI3K inhibitor combined with sitagliptin on the generation of adipocytokines.

Group	*n*	Serum samples				Visceral adipose tissue			
		Adiponectin (μg/mL)	Leptin (μg/mL)	Omentin-1 (μg/mL)	Chemerin (pg/mL)	Adiponectin/GAPDH	Leptin/GAPDH	Omentin-1/GAPDH	Chemerin/GAPDH
SP group	10	10.14 ± 1.52	6.79 ± 0.91	17.85 ± 2.86	527.85 ± 71.45	0.84 ± 0.11	1.32 ± 0.19	0.89 ± 0.12	1.52 ± 0.22
SP + LY group	10	7.14 ± 0.88*	12.84 ± 2.42*	10.33 ± 1.93*	745.23 ± 94.42*	0.49 ± 0.08*	2.10 ± 0.27*	0.44 ± 0.07*	2.21 ± 0.27*

*$P < 0.05$, compared with SP group.

group and HDL-C level was higher than that of T2DM group. This meant that there was abnormal blood lipid metabolism in diabetic models, and sitagliptin intervention could improve blood lipid metabolism.

Studies in recent years believe that adipocytokines that are synthesized and secreted by adipocytes have important regulating effect on insulin sensitivity, endothelial function and inflammation, etc, and the abnormal blood lipid metabolism and excessive fat deposition in T2DM rats can lead to abnormal synthesis and secretion of adipocytokines [15,16]. In the study, detection of adipocytokines Adiponectin, Leptin, Omentin-1 and Chemerin levels showed that Adiponectin and Omentin-1 levels in serum and visceral adipose tissue of T2DM group were significantly lower than those of NC group while Leptin and Chemerin levels were significantly higher than those of NC group. Adiponectin has anti-inflammation, anti-atherosclerosis and endothelium-protective effect [17], and Omentin-1 can increase insulin sensitivity [18]; Leptin has the function of antagonizing the biological activity of insulin and can lead to insulin resistance and cause endothelial function damage [19], and Chemerin can reduce insulin sensitivity and restrain glucose uptake and utilization of peripheral tissue [20]. The decrease of Adiponectin and Omentin-1 as well as the increase of Leptin and Chemerin in diabetic rats is associated with insulin resistance and the occurrence of endothelial injury and atherosclerosis. After sitagliptin intervention, Adiponectin and Omentin-1 levels in serum and visceral fat tissue of SP group were significantly higher than those of T2DM group while Leptin and Chemerin levels were significantly lower than those of T2DM group. This meant that sitagliptin could regulate the synthesis of adipocytokines in visceral adipose tissue of diabetic rats.

Phosphatidylcholine 3-kinase (PI3K)-protein kinase B (PKB/AKT) is an important signal pathway mediating the biological signal of insulin in vivo, the activation of PI3K can cause the phosphorylation of the third hydroxyl in inositol ring, and phosphatidyl inositol diphosphate is transformed into phosphatidyl inositol triphosphate (PIP3) that has the function of second messenger. PIP3 is combined with AKT N-terminal and causes its conformational change, and then activates downstream nuclear transcription factor NF-kB and regulates the expression of multiple genes [21,22]. In order to define whether sitagliptin regulated the expression of adipocytokines by PI3K-AKT signaling pathways, PI3K antagonist LY294002 combined with sitagliptin treatment was adopted in the study, and compared with SP group, Adiponectin and Omentin-1 levels in serum and visceral fat tissue of LY group decreased significantly while Leptin and Chemerin levels increased significantly. This meant that the effect of sitagliptin on regulating adipocytokines could be partially reversed by PI3K antagonist LY294002, and thus also confirmed that sitagliptin could regulate the expression of adipocytokines in visceral adipose tissue through PI3K-AKT signaling pathway.

To sum up, the expression of adipocytokines is significantly abnormal in visceral adipose tissue of diabetic rats, and sitagliptin can regulate the expression of adipocytokines in adipose tissue of diabetic rats through PI3K-AKT pathway.

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