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ORIGINAL CONTRIBUTIONS

Duodenojejunal Bypass Plus Sleeve Gastrectomy Reduces Infiltration of Macrophages and Secretion of TNF-α in the Visceral White Adipose Tissue of Goto-Kakizaki Rats

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Abstract

Background Current studies indicate that inflammation of white adipose tissue (WAT) is a pathogenic characteristic of insulin resistance. However, the significance of visceral WAT inflammation after bariatric surgery remains unclear.

Methods Duodenojejunal bypass plus sleeve gastrectomy (DJB-SG) was performed on Goto-Kakisaki rats. Weight, fasting blood glucose (FBG), and homeostatic model assessment of insulin resistance (HOMA-IR) in the DJB-SG group were compared to those in a sham surgery (SHAM) group every 2 weeks. The results of an oral glucose tolerance test (OGTT) and the volume of visceral adipose tissue (Visc.Fat) were compared before and 8 weeks postsurgery. Eight weeks after surgery, the rats were sacrificed and visceral WAT collected from the greater omentum. Tumor necrosis factor-α (TNF-α) and cluster of differentiation 68 (CD68) expression in the WAT were evaluated in paraffin-embedded sections by immunohistochemistry.

Results Compared with the SHAM group, the DJB-SG group demonstrated a significant reduction in weight, FBG, and HOMA-IR ($P < 0.05$), with elevation of insulin levels ($P < 0.05$) from 4 weeks after surgery. OGTT and the quantity of Visc.Fat were significantly reduced ($P < 0.05$) 8 weeks after surgery. Moreover, the expression of TNF-α and CD68 in the visceral white adipose tissue was significantly lower 8 weeks after surgery ($P < 0.05$).

Conclusions The DJB-SG model established in Goto-Kakisaki rats achieved anticipated efficacy. Reduced TNF-α-related inflammation in visceral WAT may result in improved insulin resistance.

Keywords DJB-SG · Insulin resistance · Inflammation · T2D

Introduction

The twin disorders of obesity and type 2 diabetes (T2D) characterize the most devastating healthcare crisis worldwide [1]. Bariatric surgery is currently the most effective therapy for obese patients with T2D, with Roux-en-Y gastric bypass the most accepted form of surgery worldwide [2]. As an evolving surgical process, proximal duodenal-ileal end-to-side bypass with sleeve gastrectomy has been proposed by Sanchez-Pernaute et al. [3], while duodenojejunal bypass with sleeve gastrectomy (DJB-SG) was put forward by Kasama et al. [4, 5]. The efficacy of DJB-SG has been confirmed, especially in patients with T2D [6].

Studies have revealed that the result of bariatric surgery that relieves T2D is not only weight loss but also an unidentified weight-independent mechanism. For instance, alleviation of hyperglycemia and insulin resistance were independent of weight loss, caloric restriction, and nutrient malabsorption in the early stages [7–9].

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Increased infiltration of monocytes into white adipose tissue (WAT), which differentiate into macrophages, is a basic characteristic of obesity [10, 11]. Obesity-associated insulin resistance correlates with increased numbers of macrophages and elevated levels of pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6 [12, 13]. Secretion of these factors activates several inflammatory signal transduction pathways in macrophages and adipocytes.

Insulin resistance is defined as a decreased response of peripheral tissues (e.g., liver, muscle, and adipose) to insulin [9] and is commonly associated with obesity, preceding the onset of T2D [10, 11]. Individuals with insulin resistance are predisposed to developing T2D. One hypothesis for the development of insulin resistance is the chronic low-grade inflammation in WAT of obese patients with T2D [12]. The finding that macrophage infiltration and pro-inflammatory cytokine secretion are increased in obesity may provide partly substantiate this hypothesis [5, 6].

The most accurate predictor of adverse metabolic consequences (such as diabetes and dyslipidemia) is not total body adipose mass, but specifically, the quantity of visceral adipose tissue (Visc.Fat) [13]. Thus, we hypothesize that the inflammatory status of visceral WAT plays an important role after bariatric surgery.

To test this hypothesis, we firstly performed DJB-SG surgery on Goto-Kakizaki (GK) rats, the most widely used animal model of non-obese T2D [14], and then secondly, the efficacy of the DJB surgery in the rats was verified. Finally, we evaluated the volume of Visc.Fat., its infiltration of macrophages, and secretion of TNF-α.

Materials and Methods

Animals

Twelve-week-old male GK rats ($n = 18$; Laboratory Animal Center of Cavens, China) were housed separately in independently ventilated cages at a constant temperature between 24 and 26 °C, 50–60% humidity, and with a 12-h light/dark cycle for 4 weeks prior to surgery. The rats were randomly divided into two groups: 11 in the DJB-SG group and 7 in the SHAM group. All GK rats were given free access to standard food (14% calories from fat; Southern Medical University Laboratory Animal Center).

Surgical Procedures on GK Rats

After a 12-h fast, all rats were anesthetized with 10 mg/mL (equating to 50 mg/kg) amobarbital sodium solution by intraperitoneal (i.p.) injection. After skin preparation, a 3-cm midline epigastric incision was created using sterile techniques. An initial transverse incision (approximately 0.7 cm) was created parallel to the intestine 1 cm below the pylorus, and then, a second transverse incision (approximately 0.7 cm) was made parallel to the intestine at a position 20 cm below the ligament of Treitz. The two incisions were anastomosed side to side. The intestine was then ligated at the distal end of the anastomosis. In this way, approximately 80% of the stomach was removed and the stump sutured (Fig. 1a–f).

In the SHAM group, two transverse incisions were created as in the DJB-SG group. Another transverse incision was also made in the stomach. The duration of surgery was deliberately prolonged to be similar to that experienced by the rats in the DJB-SG group to allow the animals to undergo similar surgical and anesthetic stress.

In addition, cefazolin (120 mg/kg) was administrated i.p. twice daily for 2 days. On the first postoperative day, 5 mL of normal saline was injected subcutaneously to prevent dehydration. On the second postoperative day, rats were given access only to a liquid diet comprising 5% glucose and 0.9% saline solution. Starting on the third postoperative day, the rats received a liquid diet including water, sugar water compounds, proteins, electrolytes, and other vital substances. On the seventh postoperative day, solid food was offered to the rats and gradually reinstated. On the eighth week following surgery, all GK rats were sacrificed and dissected (Fig. 1g).

Outcome Measures of GK Rats

In all groups, weight, fasting blood glucose (FBG), and homeostatic model assessment of insulin resistance (HOMA-IR) levels were measured before and twice a week after surgery for a total of 8 weeks. An OGTT and micro-CT scan were also conducted prior to and 8 weeks after surgery.

Micro-CT (Aloka Latheta LCT-200, Hitachi, Japan) scanning was conducted on all groups before and 8 weeks after surgery using a tube voltage of 50 kV and current of 0.5 mA. The anesthetized rats were scanned in an 80-mm-wide specimen holder at a resolution of 160-mm pixel. For each rat, 500 images were collected during a single 360° rotation, which were then used to measure the quantity of visceral and subcutaneous adipose tissue. Firstly, an overview scan of the whole rat was created in order to select regions of interest. The defined region of the abdomen included from the top level of the diaphragm to the top of the pelvis. Mean acquisition time for each scan was approximately 15 min. Recognition and differentiation of abdominal muscle, visceral, and subcutaneous adipose tissue (Subc.Fat) were achieved using the LaTheta software. A fat density factor of 0.92 g/cm^3 was implemented in the software and used to calculate fat mass [15].

Electronic scales (Mettler Toledo TCII-1103, Zurich, Switzerland) were employed to directly measure animal weight, and a handheld Accu-Chek Performa glucometer (Roche) was employed to directly measure blood glucose levels. Weight, FBG, and OGTT were measured following

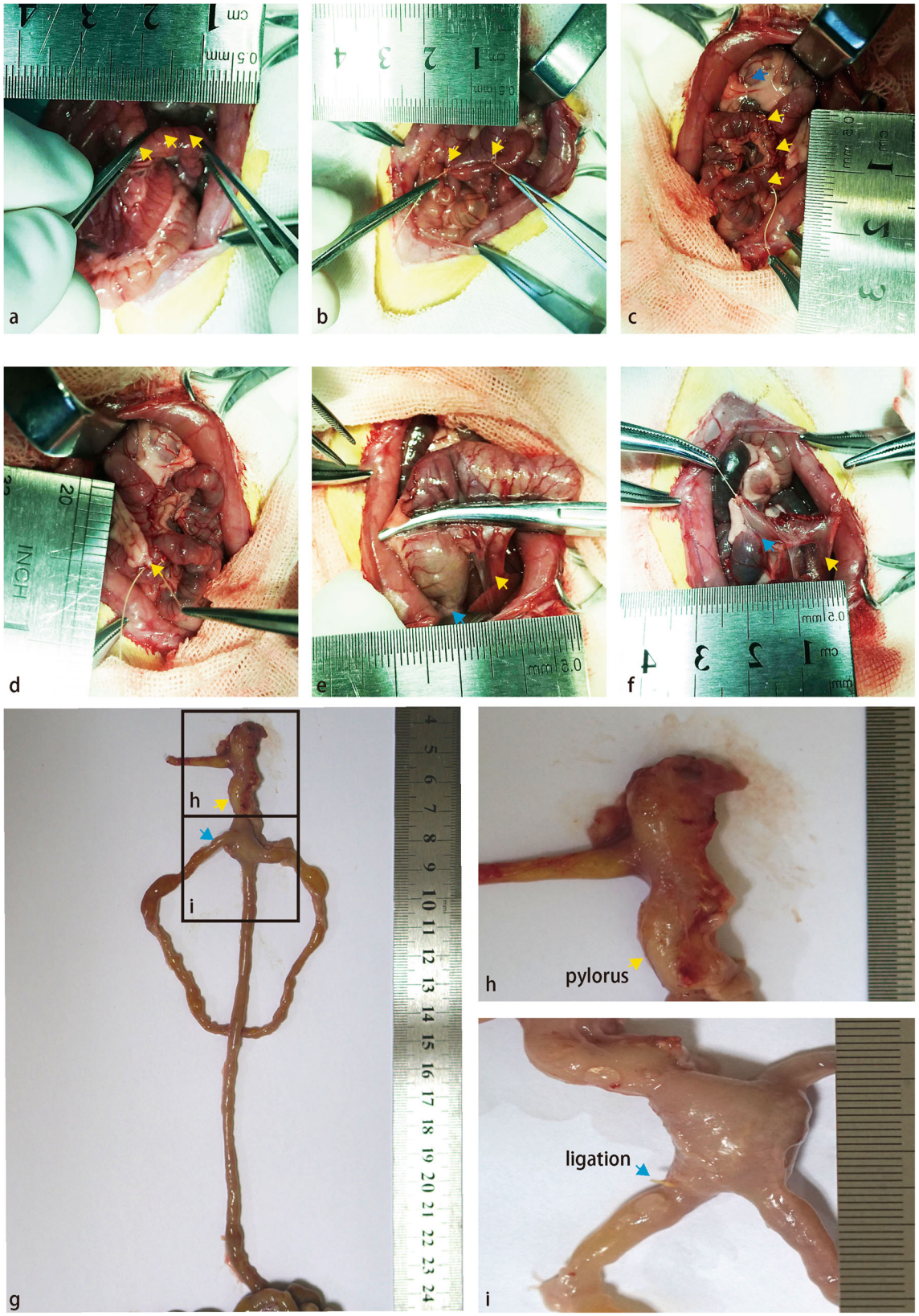

Fig. 1 Schema of DJB-SG surgery and anatomical image of DJB-SG performed on a GK rat after surgery. **a**–**f** Schema of DJB-SG surgery. **g** Anatomical image. Blue arrow: ligation, yellow arrow: pylorus

1 day of fasting. During OGTT, each rat was given a bolus of 2 g/kg hypertonic glucose (50% *w*/*v*) by oral gavage before and at 15, 30, 60, 90, and 120 min after administering the hypertonic glucose solution.

Blood samples were collected from the tail vein of the rats. The blood samples were placed into chilled 1.5-mL Eppendorf tubes (Axygen Scientific Inc., Union City, CA, USA) containing a dipeptidyl peptidase IV inhibitor (EMD Millipore, Billerica, MA, USA) in a solution of ethylenediaminetetraacetic acid (EDTA). After centrifugation at 4 °C for 10 min at 1000×*g*, the plasma supernatant was immediately extracted and stored at − 80 °C until required for analysis. Serum insulin was tested using an ELISA Kit (Bio-Rad).

Immunohistochemistry-Paraffin Embedding Protocol

WAT was isolated from the omentum majus. The samples were fixed in formalin, embedded in paraffin, and then analyzed by immunohistochemistry (IHC-P). Four-μm sections were deparaffinized and rehydrated, and their endogenous peroxidase activity inhibited using 0.3% H_2O_2 in methanol. After blocking with 5% normal goat serum for 1 h at room temperature, the slides were incubated with the following primary antibodies at 4 °C overnight: anti-CD68 and anti-TNF-α (Abcam, Cambridge, UK). The slides were incubated with an appropriate biotinylated secondary antibody (Dako, Santa Clara, CA, USA) and then streptavidin-biotin complex/horseradish peroxidase. Finally, the immunoreactive signal was developed using diaminobenzidine (DAB) (Dako) staining. The slides were counterstained using hematoxylin. Ten non-overlapping visual fields were randomly selected using a light microscope at ×40 magnification, and then, positively stained cells were manually counted and the different groups compared. Staining intensity was scored as follows: 0: negative, 1: weak, 2: moderate, and 3: strong. The frequency of positive cells was defined as follows: 0: less than 5%, 1: 5 to 25%, 2: 26 to 50%, 3: 51 to 75%, and 4: more than 75%. A staining index (values: 0–12) was calculated by multiplying the score for staining intensity with that for positive area.

Statistical Analysis

All clinical data are presented as mean ± SD and all experimental data as mean ± SEM. Parametric data were analyzed using a Student's *t* test (paired and independent). Non-parametric data were analyzed using Wilcoxon signed rank and Mann-Whitney *U* tests. Chi-squared or Fisher's exact tests were used to identify significant differences between proportions and categorical variables. A two-tailed $P < 0.05$ was considered statistically significant. Statistical analysis was performed using SPSS version 22.0 for Windows (IBM Corp., Armonk, NY, USA).

Results

The total mortality rate in the two groups (DJB-SG and SHAM) was 27.77% (5/18) 8 weeks postoperatively. Among them, seven survived in the DJB-SG group and six survived in the SHAM group. In the DJB-SG group, one rat died of anastomotic leakage 1 day postsurgery. Two rats died of diarrhea after the third and fifth days postsurgery. One rat died of severe weight loss after the first week postsurgery. In the SHAM group, one rat died of an unknown cause after the third week postsurgery.

Following surgery, the weights of the rats in the DJB-SG group declined significantly after 4 weeks compared with those of the SHAM group (Fig. 2a). The FBG of the rats in the DJB-SG group also decreased significantly after 4 weeks compared with that of the SHAM group (Fig. 2b). Additionally, the HOMA-IR and circulating insulin levels in the DJB-SG group were significantly less after 4 weeks than that of the SHAM group (Fig. 2c, e). The area under the OGTT curve (AUC_{OGTT}) of the DJB-SG group was significantly smaller than that of the SHAM group after surgery (Fig. 2d).

The number of CD68-positive cells in the adipose of rats decreased in the DJB-SG group compared with the SHAM group (Fig. 3a, b). In addition, the levels of TNF-α in the adipose tissue of the DJB-SG group also decreased (Fig. 3c, d). Furthermore, according to the micro-CT scans of the abdomen, total fat volume, namely, Subc.Fat plus Visc.Fat, decreased more significantly ($P < 0.05$) in the DJB-SG group after 8 weeks than in the SHAM group, consistent with animal weight. In particular, the dramatic decrease in fat volume was due principally to a decrease in Visc.Fat ($P < 0.05$), rather than Subc.Fat (Fig. 4).

Discussion

In this study, we observed that DJB-SG surgery improved glucose metabolism in a non-obese type 2 diabetes animal model. We provided evidence that DJB-SG surgery in an animal model caused effects similar to those observed in patients [5]. Furthermore, data demonstrated that DJB-SG surgery decreased not only Visc.Fat volume but also TNF-α expression and macrophage infiltration in the visceral adipose tissue, which may contribute to the improvement in insulin resistance.

The positive effect of bariatric surgery observed towards glycemic control has been confirmed in non-obese GK rats [14, 16], non-obese streptozotocin (STZ)-induced diabetes rats [17], and a high-fat diet plus STZ-induced rats [18]. It is important to note that divergent results have also been reported [19]. In our study, insulin resistance was well established prior to surgery. Similar to the early stage in humans, GK rats exhibit increased insulin resistance, higher insulin secretion, and pathological oral glucose tolerance. Four weeks after

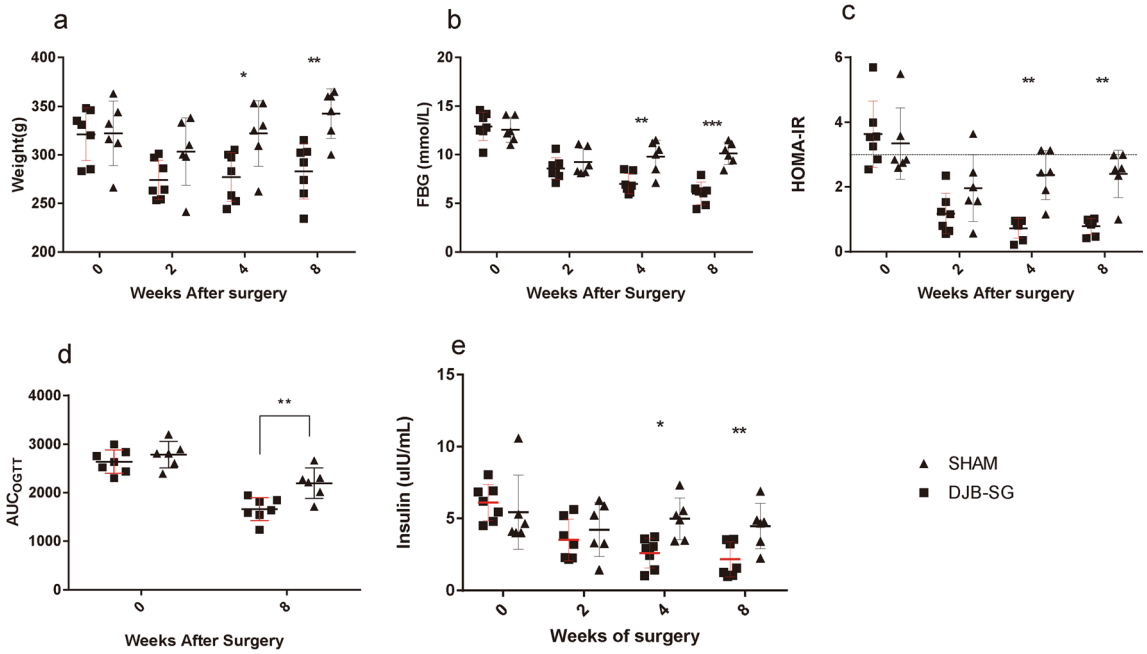

Fig. 2 Alleviation of T2D and downregulation of ghrelin observed in GK rats after DJB-SG. **a** Weight. **b** FBG. **c** HOMA-IR. **d** AUC_{OGTT}. Data represent mean ± SD, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ versus SHAM group. **e** Ghrelin. Data represent mean ± SEM, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ versus SHAM group

surgery, HOMA-IR, representing insulin resistance, decreased significantly at a confidence level of 99%. In addition, body weight did not change significantly and diabetes persisted in the SHAM group. These observations indicate that the mechanisms are partly independent of weight loss [7–9].

The present modification of DJB surgery performed on the animals was first presented by Rubino et al. [14]. One advantage is that it minimizes surgical trauma in diabetic rats. The reason that DJB-SG surgery is more difficult in humans than rats is that the duodenum is located in the posterior abdominal cavity and requires manual anastomosis using laparoscopy. Nonetheless, the duodenum was free in the abdominal cavity, and the surgery was simpler than in human patients. In all the procedures performed on the GK rats, the mortality rate was 27.7%. Together, these data suggest that DJB-SG surgery was easily performed with efficacious effects after an invasive operation.

Visceral adipose tissue is widely distributed around or in abdominal organs, such as the pancreas and omentum majus, and has been reported to be related to metabolic syndrome including insulin resistance, hyperlipidemia, and hypertension [20–23]. For individuals with identical BMI values, the Asian T2D population, such as Chinese or Japanese, have been reported to have a higher risk of metabolic syndrome [24] and higher Visc.Fat volume compared to Caucasians [25]. Clearly, for the Asian T2D population, the Visc.Fat volume is probably a more appropriate parameter of concern in bariatric surgery and requires greater attention in a comprehensive research program [22]. Harvest of postoperative visceral adipose samples in humans is limited to serious situations requiring reoperative surgery with appropriate ethical approval. Thus, clinical researchers have little opportunity to investigate human tissue and rely on animals such as the GK rats. In our study, based on a successful surgical model of DJB-SG in GK rats, we first utilized micro-CT scanning to directly confirm reported changes in visceral adipose tissue at the macrolevel and we harvested sufficient tissues samples postoperatively to explore more deeply the fundamental mechanisms including microcosmic factors such as TNF-α and CD68.

Historically, high doses of salicylates have been known to lower blood glucose concentrations [26]. As the first discovered synergistic cytokine of obesity-induced inflammation [27], TNF-α increases in the muscle and adipose tissue of obese people and rats [28]. Insulin resistance occurred in cells and animal models when a certain quantity of TNF-α has been administered [29]. Alleviation of insulin resistance has been observed in animal models that have an absence of TNF-α or TNF-α receptor [30]. Those findings suggest that TNF-α has a strong association with insulin resistance.

TNF-α could increase insulin resistance in a variety of ways. It could upregulate phosphorylation of Ser307 in insulin receptor substrate-1, which can inhibit insulin action and block the interaction of insulin with insulin receptors [31]. Infusion of TNF-α increases phosphorylation of p70 S6 kinase, extracellular signal-regulated kinase-1/2, and c-Jun NH(2)-terminal kinase, concomitant with increased serine and reduced tyrosine phosphorylation of insulin receptor substrate-1. These signaling effects are associated with impaired phosphorylation of Akt substrate 160, the most proximal step identified in the canonical

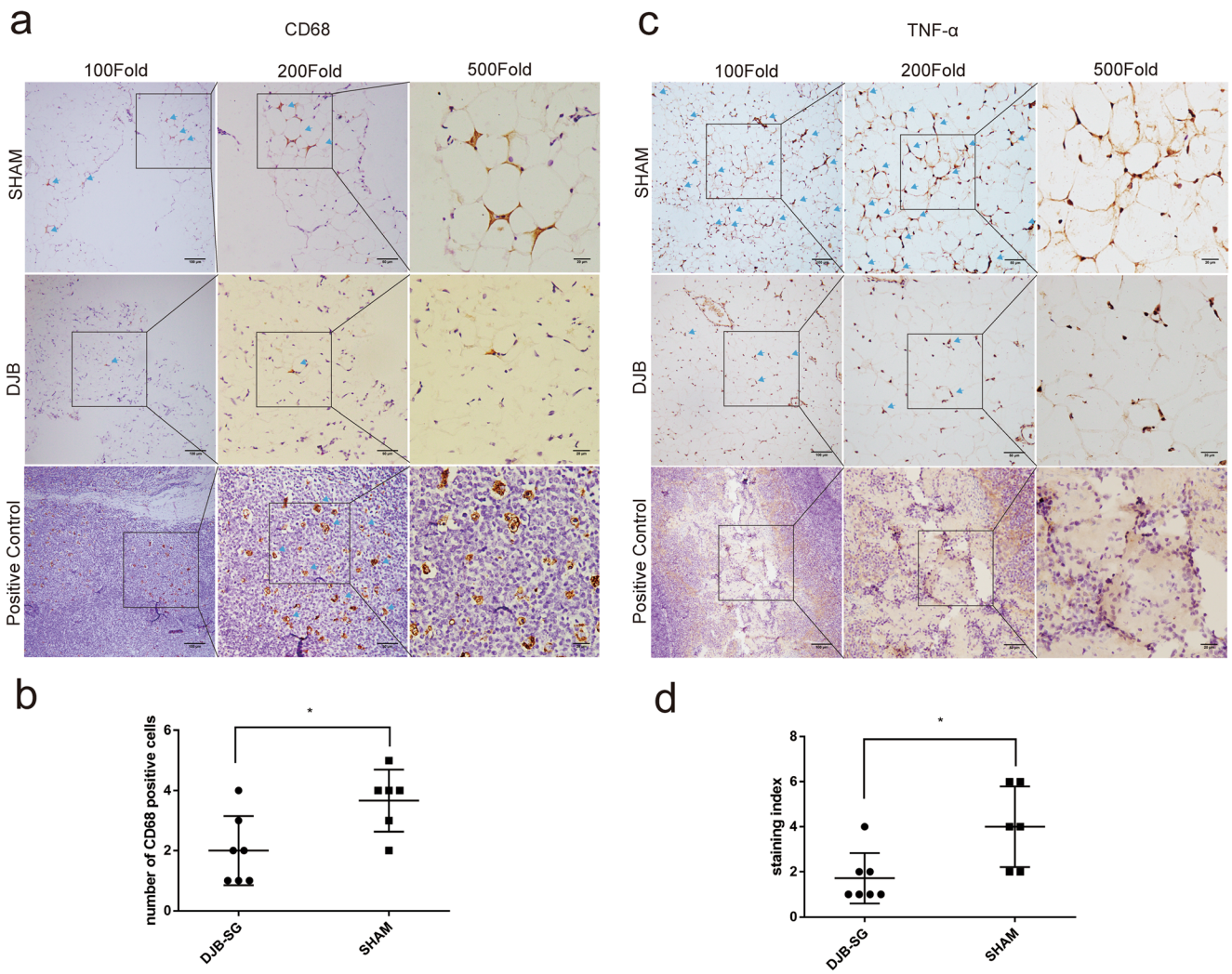

Fig. 3 Reduced inflammation in adipose tissue of GK rats. **a** Immunohistochemical staining of CD68 in epididymal WAT from GK rats. Human tonsil specimens were positive controls. Blue arrows indicate macrophages. **b** Number of CD68-positive cells. Data represent mean ± SEM, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ versus the SHAM group. **c** Immunohistochemical staining of TNF-α in epididymal WAT. Human tonsil specimens were positive controls. Blue arrows indicate TNF-α. **d** TNF-α staining index. Data represent mean ± SD, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ versus the SHAM group

insulin signaling cascade regulating GLUT4 translocation and glucose uptake [32, 33]. TNF-α could activate NF-κB, as IKKβ/NF-κB-mediated impairment of hypothalamic adult neural stem cells is a critical neurodegenerative mechanism in obesity and related diabetic disorders [34].

The theory of low-grade systemic inflammation generates a fresh research orientation for the pathogenesis of diabetes [35, 36]. It is unclear whether inflammation and immunity are the cause of type 2 diabetes or just a pathological marker. Interestingly, in the same way that tumor markers serve as a prediction of cancer [37], C-reactive protein serves as a predictive indicator for diabetes and cardiovascular diseases, and has wide acceptance [38]. As for bariatric surgery, several nominated blood stream markers are available that are reported to predict the therapeutic effect of surgery [39], but predictive pathological markers are scarce.

This study investigated the expression of CD68 and TNF-α in visceral WAT, the results suggesting that DJB-SG surgery may possibly influence TNF-α-related inflammation in visceral adipose tissue. We could investigate WAT as a research object: for instance, clinicians could routinely remove a sample of WAT from the omentum majus during surgery and quantify inflammatory indicators (such as TNF-α expression) using pathological examination (such as IHC-P). If a strong correlation between high expressions of TNF-α preoperatively and high postoperative T2D remission rate was observed, pathological examination of WAT may become a new biomarker for predicting the therapeutic effect of the procedure.

Occasionally, we observed that although the number of macrophages in adipose tissue was reduced, the total number was considerably fewer than fat cells (less than 5%). Furthermore, TNF-α expression was positive in a large

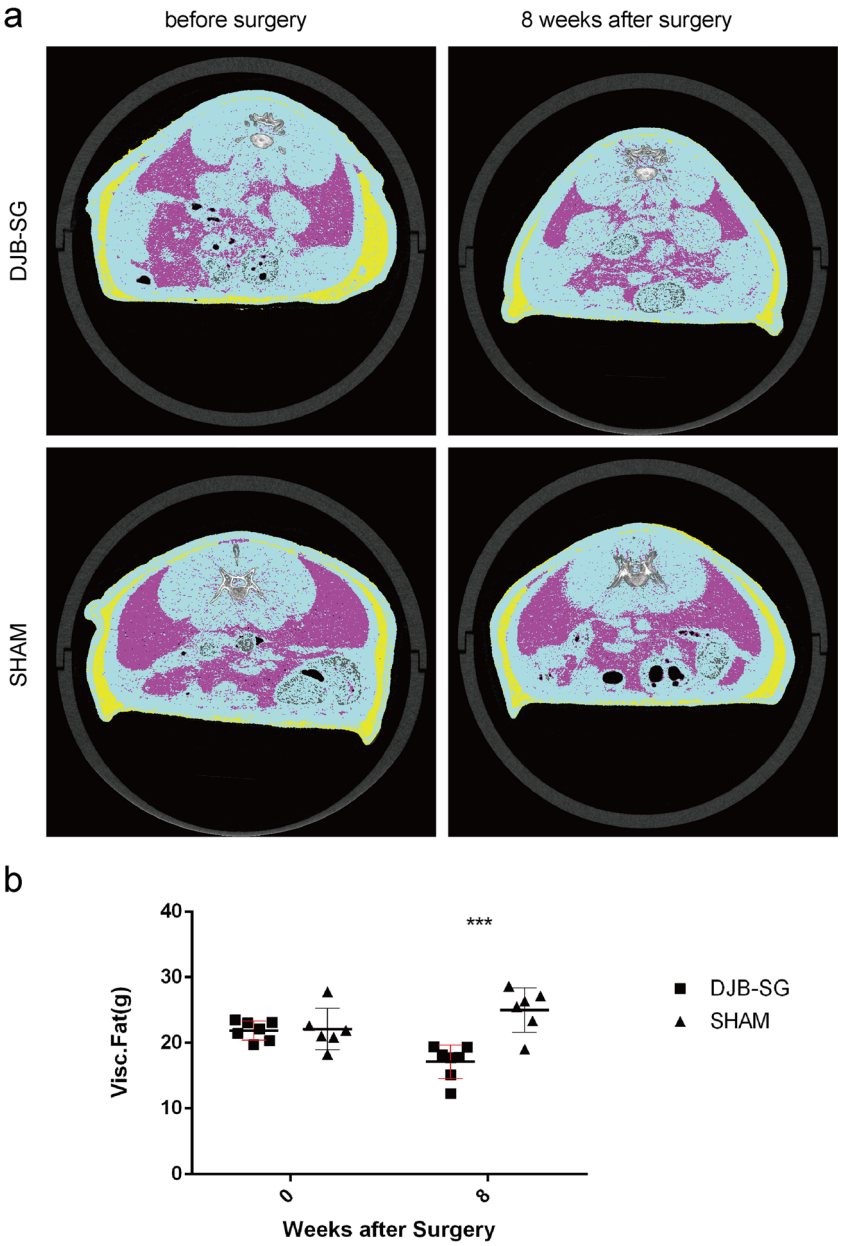

Fig. 4 Reduction of Visc.Fat observed in GK rats after DJB-SG. **a** Visceral and subcutaneous adipose tissue perpendicular to the longitudinal axis of the rat were scanned by micro-CT. Red area: visceral adipose tissue, yellow area: subcutaneous adipose tissue. **b** Volume of visceral adipose tissue (Visc.Fat). Data represent mean ± SD, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ versus the SHAM group

proportion of the adipose tissue (approximately 40~50%). The question remains as to the principal source of TNF. Does it derive from adipocytes or macrophages, or some other source? Thus, our continued research anticipates making progress in this regard.

Conclusions

The DJB-SG-model established in Goto-Kakisaki rats achieved anticipated efficacy. Reduced TNF-α-related inflammation in visceral WAT may result in improved insulin resistance.

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Compliance with Ethical Standards

Conflict of Interest Hao Yu, Zhigao Song, Hongbin Zhang, Kehong Zheng, Junfang Zhan,Jingbo Sun, Zhizhi Wang, Lucas Zellmer, Xiaojiang Dai, Wu Liangping declare that they have no conflicts of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of Guangzhou General Hospital of Guangzhou Military Command's research committee

and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

All applicable Guangzhou General Hospital of Guangzhou Military Command's guidelines for the care and use of animals were followed.

Statement of Animal Rights All animal experimental procedures involved in this study were approved by the Animal Care and Utilization Committee of Guangzhou General Hospital of Guangzhou Military Command.

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References

1. Mokdad AH, Ford ES, Bowman BA, et al. Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. JAMA. 2003;289:76.
2. Adams TD, Davidson LE, Litwin SE, et al. Weight and metabolic outcomes 12 years after gastric bypass. N Engl J Med. 2017;377:1143–55.
3. Sanchez-Pernaute A et al. Proximal duodenal-ileal end-to-side bypass with sleeve gastrectomy: proposed technique. Obes Surg. 2007;17:1614–8.
4. Kasama K, Tagaya N, Kanehira E, et al. Laparoscopic sleeve gastrectomy with duodenojejunal bypass: technique and preliminary results. Obes Surg. 2009;19:1341–5.
5. Lee WJ, Lee KT, Kasama K, et al. Laparoscopic single-anastomosis duodenal-jejunal bypass with sleeve gastrectomy (SADJB-SG): short-term result and comparison with gastric bypass. Obes Surg. 2014;24:109–13.
6. Navarrete SA, Leyba JL, Llopis SN. Laparoscopic sleeve gastrectomy with duodenojejunal bypass for the treatment of type 2 diabetes in non-obese patients: technique and preliminary results. Obes Surg. 2011;21:663–7.
7. Astiarraga B, Gastaldelli A, Muscelli E, et al. Biliopancreatic diversion in nonobese patients with type 2 diabetes: impact and mechanisms. J Clin Endocrinol Metab. 2013;98:2765–73.
8. Heneghan HM, Nissen S, Schauer PR. Gastrointestinal surgery for obesity and diabetes: weight loss and control of hyperglycemia. Curr Atheroscler Rep. 2012;14:579–87.
9. Severino A, Castagneto-Gissey L, Raffaelli M, et al. Early effect of roux-en-Y gastric bypass on insulin sensitivity and signaling. Surg Obes Relat Dis. 2016;12:42–7.
10. Masoodi M, Kuda O, Rossmeisl M, et al. Lipid signaling in adipose tissue: Connecting inflammation & metabolism. Biochim Biophys Acta. 2015;1851:503–18.
11. Kanda H, Tateya S, Tamori Y, et al. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. J Clin Invest. 2006;116:1494–505.
12. Moraes-Vieira PM, Yore MM, Dwyer PM, et al. RBP4 activates antigen-presenting cells, leading to adipose tissue inflammation and systemic insulin resistance. Cell Metab. 2014;19:512–26.
13. Borst SE. The role of TNF-alpha in insulin resistance. Endocrine. 2004;23:177–82.
14. Rubino F, Forgione A, Cummings DE, et al. The mechanism of diabetes control after gastrointestinal bypass surgery reveals a role of the proximal small intestine in the pathophysiology of type 2 diabetes. Ann Surg. 2006;244:741–9.
15. Hill AM, LaForgia J, Coates AM, et al. Estimating abdominal adipose tissue with DXA and anthropometry. Obesity (Silver Spring). 2007;15:504–10.
16. Rubino F, Marescaux J. Effect of duodenal-jejunal exclusion in a non-obese animal model of type 2 diabetes: a new perspective for an old disease. Ann Surg. 2004;239(1):1–11.
17. Breen DM, Rasmussen BA, Kokorovic A, et al. Jejunal nutrient sensing is required for duodenal-jejunal bypass surgery to rapidly lower glucose concentrations in uncontrolled diabetes. Nat Med. 2012;18:950–5.
18. Jurowich CF, Rikkala PR, Thalheimer A, et al. Duodenal-jejunal bypass improves glycemia and decreases SGLT1-mediated glucose absorption in rats with streptozotocin-induced type 2 diabetes. Ann Surg. 2013;258:89–97.
19. Gavin TP, Sloan RC, Lukosius EZ, et al. Duodenal-jejunal bypass surgery does not increase skeletal muscle insulin signal transduction or glucose disposal in Goto-Kakizaki type 2 diabetic rats. Obes Surg. 2011;21:231–7.
20. Elffers TW et al. Body fat distribution, in particular visceral fat, is associated with cardiometabolic risk factors in obese women. PLoS One. 2017;e185403:12.
21. Jablonowska-Lietz B, Wrzosek M, Wlodarczyk M, et al. New indexes of body fat distribution, visceral adiposity index, body adiposity index, waist-to-height ratio, and metabolic disturbances in the obese. Kardiol Pol. 2017;75:1185.
22. Lee SW, Son JY, Kim JM, et al. Body fat distribution is more predictive of all-cause mortality than overall adiposity. Diabetes Obes Metab. 2018;20:141–7.
23. Taniguchi A, Nakai Y, Sakai M, et al. Relationship of regional adiposity to insulin resistance and serum triglyceride levels in nonobese Japanese type 2 diabetic patients. METABOLISM. 2002;51:544–8.
24. Ko GTC, Tang JSF. Waist circumference and BMI cut-off based on 10-year cardiovascular risk: evidence for "central pre-obesity". OBESITY. 2007;15:2832–9.
25. Wang J, Thornton JC, Russell M, et al. Asians have lower body mass index (BMI) but higher percent body fat than do whites: comparisons of anthropometric measurements. Am J Clin Nutr. 1994;60:23–8.
26. Baron SH. Salicylates as hypoglycemic agents. Diabetes Care. 1982;5:64–71.
27. Allison MA, Jensky NE, Marshall SJ, et al. Sedentary behavior and adiposity-associated inflammation: the Multi-Ethnic Study of Atherosclerosis. *Am J Prev Med*. 2012;42:8.
28. Ctoi AF et al. Metabolically healthy versus unhealthy morbidly obese: chronic inflammation, nitro-oxidative stress, and insulin resistance. Nutrients. 2018;10
29. Hotamisligil GS. Mechanisms of TNF-alpha-induced insulin resistance. Exp Clin Endocrinol Diabetes. 1999;107:119.
30. Uysal KT, Wiesbrock SM, Marino MW, et al. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. Nature. 1997;389:610.
31. Hotamisligil GS et al. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. Science. 1996;271:665–70.
32. Plomgaard P, Bouzakri K, Krogh-Madsen R, et al. Tumor necrosis factor-alpha induces skeletal muscle insulin resistance in healthy human subjects via inhibition of Akt substrate 160 phosphorylation. DIABETES. 2005;54:2939–45.
33. Bouzakri K, Zierath JR. MAP4K4 gene silencing in human skeletal muscle prevents tumor necrosis factor-alpha-induced insulin resistance. J Biol Chem. 2007;282:7783–9.
34. Li J, Tang Y, Cai D. IKKbeta/NF-kappaB disrupts adult hypothalamic neural stem cells to mediate a neurodegenerative mechanism of dietary obesity and pre-diabetes. Nat Cell Biol. 2012;14:999–1012.
35. van Greevenbroek MM, Schalkwijk CG, Stehouwer CD. Obesity-associated low-grade inflammation in type 2 diabetes mellitus: causes and consequences. Neth J Med. 2013;71:174.

36. Leon-Pedroza JI et al. Low-grade systemic inflammation and the development of metabolic diseases: from the molecular evidence to the clinical practice. Cir Cir. 2015;83:543–51.
37. Kelloff GJ, Sigman CC. Cancer biomarkers: selecting the right drug for the right patient. Nat Rev Drug Discov. 2012;11:201–14.
38. Sattar N, Gaw A, Scherbakova O, et al. Metabolic syndrome with and without C-reactive protein as a predictor of coronary heart disease and diabetes in the west of Scotland coronary prevention study. Circulation. 2003;108:414–9.
39. Trak-Smayra V, Dargere D, Noun R, et al. Serum proteomic profiling of obese patients: correlation with liver pathology and evolution after bariatric surgery. Gut. 2009;58:825–32.