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Original Article

Ileal interposition reduces blood glucose levels and decreases insulin resistance in a type 2 diabetes mellitus animal model by up-regulating glucagon-like peptide-1 and its receptor

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Abstract: This study is to explore the possible mechanism of ileal interposition (IT) treatment of glycemic control of the type 2 diabetes mellitus (T2DM) by establishing an IT animal model. Twelve T2DM rats (GK rats) of 8-week old were divided into GK IT surgery group (GK-IT) and GK sham group (GK-Sham). Six Wistar rats were used as the non-T2DM sham group (WS-Sham). Enzyme-linked immunosorbent assay was used to detect plasma insulin concentration and fasting pancreas glucagon-like peptide-1 (GLP-1) concentration changes. Homeostasis model assessment of insulin resistance was used to quantitatively measure insulin resistance. Glucagon-like peptide-1 receptor (GLP-1R) expression was detected by Western blotting. IT significantly decreased fasting blood glucose level and the oral glucose tolerance, and reduced insulin resistance of GK rats by increasing GLP-1 concentration and GLP-1R levels. The postoperative pancreatic β-cell apoptosis rate of GK-Sham group was significantly higher than those in the GK-IT group and the WS-Sham group. IT significantly reduces blood glucose and decreases insulin resistance by up-regulating GLP-1 concentrations and GLP-1R levels, which may contribute to insulin secretion of pancreatic β-cells and decreases apoptosis of pancreatic β-cell.

Keywords: Ileal interposition, type 2 diabetes mellitus (T2DM), glucagon-like peptide-1 (GLP-1), glucagon-like peptide-1 receptor (GLP-1R)

Introduction

The type 2 diabetes mellitus (T2DM) is one of the chronic diseases that seriously threaten human health. However, etiology and pathogenesis of T2DM is still unclear. From some obesity surgeries, it is found that in addition to effective control of patient weight, their T2DMs are also effectively controlled by the surgeries [1, 2]. Recently, gastrointestinal tract reconstruction for treatment of T2DM is a research hotspot. It is considered that the Roux-en Y gastric bypass (RYGB) surgery is shown to be effective in the treatment of T2DM [3]. Although there are two types of hypotheses related to RYGB, termed as "foregut hypothesis" [4] and "hindgut hypothesis" [5], respectively, the exactly mechanism is still unclear. In this study, ileal interposition (IT) was performed to explore the potential possibility of "hindgut hypothesis".

IT may induce changes in the gastrointestinal hormone concentration in the blood [6, 7]. The incretin hormones are the polypeptide secreted by intestinal. GLP-1 is a particularly important incretin hormone, since it enhances glucose-stimulated insulin secretion, controls absorption of oral sugar, protein and fat [8], and promotes regeneration of pancreatic β cells [9]. GLP-1 is secreted by L cells of distal intestine and colon [8]. GLP-1 functions by binding to specific G protein-coupled receptor of β cells in pancreas (GLP-1R) [8].

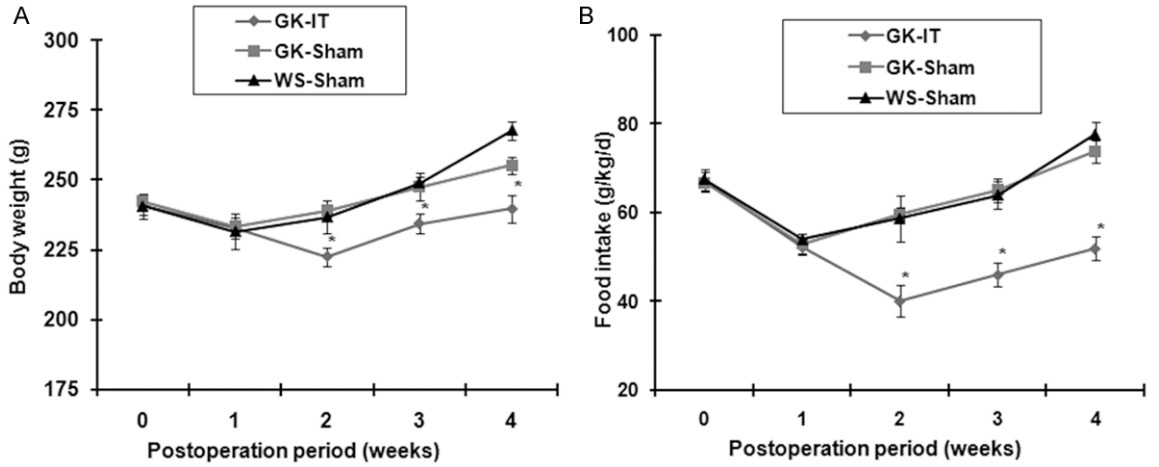

Figure 1. Changes in the body weight values and food intakes among the three groups (WS-Sham, GK-Sham, and GK-IT). A. The rat body weight was measured one week before and every week after operation in each group. *, $P < 0.05$ compared with GK-Sham group and WS-Sham group. B. The rat food intake rates were measured 1 week before and every week after operation in each group. *, $P < 0.05$ compared with the GK-Sham group and WS-Sham groups.

In this study, the GK rat model of T2DM was used to investigate the effects of IT on glycemic controlling, insulin resistance, GLP-1, GLP-1R, and β cell apoptosis, in order to further elucidate its possible mechanisms.

Materials and methods

Animals and grouping

Twelve male GK rats of 8-week old (provided by Slac Laboratory Animal Co., Shanghai Laboratory Animal Center, Chinese Academy of Sciences) were randomly divided into 2 groups: IT group (GK-IT group) and sham group (GK-Sham group). Each group contains of 6 rats. Six Wistar rats of the same age were used as the non-diabetes sham group (WS-Sham group). Rats were fed for one week to adapt to the environment with 12-hour light/dark cycle, and free access to standard feed and water. All animal experiments were carried out in accordance with the requirements of Capital Medical University Animal Committee.

Surgical procedures

After fasting of 12 hours before surgery, 10% chloral hydrate (0.3 ml/100 g body weight) was injected intraperitoneally for anesthesia. IT was performed according to Culnan *et al* [10] as following. Firstly, midline longitudinal incision of abdominal was taken. Then, 10 cm of ileum in distance of 5-15 cm to ileocecum was intercepted with mesenteric reserved. The 10 cm of ileum was moved to jejunum 5 cm from the flexor ligament, and end-to-end anastomosis was performed. The 5-0 silk threads were used for anastomosis. Bleeding and intestinal leakage was checked and 3-0 threads were used for abdomen closure. In the GK-sham and WS-sham groups, enterotomy and intestinal anastomosis were performed in the corresponding sites. Postoperative treatment was in accordance with Li *et al* [11].

Experiments

Rats body weight and food intake were recorded one week before surgery, and weekly after surgery in four weeks. Blood glucose meter was used to detect the fasting (fasting for 12-14 hours usually) blood glucose levels one week before surgery and the levels of the weekly fasting blood glucoses within 4 weeks post-surgery. Glucose meter SureStep Plus was purchased from Lifescan, Johnson & Johnson (Lifescan, Johnson & Johnso, New Brunswick, New Jersey, USA). Oral glucose tolerance test (OGTT) was performed before surgery and the fourth week after surgery. After fasting for 12-14 hours, rats were given orally glucose solution gavage in sober state (1 g/kg). Glucose values were measured before gavage (fasting) and at 30 min, 60 min, or 120 min after gavage. Trapezoidal method was used to calculate the area under

Table 1. Fasting glucose values of all groups one week pre-surgery and weekly post-surgery (mg/dl)

	GK-Sham	GK-IT	WS-Sham
1 week pre-surgery	118 ± 4*	123 ± 6*	93 ± 3
1 week post-surgery	115 ± 5*	97 ± 5*,**	88 ± 6
2 weeks post-surgery	120 ± 6*	101 ± 7*,**	95 ± 2
3 weeks post-surgery	124 ± 10*	96 ± 4**	89 ± 7
4 weeks post-surgery	130 ± 11*	94 ± 5**	89 ± 8

Note: *, $P < 0.05$ compared with the WS-Sham group; **, $P < 0.05$ compared with the GK-Sham group.

the curve (AUC). Plasma insulin concentration and fasting GLP-1 concentration were determined by ELISA assay before gavage (fasting) and at 30 min after gavage one week before surgery and weekly after surgery in four weeks. Rat insulin enzyme-linked immunosorbent (ELISA) kit and rat GLP-1 ELISA kit was purchased from IBL Company (Hamburg, Germany). According to the fasting glucose and fasting insulin, insulin resistance levels were measured by homeostasis model assessment value (HOMA-IR), which is calculated as fasting glucose (mmol/l) × fasting insulin (mU/ml)/22.5.

Rats were sacrificed after 28 days, and pancreas tissues were taken along the longitudinal axis of incision for GLP-1R protein expression by Western blotting. Some pancreas tissue was sliced and detected in accordance with apoptosis detection kit. In situ end labeling (TUNEL) was used for detection of pancreatic β-cell apoptosis. Apoptosis Detection Kit was purchased from Roche Company (Branford, CT, USA). TUNEL result is apoptosis rate in five high fields of each slice (× 400) = (number of apoptosis cells/number of total cells) × 100%.

Western blotting

Total proteins were harvested and separated on 10% SDS/PAGE gels, and then subjected to immunoblot analyses. PVDF membrane was purchased from Millipore Corporation (Billerica, MA, USA). Rabbit anti-mouse GLP-1R polyclonal antibody was purchased from Abcam Company, UK (Cambridge, UK). Rabbit anti-mouse β-actin polyclonal antibody was purchased from Tianjin Saier Biotechnology Company (Tianjin, China). Bound antibodies were detected using the ECL system (Perkin Elmer, NEL100001EA). The immunoblot experiments were repeated at least 3 times. Image quantifications were performed using ImageQuant software.

Statistical analysis

Data are expressed as mean ± standard deviation (SD). One-way ANOVA was analyzed by SPSS13.0 software. $P < 0.05$ was considered statistically significant.

Results

Surgery reduces body weight and food intake in the animal model

Rats in all three groups (the GK-Sham group, WS-Sham group, and GK-IT group) survived after surgery and four weeks of experiment. One week before surgery, there was no significant difference of rat body weight and food intake per kilogram between groups ($P > 0.05$). Average body weight decreased after surgery in all groups. In the GK-Sham group and WS-Sham group, body weight slowly increased one week after surgery. In the GK-IT group, body weight decreased two weeks after surgery and then rebounded. Postoperatively, food intake was significantly reduced, and gradually increased afterwards. In 2, 3, and 4 weeks after surgery, the body weights (**Figure 1A**) and food intakes (**Figure 1B**) of the GK-IT group were significantly reduced compared with those in the GK-Sham group and the WS-Sham group ($P < 0.05$).

IT decreases glucose tolerance and insulin resistance

The influence of IT on fasting glucose and oral glucose tolerance of type 2 diabetes rats is shown in **Table 1**. One week before surgery, fasting glucose of GK rats was significantly higher than that of Wistar rats ($P < 0.05$). After IT, fasting blood glucose of the GK-IT group was significantly decreased compared with the GK-Sham group ($P < 0.05$). Three week after surgery, the fasting glucose of GK-IT rats were reduced to levels similar to those WS sham group level ($P > 0.05$).

To further study the influence of IT on glucose tolerance, oral glucose tolerance test (OGTT) was detected one week before surgery and weekly after surgery for four weeks in each group (**Table 2**). The AUC of OGTT in GK-IT and GK-Sham group was significantly greater than

Table 2. OGTT and AUC values of all groups one week pre-surgery and four weeks post-surgery

	0 min	30 min	60 min	120 min	AUC [mg/dL(min)]
Pre-surgery					
GK-IT	123 ± 6	253 ± 9	224 ± 13	155 ± 4	24,215 ± 795*
GK-Sham	118 ± 4	242 ± 11	217 ± 13	151 ± 4	23,378 ± 791*
WS-Sham	93 ± 3	142 ± 8	107 ± 10	95 ± 15	13,302 ± 869
Post-suegery					
GK-IT	94 ± 5	162 ± 19	136 ± 7	110 ± 18	15,696 ± 689*,**,#
GK-Sham	130 ± 11	260 ± 22	232 ± 17	136 ± 13	24,260 ± 1544*
WS-Sham	89 ± 8	140 ± 4	108 ± 10	95 ± 7	13,221±640

Note: *, $P < 0.05$ compared with WS-Sham; **, $P < 0.05$ compared with GK-Sham; #, $P < 0.05$ compared with pre-operation.

Table 3. GLP-1 concentration changes of all groups pre-surgery and four weeks post-surgery (pmol/L)

	Pre-surgery	4 weeks post-surgery
GK-IT	20.89 ± 2.56*	53.53 ± 1.87**,#
GK-Sham	20.06 ± 2.00*	19.70 ± 2.25*
WS-Sham	50.39 ± 3.08	50.36 ± 3.14

Note: *, $P < 0.05$ compared with WS-Sham group; **, $P < 0.05$ compared with GK-Sham group; #, $P < 0.05$ compared to the values of the relative pre-operative group.

that in WS-Sham group ($P < 0.05$). Fourth weeks after surgery, OGTT was also detected in all groups, and oral glucose tolerance AUC of GK-IT group was significantly less than that in GK-Sham group ($P < 0.05$) with 35% decrease compared with that before surgery. It showed IT effectively increased oral glucose tolerance in GK rats. Our results also showed that glucose-stimulated insulin secretion of GK-IT rats after surgery increased significantly compared with GK-Sham (data not shown). Four weeks after surgery, HOMA-IR value of GK-IT group was significantly lower than that in the GK-Sham group (data not shown).

IT increases pancreas GLP-1R expression levels of GK rats

To further explore the mechanism of IT treatment for diabetes, plasma GLP-1 concentration was detected one week before and 4 weeks after surgery. Rats were sacrificed four weeks after surgery, and pancreas GLP-1R protein expression was determined. One week before surgery, plasma GLP-1 concentration of diabetes rats was significantly lower than that of Wistar rats ($P < 0.05$). Four weeks after surgery, plasma GLP-1 concentration of GK-IT group was significantly increased, but there was no significant difference with WS-Sham group (**Table 3**). After IT, pancreas GLP-1R expression of GK rats was significantly increased and significantly higher than those in the GK-Sham group and WS-Sham group ($P < 0.05$) (**Figure 2**). It showed that IT increased plasma GLP-1 concentrations and pancreas GLP-1 receptor expression. The increase of GLP-1 concentration and GLP-1R level is the possible mechanism of IT treatment for improved glucose tolerance and type 2 diabetes.

IT decreases β-cell apoptosis rates of GK-IK rats

Four weeks after operation, TUNEL apoptosis kit was used to detect rat β-cell apoptosis. The apoptosis rate was 7.18 ± 0.49% in GK-IT group, 25.02 ± 1.57% in GK-Sham group, 5.98 ± 0.87% in WS-sham group. After IT, β-cell apoptosis rate of GK-IT rat was significantly lower than that in the GK-Sham group ($P < 0.05$), and there was no significant difference when compared levels in the WS-Sham group (**Figure 3A** and **3B**).

Discussion

In this study, body weight and food intake of GK rats after IT were gradually reduced, and fasting glucose also decreased. Fasting glucose decreased to the same level of non-diabetic rats in WS-Sham group level three weeks after surgery. Though body weight and food intake of GK-IT rats increased three weeks after surgery, fasting glucose remained same. It indicated that after IT, improved glucose tolerance was not associated with body weight and food intake, which is consistent with the previous studies [12, 13]. Therefore, decreased fasting glucose is independent of weight loss after IT. Four weeks after surgery, OGTT AUC of GK-IT

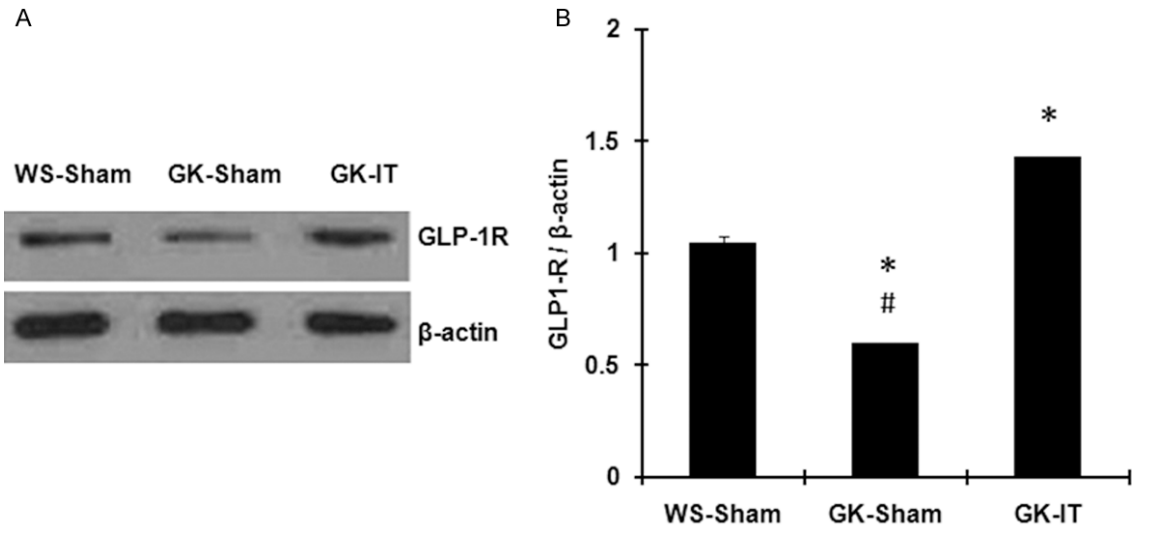

Figure 2. Expression of GLP-1R protein in rats of the three groups. A. Western blot analysis of the expression of GLP-1R protein. The GLP-1R protein was isolated from tissues four weeks after surgery. β-actin was used as a loading control. B. The expression levels of GLP-1R protein relative to β-actin in the three groups were calculated. *, $P < 0.05$ compared with WS-Sham group; #, $P < 0.05$ compared with the GK-IT group.

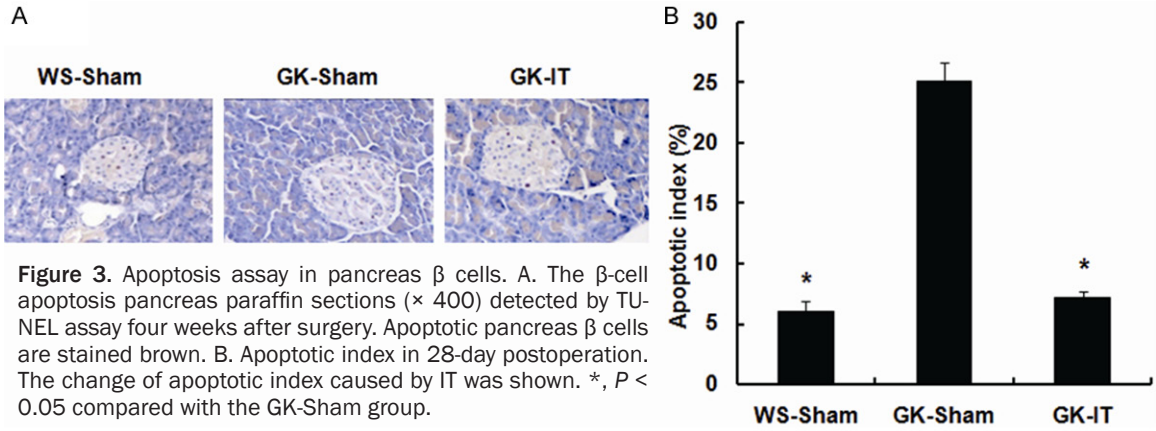

Figure 3. Apoptosis assay in pancreas β cells. A. The β-cell apoptosis pancreas paraffin sections (× 400) detected by TUNEL assay four weeks after surgery. Apoptotic pancreas β cells are stained brown. B. Apoptotic index in 28-day postoperation. The change of apoptotic index caused by IT was shown. *, $P < 0.05$ compared with the GK-Sham group.

group was significantly lower than that in GK-Sham group, which indicated surgery effectively controlled blood glucose [13, 14].

Before and 4 weeks after surgery, there was no significant difference of fasting plasma insulin levels in GK-IT group and GK-Sham group. After IT, insulin resistance of GK-IT group was significantly improved than that in GK-Sham group. Four weeks after surgery, plasma insulin concentration of GK-IT group was significantly higher than that in GK-Sham group 30 minutes after gavage, indicating that IT can reduce insulin resistance of type 2 diabetes in rats and promote pancreas β-cell insulin secretion after meal.

GLP-1 is polypeptide with 30 amino acids secreted by L cells in distal small intestine and colon [15]. GLP-1 increases glucose-induced insulin secretion, inhibits glucagon secretion, reduces food absorption, improves insulin sensitivity, promotes β-cell regeneration, and reduces its apoptosis [6]. When excessive chyme contacts with the distal small intestine, gastrointestinal hormone secretion was increased [13]. IT inserts distal ileum into proximal intestine, so food that is not completely digested or undigested would prematurely reach the ileum, thus stimulates intestinal L cells to increase GLP-I secretion. In our study, it shows that 4 weeks after surgery, plasma GLP-1 concentration increased to normal levels. GLP-1 is

effective only after binding to its receptor. Therefore four weeks after surgery, pancreas GLP-1R protein expression was detected. GLP-1R is membrane protein located on pancreas β cells [16, 17]. It shows that, four weeks after surgery, GLP-1R protein expression level in IT group was higher than that in GK-Sham group and WS-Sham group.

Apoptosis is the main reason for reduced pancreas β-cell in type 2 diabetes [18], it is found that β-cell apoptosis was less in GK-IT group than that in GK-Sham group. Reduced pancreas β-cell apoptosis by IT is one reason for the increased plasma insulin concentration after glucose gavage. GLP-1 regulates the expression of apoptosis-related gene Bcl-2/Bax, reduces β-cell apoptosis and increases β-cell number to increase insulin secretion for control of diabetes [19]. Improved GLP-1 concentration and GLP-1R level may be the possible mechanism to reduce β-cell apoptosis.

In conclusion, it shows that IT effectively reduced the blood glucose levels, improved insulin resistance and increased insulin concentrations after glucose gavage of diabetes GK rats. The increased plasma GLP-1 concentration and pancreas GLP-1R protein expression, as well as reduced pancreas β-cell apoptosis rate, may be the possible mechanism of IT for the treatment of type 2 diabetes.

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Disclosure of conflict of interest

None.

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