



The Changes of Serum Metabolites in Diabetic GK Rats after Ileal Transposition Surgery

Kemin Yan¹ · Weijie Chen² · Huijuan Zhu¹ · Guole Lin² · Wei Sun³ · Xiaoyan Liu³ · Hui Pan¹ · Linjie Wang¹ · Hongbo Yang¹ · Meijuan Liu¹ · Fengying Gong¹

Published online: 6 November 2018

© Springer Science+Business Media, LLC, part of Springer Nature 2018

Abstract

Background Ileal transposition (IT) surgery could improve metabolism. Metabolomics has been applied comprehensively in analyzing the global dynamic alterations of metabolites. In the present study, we aimed to investigate serum metabolite alterations in diabetic Goto-Kakizaki (GK) rats after IT surgery.

Methods Male GK rats were subjected to IT and Sham-IT surgery. Six weeks later, body weight, food intake, fat mass, and serum biochemical parameters were measured. The serum metabolomic fingerprint was analyzed using ultra-performance liquid chromatography–mass spectrometry (LC-MS)-based, non-targeted metabolomic approach. The differential metabolites were identified using principal component analysis and orthogonal partial least squares discriminant analysis. Metabolic pathway analysis was performed using HMDB and KEGG databases.

Results The body weight, food intake, fat mass, serum levels of glucose and insulin, and homeostasis model assessment of insulin resistance (HOMA-IR) of IT rats were significantly decreased when compared with Sham-IT rats (all $P < 0.05$). In the metabolomics analysis, ten serum differential metabolites were identified. Compared with Sham-IT rats, serum LysoPC(O-18:0) and PG(20:4/20:0) of IT rats were decreased, while genistein 4'-O-glucuronide, 5,6:8,9-Diepoxyergost-22-ene-3,7beta-diol, PI(16:0/18:2(9Z,12Z)), docosapentaenoic acid, 3-Oxo-4,6-choladienoic acid, 3-Oxocholeic acid, and TG were increased. Pathway analysis highlighted the following pathways: ether lipid metabolism, alpha linolenic acid and linolenic acid metabolism, incretin synthesis and secretion, free fatty acid receptors, and biosynthesis of unsaturated fatty acids.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11695-018-3582-4>) contains supplementary material, which is available to authorized users.

✉ Fengying Gong
fygong@aliyun.com; fygong@sina.com

Kemin Yan
yankemin1@sina.com

Weijie Chen
wedge.c@163.com

Huijuan Zhu
shengxin2004@163.com

Guole Lin
guolelin2002@163.com

Wei Sun
sunwei1018@sina.com

Xiaoyan Liu
xiaoyanliu.good@163.com

Hui Pan
panhui20111111@163.com

Linjie Wang
eileenwood@163.com

Hongbo Yang
hongbo_yang7@126.com

Meijuan Liu
1355417772@qq.com

¹ Key Laboratory of Endocrinology of National Health Commission, Department of Endocrinology, Peking Union Medical College Hospital, Chinese Academy of Medical Science and Peking Union Medical College, Beijing 100730, China

² Department of Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Science and Peking Union Medical College, Beijing 100730, China

³ Core Facility of Instrument, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences/School of Basic Medicine, Peking Union Medical College, Beijing 100730, China

Conclusions IT surgery could significantly decrease body weight and fat mass and improve glucose metabolism in diabetic GK rats. These beneficial effects might be related to the changes of serum metabolites which involved in lipid metabolism, bile acids, and incretin.

Keywords Ileal transposition (IT) surgery · Metabolomics · Fat mass · Liquid chromatography–mass spectrometry (LC-MS) · Goto-Kakizaki (GK) rats

Introduction

Bariatric or metabolic surgery has been reported to effectively improve type 2 diabetes mellitus (T2DM) [1–3]. Ileal transposition (IT) surgery is a promising bariatric surgery, in which a distal ileum segment is translocated to the upper jejunum distal to the ligament of Treitz [4]. Recently, IT surgery has been shown to lose weight, improve glucose and lipid metabolism, and increase insulin sensitivity in diabetic or obese rats [4–10]. The beneficial effects of IT surgery have been indicated to be associated with the increased secretion of hindgut peptides, the alterations in enterohepatic bile acid metabolism, the changes in host-gut microbial metabolic crosstalk, and the improvements in glucose and lipid metabolism in liver, muscle, and adipose tissue [4, 11]. However, there are no reports about serum metabolite alterations in animals after IT surgery.

Metabolomics, one of the newer omics fields, has been widely applied in comprehensively analyzing the changes of small molecular metabolites (< 1 kDa) in living organisms. It is becoming an effective and important tool in pharmacological researches, biology studies, biomarker discovery, disease diagnosis, and toxicity evaluations [12–14]. Metabolomics studies mainly rely on the high-throughput and highly sensitive technology platforms such as nuclear magnetic resonance (NMR), liquid chromatography–mass spectrometry (LC-MS), and gas chromatography–mass spectrometry (GC-MS). In general, metabolomics can be roughly divided into the targeted metabolomics and the untargeted metabolomics [15]. In the targeted metabolomics, specific compounds are quantified and compared to established reference ranges. In the untargeted metabolomics, all detectable metabolites are analyzed and compared between groups of samples. Therefore, the untargeted metabolomics is a semi-quantitative way which has been used in the research without any priori metabolic hypothesis. LC-MS method has been the preferred choice for untargeted metabolomics due to its versatility in metabolite coverage and sensitivity of the instruments [15]. In recent years, several metabolomics studies have been performed to explore the metabolic effects of bariatric surgery such as Roux-en-Y gastric bypass (RYGB), sleeve gastrectomy (SG), and gastric banding (GB) [16–23]. For example, clinical improvements after RYGB surgery have been reported to be associated with the metabolic alterations of free fatty acids, amino acids, bile acids, and lipids species [20, 21]. Both plasma and cardiac metabolic shifts also confirmed the

enhancement of cardiac energy metabolism after RYGB surgery [19]. In addition, metabolic improvements after SG and GB surgery have been found to be correlated with lipid metabolites [23].

In the present study, we firstly investigated serum metabolome alterations in diabetic Goto-Kakizaki (GK) rats after IT surgery using the untargeted LC-MS metabolomics approach. Our preliminary results would provide a basic metabolomics evidence for understanding the beneficial effects of IT surgery.

Materials and Methods

Animals

Male GK T2DM rats (10 weeks old, weighing 279.7 ± 4.0 g) were obtained from National Rodent Laboratory Animal Resources (Shanghai, China). Rats were housed individually in standard cages under controlled temperature (22–25 °C) and humidity (about 60%), and maintained on a 12-h dark/light cycle. The rats were fed with high-fat diets (total energy 45% fat, 35% carbohydrates, and 20% protein; H10045, Beijing HFK Bioscience Co. Ltd., Beijing, China) and have free access to water before the surgery. After 1 week of acclimation, rats were randomly assigned to IT and Sham-IT groups ($n = 7$ per group). The surgical operations were performed as the following. All animal experimental procedures were approved by the ethics committee of Peking Union Medical College Hospital.

Surgical Procedures

The surgical operations were performed following an overnight fasting as previously described [24]. In brief, the rats were anesthetized with gaseous anesthesia (1.5–3% isoflurane), and ileal transposition was performed in rats of the IT group. A 10-cm segment of ileum 5 cm proximal to the ileocecal valve was transected, transposed, and anastomosed isoperistaltically with the jejunum 5 cm distal to the Treitz ligament. An anastomosis was made with the remaining ends of the ileal segments using 7–0 silk sutures. Sham-IT surgery was performed by making transections in the same locations as in the IT surgery and the gastrointestinal tract was reattached by anastomosis in the original position. Sham surgeries were prolonged to achieve similar operative times to IT surgeries.

The Measurements of Body Weight, Food Intake, Fat Mass, and Serum Biochemical Parameters

After surgery, rats were fed with non-residue diet (Ensure, Abbott Laboratories, Chicago, USA) for 1 week and then were fed with high-fat diet ad libitum. The body weights and food intake of the rats were measured weekly for 6 weeks. Subsequently, the rats were administered an inhalation anesthesia using 2% isoflurane after a 12-h fasting. Blood samples were obtained by cardiac puncture. Serum was extracted after centrifuging at 3000 rpm and 4 °C for 10 min. Then, serum fasting blood glucose (FBG), high-sensitivity C reactive protein (hsCRP), triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), free fatty acids (FFAs), lipoprotein(a) [Lp(a)], and uric acid (UA) were measured by routine automated laboratory methods. Serum insulin levels were measured by ELISA kits (CEA448Ra, Wuhan USCN Business Co., Ltd., Wuhan, China) following the manufacturers' instruction and the coefficients of variation was 2.9%. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated according to the following formula: fasting serum insulin (pmol/L) × FBG (mmol/L)/135 [24]. White adipose tissue (WAT), including epididymal adipose tissue (eWAT), perirenal adipose tissue (pWAT), and inguinal subcutaneous adipose tissue (sWAT), were dissected and weighted, respectively. The WAT mass percentage was calculated by the percentage of total body weight occupied by the total WAT mass.

Metabolomic Analysis

Serum samples were thawed at 4 °C. One hundred microliters serum samples were mixed with 100 µL distilled water and then homogenized with 400 µL pre-chilled acetonitrile, followed by vigorous vortex, let stand for 60 min at −4 °C and centrifuged at 14,000g and 4 °C for 10 min. The supernatant was dehydrated under vacuum and then dissolved with 100 µL of 2% acetonitrile. To test the stability and reproducibility of metabolomic analysis, the quality control (QC) sample was used. A pool of equal volumes of serum from each sample was used to prepare the QC sample. Two microliters of mixture were sent to the ultra-performance LC-MS measurements using a Waters ACQUITY H-class LC system coupled with a linear ion-trap quadrupole (LTQ) Orbitrap mass spectrometer (Thermo Fisher Scientific, MA). Serum metabolites separation was performed using a Waters HSS C18 column (3.0 × 100 mm, 1.7 µm) with a 36-min gradient at a flow rate of 0.3 mL/min. The 0.1% formic acid in water and acetonitrile were used as mobile phases A and B, respectively. The gradient was set as follows: 0–2 min, 2% solvent B; 2–5 min, 2–55% solvent B; 5–15 min, 55–100% solvent B; 15–25 min, 100% solvent B; 20–25.1 min, 100–2% solvent B; and 25.1–36 min, 2% solvent B. The column temperature was set at

40 °C. Mass spectra were acquired with ion spray voltage at 4.2 kV in a positive mode, capillary temperature at 350 °C, sheath gas nitrogen at 45 arbitrary units, and auxiliary gas at 10 arbitrary units. The mass range was set from 100 to 1000 *m/z*.

Data Processing

The original data files were processed using the Progenesis QI software (Waters, Milford, MA) [25]. Further data processing was performed using the MetaAnalyst 3.0 (<http://www.metaboanalyst.ca>). Variables with relative standard deviation lower than 50% of the samples were kept for further statistical analysis. After the pareto scaling, principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were performed using SIMCA (version 14.0, Umetrics, Sweden). Further, according to the variable importance plot (VIP) value obtained from OPLS-DA and *P* values in non-parametric tests (Wilcoxon rank-sum test), variables with VIP > 1.0 and *P* < 0.05 were selected. Metabolic pathway analysis was searched on free databases: HMDB (<http://www.hmdb.ca/>) and KEGG (<http://www.genome.jp/kegg/>).

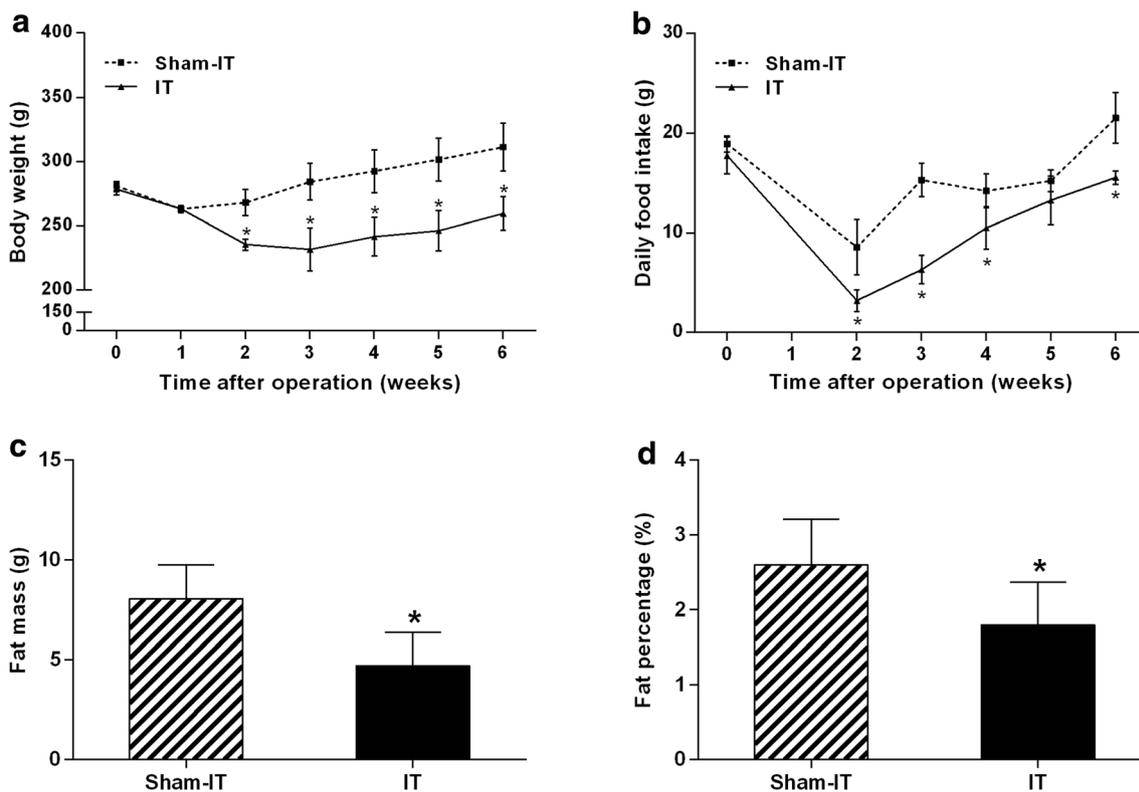
Statistical Analysis

Data were presented as mean ± standard deviation (SD) or the median (25–75%). The *t* test was used for data analysis. Skewed data were ln-transformed, and Mann-Whitney *U* test was used if the data were still not normally distributed. All of the statistical computations were run using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). The significance level was set at *P* < 0.05.

Results

Effects of IT Surgery on Body Weight, Food Intake, Fat Mass, and Serum Biochemical Parameters in Diabetic GK Rats

The surgical operations were progressed smoothly. Two rats in IT group and one in Sham-IT group were died due to the intestinal obstruction after surgery. The initial body weight and food intake of rats in IT and Sham-IT groups were similar before surgery. At the beginning of the 2nd week after surgery, the body weight of the rats in IT group were significantly decreased by 12.2% compared with the Sham-IT group (Fig. 1a, *P* < 0.05). This decreasing trend was continued to the 6th week after surgery, when the body weight of the IT rats was decreased to 83.4% of the Sham-IT rats (*P* < 0.05). The similar change was also found in the food intake of IT rats which was decreased to 72.2% of the Sham-IT rats at the 6th



* $P < 0.05$ vs. Sham-IT group

Fig. 1 Effects of IT surgery on the body weight, food intake, and fat mass of diabetic GK rats. GK rats underwent IT ($n = 5$) or Sham-IT surgery ($n = 6$), and the changes in body weight (a) and food intake (b) were weekly observed for 6 weeks after IT surgery. Six weeks later, white adipose tissue (WAT), including inguinal subcutaneous adipose tissue

(sWAT), epididymal adipose tissue (eWAT), and perirenal adipose tissue (pWAT), was obtained from the rats and weighed (c). The WAT mass percentage was calculated by the percentage of body weight occupied by the total WAT mass (d). The data are represented as the mean \pm SD. * $P < 0.05$ vs. the Sham-IT group

week after surgery (Fig. 1b, $P < 0.05$). Meanwhile, total WAT mass of IT rats showed a 41.5% reduction compared with the Sham-IT rats at the 6th week after surgery (Fig. 1c, $P < 0.05$). Similarly, the WAT mass percentage of the IT rats was also decreased by 30.9% in comparison with the Sham-IT rats (Fig. 1d, $P < 0.05$).

Besides, there was significant improvement in glucose metabolism of the rats after IT surgery. As presented in Table 1, serum levels of FBG and insulin of the IT rats were remarkably decreased by 51.0% and 22.0%, respectively, compared with the Sham-IT rats ($P < 0.05$). HOMA-IR, the indicator of insulin resistance, was also decreased in the rats after IT surgery. However, there were no significant differences in the serum levels of hsCRP, TG, TC, LDL-c, HDL-c, FFA, Lp(a), and UA between the two groups.

Metabolomics Analysis of Serum Samples

Positive ion full-scan chromatograms of the representative serum samples from QC, Sham-IT group and IT group were shown in the Supplementary Figure S1. Although some

Table 1 Effects of IT surgery on serum biochemical parameters of diabetic GK rats

	Sham-IT ($n = 6$)	IT ($n = 5$)
FBG (mmol/L)	14.05 \pm 0.76	6.88 \pm 0.35*
Insulin (ng/mL)	1.86 \pm 0.40	1.45 \pm 0.71*
HOMA-IR	30.55 (26.32, 39.15)	11.01 (7.50, 16.50)*
hsCRP (mg/L)	0.06 \pm 0.01	0.05 \pm 0.01
TG (mmol/L)	0.57 \pm 0.38	0.67 \pm 0.22
TC (mmol/L)	1.97 \pm 0.63	2.22 \pm 0.41
LDL-c (mmol/L)	0.23 \pm 0.06	0.22 \pm 0.04
HDL-c (mmol/L)	0.53 \pm 0.22	0.63 \pm 0.12
FFA (μ mol/L)	435.18 \pm 91.78	434.76 \pm 169.35
Lp(a) (mg/L)	2.32 \pm 1.32	2.38 \pm 1.69
UA (μ mol/L)	113.68 \pm 24.17	125.18 \pm 32.55

Values are the mean \pm SD or the median (25–75%). FBG, fasting blood glucose; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity C reactive protein; TG, triglycerides; TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; FFA, free fatty acid; Lp(a), lipoprotein(a); UA, uric acid. * $P < 0.05$ vs. the Sham-IT group

changes of the metabolites in IT and Sham-IT groups could be observed from the chromatograms, a clear detailed result could only be provided by means of pattern recognition approach.

Metabolomics technical stability was assessed by analyzing the QC sample variations with time. The scoring plots in PCA mode showed a good reproducibility of QC (Fig. 2), suggesting that the platform had essential stability throughout the analytical run. Then, unsupervised PCA was performed to explore the tendency of serum metabolic profiling variations between IT and Sham-IT rats. The resulting score plot as presented in Fig. 3 shows that the metabolic profiling in IT rats could be obviously separated from the Sham-IT rats, with IT rats' samples clustering to the right of the PCA plot while Sham-IT rats to the left. To highlight the metabolite differences between the IT and Sham-IT rats, feature selections were performed by using an OPLS-DA model as shown in Fig. 4a. This OPLS-DA model was considered to have good predictive capability. The result indicated that there was a significant separation in serum metabolites between IT and Sham-IT rats. A permutation test ($n = 20$), with $R^2X = 0.602$, $R^2Y = 0.921$, $Q^2 = 0.812$, Q^2 -incept = -0.292 (Fig. 4b), confirmed the validity of the model.

In the OPLS-DA model and the non-parametric tests, variables with VIP values > 1.0 and $p < 0.05$ were selected as potential biomarkers for the effects of IT surgery. Finally, a total of ten characteristic serum metabolites were identified to be significantly different between IT and Sham-IT rats (Table 2, $P < 0.05$). Among them, compared with the rats in Sham-IT group, serum LysoPC(O-18:0) and PG(20:4/20:0) of the rats in IT group were decreased, while genistein 4'-O-glucuronide, 5,6:8,9-Diepoxyergost-22-ene-3,7beta-diol, PI(16:0/18:2(9Z,12Z)), docosapentaenoic acid, 3-Oxo-4,6-choladienoic acid, 3-Oxochoolic acid, and TG of the rats in IT group were increased. Pathway analysis of these metabolites showed that the effects of IT surgery might be associated with ether lipid metabolism, alpha linolenic acid and linolenic acid

metabolism, incretin synthesis, secretion and inactivation, free fatty acid receptors, and biosynthesis of unsaturated fatty acids.

Discussion

In the present study, IT surgery was demonstrated to significantly reduce body weight, food intake and fat mass, and decrease serum levels of FBG and insulin, as well as HOMA-IR in diabetic GK rats. In accordance with our results, these beneficial effects of IT surgery have also been described by several other scientists in GK rats or other diabetic rats [5–10, 26–29]. Further, the possible mechanisms that might contribute to these beneficial effects of IT surgery have also been reported. The anatomic changes in intestinal tract and the alterations of gut hormone, including enhanced secretion of glucagon-like peptide-1 (GLP-1) and increased activation of GLP-1 signaling, played a role in the improvements in glucose and lipid metabolism after IT surgery [5, 9, 10, 27, 28]. Besides, changes in bile acids metabolism, improvements in brown adipose tissue function, and upregulation of the WNT signaling pathway may mediate the metabolic benefits seen after IT surgery [7, 8, 26]. Although there were no significant differences in the levels of serum lipid profiles between IT and Sham-IT groups in our present study, IT surgery has been found to have an effect on lipid metabolism in some researches. Studies conducted by Cummings et al. and Kohli R et al. showed that fasting plasma TG and cholesterol concentrations were significantly lower after IT surgery in diabetic or diet-induced obese rats [10, 26].

To the best of our knowledge, this is the first time that metabolomics methodology was employed in investigating the serum metabolites alterations associated with the effects of IT surgery. As a result, the differential metabolites between the rats after IT surgery and Sham-IT surgery could be summarized as bile acids, ergostane steroid, ether phospho-ether lipid, glycerophospholipid, polyphenol

Fig. 2 Assessment of QC samples. Trend plot showing the variation of $t[1]$ over the QC sample. X-axis numbers represented the number of times for QC sample assessment; Y-axis was arbitrary (3 SD)

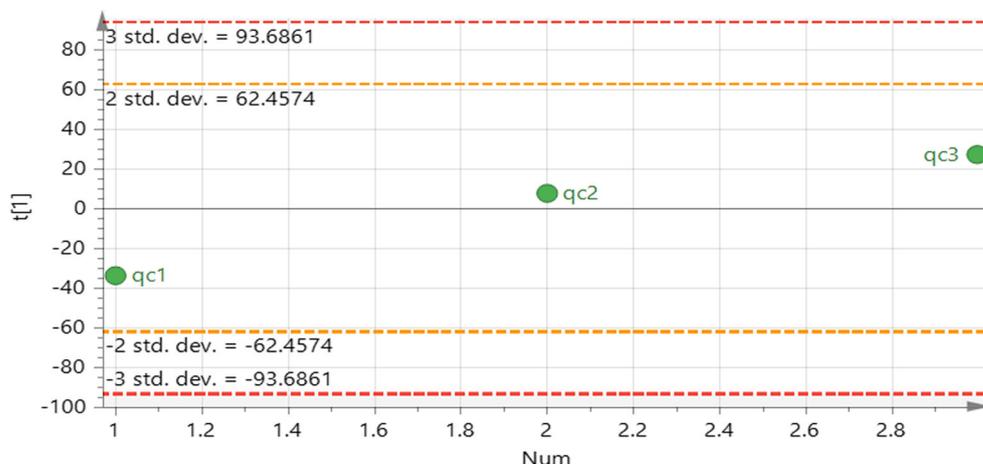
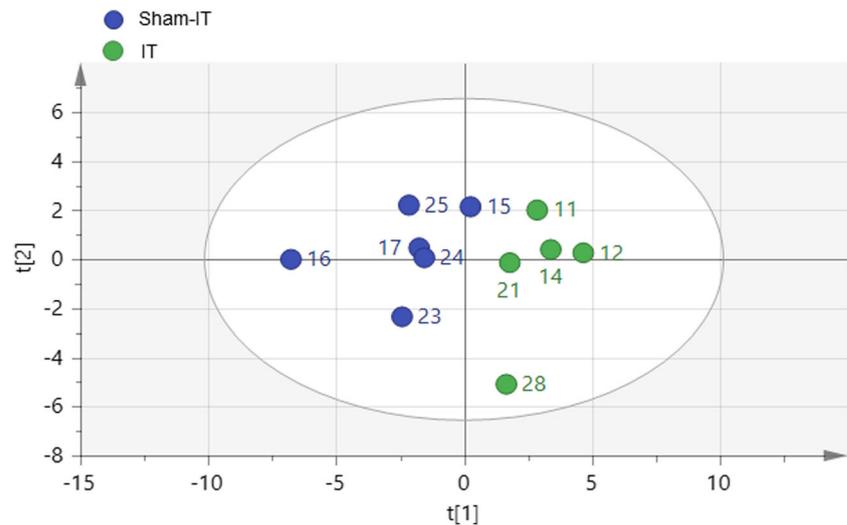


Fig. 3 PCA score plot of metabolic profiling variation of IT and Sham-IT rats. $R^2X = 0.192$



metabolite, triglyceride, and unsaturated fatty acid. Metabolic pathway analysis of these differential metabolites between IT and Sham-IT rats showed that they might probably be involved in lipid metabolism and incretin secretion. Previous metabolomic studies using plasma, serum, urine, and different types of tissue samples also displayed the marked metabolic alterations associated with bariatric surgeries such as RYGB, SG, and some other surgery types, including duodenal-jejunal endoluminal bypass, laparoscopic gastric banding, biliopancreatic diversion, and duodenal-jejunal endoluminal bypass liner [16–18, 30–32]. In these studies, metabolomics techniques have been applied in patients or animals suffering from obesity, type 2 diabetes, and non-alcoholic fatty liver disease. Consistent with our findings, pathway analysis of metabolites from these metabolomic studies also showed that the following pathways, including linoleic acid metabolism, alpha-linolenic acid metabolism, primary bile acid

biosynthesis, and fatty acid biosynthesis, were associated with bariatric surgeries [16].

Triglycerides (TGs), the major components of very low-density lipoprotein and chylomicrons, play an important role in metabolism as energy sources and transporters of dietary fat [33]. It has been demonstrated that some kinds of TGs, such as those with long-chain fatty acids, correlated negatively with insulin levels after RYGB surgery [34]. Therefore, the increased of TGs after IT surgery in the present study might contribute to the decrease of insulin levels. Docosapentaenoic acid, also known as clupanodonic acid, is a long-chain omega-3 polyunsaturated fatty acid which is abundant in fish oils [35]. The beneficial effects of long-chain omega-3 polyunsaturated fatty acids on insulin sensitivity and glucose metabolism have been documented previously [36]. The increase of this omega-3 polyunsaturated fatty acid in rats after IT surgery indicated that it might be associated with the improvement of glucose metabolism after IT surgery.

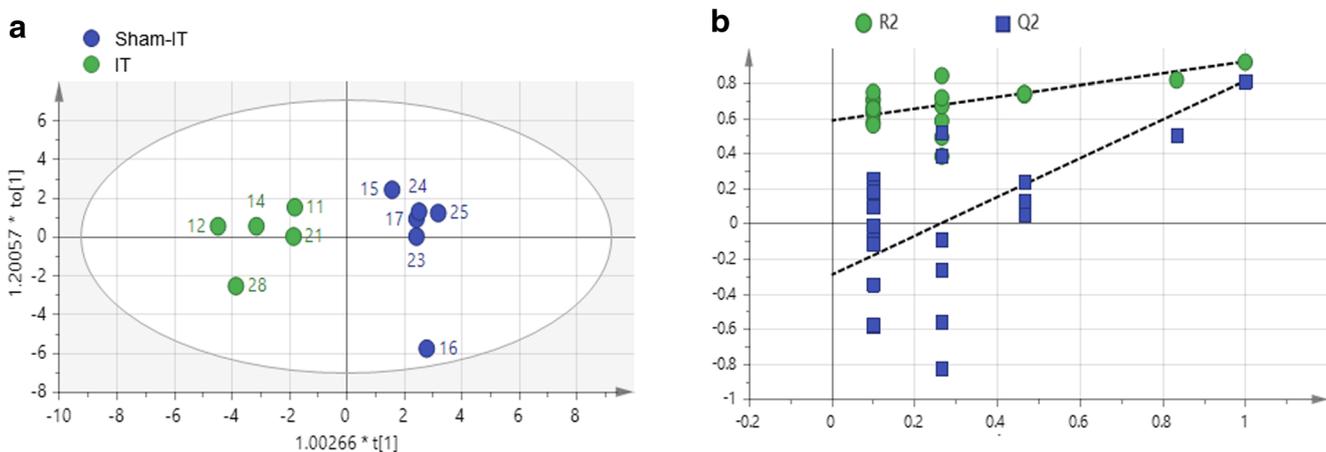


Fig. 4 OPLS-DA analysis of serum metabolic profiling variation of IT and Sham-IT rats. **a** Score plot of OPLS-DA based on serum profiling of IT and Sham-IT rats. $R^2X = 0.602$, $R^2Y = 0.921$, $Q^2 = 0.812$. **b** Permutation test plot for the PLS-DA model (number of permutations, 20; Q^2 -incept = -0.292)

Table 2 Identification of characteristic serum metabolites between IT and Sham-IT rats

Compound	ID	Description	VIP	Fold change [#]	P value
17.32_ 510.3906 <i>m/z</i>	HMDB11149	LysoPC(O-18:0)	2.73	0.07	0.029
13.89_ 844.5461 <i>m/z</i>	–	PG(20:4/20:0)	1.53	0.27	0.011
7.43_447.0915 <i>m/z</i>	HMDB41737	Genistein 4'-O-glucuronide	1.86	17.65	0.029
9.85_467.3110 <i>m/z</i>	HMDB33647	5,6:8,9-Diepoxyergost-22-ene-3,7beta-diol	1.45	7.29	<0.001
9.36_835.5322 <i>m/z</i>	HMDB09784	PI(16:0/18:2(9Z,12Z))	1.39	31.52	0.039
9.36_353.2471 <i>m/z</i>	HMDB06528	Docosapentaenoic acid	1.35	10.02	0.045
9.85_371.2577 <i>m/z</i>	HMDB00476	3-Oxo-4,6-choladienoic acid	1.28	7.94	0.014
9.86_424.3052 <i>m/z</i>	HMDB00502	3-Oxochoolic acid	1.26	6.92	0.002
9.79_897.5224 <i>m/z</i>	–	TG	1.10	3.42	0.024
9.79_816.5747n	–	TG	1.01	3.47	0.016

[#] Fold change was obtained by comparing those metabolites in IT group to Sham-IT group

Besides, the differential metabolites PG(20:4/20:0) and PI(16:0/18:2(9Z,12Z)) belong to glycerophospholipids, a kind of the glycerol-based phospholipids which are known as major sources of fatty acid-derived lipid mediators [37]. The opposite trends of PG(20:4/20:0) and PI(16:0/18:2(9Z,12Z)) after IT surgery in our present study might be due to their structural differences. LysoPC(O-18:0), that is, 1-octadecyl-sn-glycero-3-phosphocholine, is an ether phospho-ether lipid. It is the intermediate in the ether lipid metabolism pathway. The decrease of LysoPC(O-18:0) after IT surgery indicated the reduction in ether lipid metabolism.

Additionally, the increased 3-Oxo-4,6-choladienoic acid and 3-Oxochoolic acid are bile acids, which are steroid acids found predominantly in bile of mammals. Bile acids are also steroidal amphipathic molecules derived from the catabolism of cholesterol and physiological detergents that facilitate excretion, absorption, and transport of fats and sterols in the intestine and liver [38, 39]. Coincidentally, previous studies have demonstrated that circulating bile acid concentrations were higher after IT surgery than after sham surgery in obese or diabetic rats [9, 10, 26]. The postoperative increases of circulating bile acids have been considered to contribute to the decreases of plasma TG and cholesterol concentrations after IT surgery [10, 26].

Incretin are gut peptides that are secreted after nutrient intake and stimulate insulin secretion to decrease blood glucose levels [40]. Glucose-dependent insulintropic polypeptide (GIP) and GLP-1 are the well-known incretin secreted from gut. Pathway analysis of the metabolites in our present study showed that the effects of IT surgery might be associated with incretin synthesis, secretion, and inactivation. Consistently, it has been demonstrated that circulating levels of GIP and GLP-1 significantly increased after IT surgery, which might contribute to the hypoglycemic action of IT surgery [5, 6, 9, 10, 29, 41].

In addition, the 5,6:8,9-Diepoxyergost-22-ene-3,7beta-diol, found in mushrooms, belongs to the class of

chemical entities known as ergostane steroids. Its biofunctions involve cell signaling, fuel and energy storage, and membrane integrity and stability. Genistein 4'-O-glucuronide is a polyphenol metabolite detected in biological fluids. Polyphenols are secondary plant metabolites and many kinds of polyphenols have been described in common foods and beverages [42]. The changes of these two metabolites after IT surgery might be associated with the diet and food intake of rats.

In summary, our data manifested that IT surgery significantly decreased the body weight, food intake, and fat mass and improved glucose metabolism in diabetic GK rats. The serum metabolomics analysis after IT surgery showed that these beneficial effects of IT surgery were probably associated with lipid metabolism, bile acids, and incretin. Nowadays, IT surgery has been applied in the treatment of obesity and diabetes for humans only when combined with other surgical procedure like duodenal diverted sleeve gastrectomy [43, 44]. The results of this paper reveal the metabolite changes associated with the beneficial effects of IT surgery, which would possibly promote IT surgery to the treatment of obesity and diabetes for humans. However, this is only a preliminary study to investigate the effects of IT surgery by means of the metabolomic analysis. Further studies must be done to investigate the characteristic metabolites after IT surgery and then further explore its precise mechanism.

Funding The study was supported by grants from the National Natural Science Foundation of China (No. 81400797 for Weijie Chen, No. 30600836, 81471024 for Huijuan Zhu, No. 30540036, 30771026, 81370898 for Fengying Gong), the Beijing Natural Science Foundation (No. 7082079, 7182130 for Fengying Gong), the National Key Program of Clinical Science (WBYZ2011-873 for Fengying Gong and Huijuan Zhu), and the PUMCH Foundation (2013-020 for Fengying Gong).

Compliance with Ethical Standards

All animal experimental procedures were approved by the ethics committee of Peking Union Medical College Hospital.

Conflict of Interest The authors declare that they have no conflict of interest.

References

- Dixon JB, le Roux CW, Rubino F, et al. Bariatric surgery for type 2 diabetes. *Lancet*. 2012;379(9833):2300–11.
- Koliaki C, Liatis S, le Roux CW, et al. The role of bariatric surgery to treat diabetes: current challenges and perspectives. *BMC Endocr Disord*. 2017;17(1):50.
- Pok EH, Lee WJ. Gastrointestinal metabolic surgery for the treatment of type 2 diabetes mellitus. *World J Gastroenterol*. 2014;20(39):14315–28.
- Oh TJ, Ahn CH, Cho YM. Contribution of the distal small intestine to metabolic improvement after bariatric/metabolic surgery: lessons from ileal transposition surgery. *J Diabetes Investig*. 2016;7(Suppl 1):94–101.
- Sun X, Zheng M, Song M, et al. 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. *Int J Clin Exp Pathol*. 2014;7(7):4136–42.
- Ramzy AR, Nausheen S, Chelikani PK. Ileal transposition surgery produces ileal length-dependent changes in food intake, body weight, gut hormones and glucose metabolism in rats. *Int J Obes*. 2014;38(3):379–87.
- Ikezawa F, Shibata C, Kikuchi D, et al. Effects of ileal interposition on glucose metabolism in obese rats with diabetes. *Surgery*. 2012;151(6):822–30.
- Sun X, Song M, Bai R, et al. Ileal interposition surgery-induced improvement of hyperglycemia and insulin resistance in Goto-Kakizaki rats by upregulation of TCF7L2 expression. *Exp Ther Med*. 2013;5(5):1511–5.
- Culnan DM, Albaugh V, Sun M, et al. Ileal interposition improves glucose tolerance and insulin sensitivity in the obese Zucker rat. *Am J Physiol Gastrointest Liver Physiol*. 2010;299(3):G751–60.
- Cummings BP, Strader AD, Stanhope KL, et al. Ileal interposition surgery improves glucose and lipid metabolism and delays diabetes onset in the UCD-T2DM rat. *Gastroenterology*. 2010;138(7):2437–46, 2446.e1.
- Chelikani PK. Ileal transposition surgery: mechanisms of weight loss and diabetes improvements. In: Faintuch J, Faintuch S, editors. *Obesity and diabetes*. Cham: Springer; 2015. p. 143–52.
- Guo Q, Zhang Q-Q, Chen J-Q, et al. Liver metabolomics study reveals protective function of *Phyllanthus urinaria* against CCl₄-induced liver injury. *Chin J Nat Med*. 2017;15(7):525–33.
- Zhao G, Hou X, Li X, et al. Metabolomics analysis of alloxan-induced diabetes in mice using UPLC-Q-TOF-MS after *Crassostrea gigas* polysaccharide treatment. *Int J Biol Macromol*. 2018;108:550–7.
- Hollywood K, Brison DR, Goodacre R. Metabolomics: current technologies and future trends. *Proteomics*. 2006;6(17):4716–23.
- Gertsman I, Barshop BA. Promises and pitfalls of untargeted metabolomics. *J Inherit Metab Dis*. 2018;41(3):355–66.
- Samczuk P, Ciborowski M, Kretowski A. Application of metabolomics to study effects of bariatric surgery. *J Diabetes Res*. 2018;2018:6270875.
- Liu R, Hong J, Xu X, et al. Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention. *Nat Med*. 2017;23(7):859–68.
- Zhao L, Ni Y, Yu H, et al. Serum stearic acid/palmitic acid ratio as a potential predictor of diabetes remission after Roux-en-Y gastric bypass in obesity. *FASEB J*. 2017;31(4):1449–60.
- Ashrafian H, Li JV, Spagou K, et al. Bariatric surgery modulates circulating and cardiac metabolites. *J Proteome Res*. 2014;13(2):570–80.
- Lopes TI, Geloneze B, Pareja JC, et al. Blood metabolome changes before and after bariatric surgery: a (1) H NMR-based clinical investigation. *OMICS*. 2015;19(5):318–27.
- Luo P, Yu H, Zhao X, et al. Metabolomics study of Roux-en-Y Gastric Bypass Surgery (RYGB) to treat type 2 diabetes patients based on ultraperformance liquid chromatography–mass spectrometry. *J Proteome Res*. 2016;15(4):1288–99.
- Sarosiek K, Pappan KL, Gandhi AV, et al. Conserved metabolic changes in nondiabetic and type 2 diabetic bariatric surgery patients: global metabolomic pilot study. *J Diabetes Res*. 2016;2016:3467403.
- Nemati R, Lu J, Tura A, et al. Acute changes in non-esterified fatty acids in patients with type 2 diabetes receiving bariatric surgery. *Obes Surg*. 2017;27(3):649–56.
- Yan K, Chen W, Zhu H, et al. Ileal transposition surgery decreases fat mass and improves glucose metabolism in diabetic GK rats: possible involvement of FGF21. *Front Physiol*. 2018;9:191.
- Liu X, Cheng X, Liu X, et al. Investigation of the urinary metabolic variations and the application in bladder cancer biomarker discovery. *Int J Cancer*. 2018;143(2):408–18.
- Kohli R, Kirby M, Setchell KD, et al. Intestinal adaptation after ileal interposition surgery increases bile acid recycling and protects against obesity-related comorbidities. *Am J Physiol Gastrointest Liver Physiol*. 2010;299(3):G652–60.
- Gaitonde S, Kohli R, Seeley R. The role of the gut hormone GLP-1 in the metabolic improvements caused by ileal transposition. *J Surg Res*. 2012;178(1):33–9.
- Nausheen S, Shah IH, Pezeshki A, et al. Effects of sleeve gastrectomy and ileal transposition, alone and in combination, on food intake, body weight, gut hormones, and glucose metabolism in rats. *Am J Physiol Endocrinol Metab*. 2013;305(4):E507–18.
- Yan Z, Chen W, Liu S, et al. Myocardial insulin signaling and glucose transport are up-regulated in Goto-Kakizaki type 2 diabetic rats after ileal transposition. *Obes Surg*. 2012;22(3):493–501.
- Calvo N, Beltran-Debon R, Rodriguez-Gallego E, et al. Liver fat deposition and mitochondrial dysfunction in morbid obesity: an approach combining metabolomics with liver imaging and histology. *World J Gastroenterol*. 2015;21(24):7529–44.
- Jung J, Ha TK, Lee J, et al. Changes in one-carbon metabolism after duodenal-jejunal bypass surgery. *Am J Physiol Endocrinol Metab*. 2016;310(8):E624–E32.
- Friedrich N, Budde K, Fau-Wolf T, et al. Short-term changes of the urine metabolome after bariatric surgery. *OMICS*. 2012;16(11):612–20.
- Kockx M, Kritharides L. Triglyceride-rich lipoproteins. *Cardiol Clin*. 2018;36(2):265–75.
- Arora T, Velagapudi V, Pournaras DJ, et al. Roux-en-Y gastric bypass surgery induces early plasma metabolomic and lipidomic alterations in humans associated with diabetes remission. *PLoS One*. 2015;10(5):e0126401.
- Kaur G, Guo XF, Fau-Sinclair AJ, et al. Short update on docosapentaenoic acid: a bioactive long-chain n-3 fatty acid. *Curr Opin Clin Nutr Metab Care*. 2016;19(2):88–91.
- Flachs P, Rossmeisl M, Fau-Kopecky J, et al. The effect of n-3 fatty acids on glucose homeostasis and insulin sensitivity. *Physiol Res*. 2014;63(Suppl 1):S93–118.

37. Hishikawa D, Hashidate T, Fau-Shimizu T, et al. Diversity and function of membrane glycerophospholipids generated by the remodeling pathway in mammalian cells. *J Lipid Res.* 2014;55(5):799–807.
38. Shapiro H, Kolodziejczyk AA, Halstuch D, et al. Bile acids in glucose metabolism in health and disease. *J Exp Med.* 2018;215(2):383–96.
39. Molinaro A, Wahlstrom A, Marschall HU. Role of bile acids in metabolic control. *Trends Endocrinol Metab.* 2018;29(1):31–41.
40. Nauck MA, Meier JJ. Incretin hormones: their role in health and disease. *Diabetes Obes Metab.* 2018;20(Suppl 1):5–21.
41. Chen W, Xu Q, Xiao Y, et al. Blockade of central GLP-1 receptors deteriorates the improvement of diabetes after ileal transposition. *Int J Med Sci.* 2016;13(12):955–62.
42. Neveu V, Perez-Jimenez J, Vos F, et al. Phenol-explorer: an online comprehensive database on polyphenol contents in foods. *Database (Oxford).* 2010;2010:bap024.
43. Yormaz S, Yilmaz H, Ece I, et al. Laparoscopic ileal interposition with diverted sleeve gastrectomy versus laparoscopic transit bipartition with sleeve gastrectomy for better glycemic outcomes in T2DM patients. *Obes Surg.* 2018;28(1):77–86.
44. Celik A, Cagiltay E, Ugale S, et al. Diverted sleeve gastrectomy with ileal transposition in overweight, obese, and morbidly obese patients with type 2 diabetes: results of 1-year follow-up. *Surg Obes Relat Dis.* 2016;12(3):541–9.