

Original Article

Effects of sleeve gastrectomy on lipid and energy metabolism in ZDF rats via PI3K/AKT pathway

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Abstract: Sleeve Gastrectomy (SG), as the most effective bariatric surgery, has been using to chronically lose weight and control glucose metabolism in Type 2 diabetes mellitus patients. However, the underlining mechanism is still unclear. In this study, we performed SG on Zucker diabetes fatty (ZDF) rat and investigated visceral lipid metabolism and energy metabolism. After performance of SG, weight, food intake, fasting plasma glucose (FPG) and oral glucose tolerance test (OGTT) of rats were measured. Furthermore, whole-body metabolic parameters were obtained through TSE LabMaster. Blood lipid and renal function were analyzed by serum from rats' tail vein. Furthermore, the renal genes expression was either detected by real-time PCR, while western blotting was employed to detect the AKT/PI3K proteins level in rats' kidney. Compared to control groups, body weight of ZDF rats treated with SG were significantly reduced, simultaneously with glucose homeostasis and energy metabolism improved including RER ($P<0.05$), energy expenditure ($P<0.05$) at night and activity of animal. Meanwhile, serum lipid of ZDF rats after SG was decreased, and renal function recovered. Histology analysis confirmed that the size of perirenal adipose from SD treated ZDF rats obviously decreased ($P<0.001$), effectively stimulating up-regulation of lipogenesis genes ($P<0.05$), while adipogenesis genes ($P<0.05$) in kidney was down-regulated. In addition, phosphorylation of PI3K (p-PI3K) and AKT (p-AKT) in rats kidney were significantly decreased in SG group ($P<0.05$). Weight loss, food intake, fasting plasma glucose and glucose tolerance in SG surgery rats were improved, which were coincident with energy metabolism changes. In conclusion, SG improves lipid and energy metabolism in ZDF rats model due to activating PI3K/Akt signaling pathway, which was contributed to the mechanism of bariatric surgery toward kidney.

Keywords: Sleeve gastrectomy, Zucker diabetes fatty rats, lipid metabolism, energy metabolism, PI3K/Akt pathway

Introduction

Sleeve Gastrectomy (SG), compared with conventional medical treatment and dieting, was an effective method for losing weight and accelerating glucose metabolism in both humans [1, 2] and rodents [3-5] in clinics.

Recently, meta-analysis [6] demonstrated that bariatric surgery significantly alleviated diabetes and its complications including microangiopathy [7, 8], macrovascular disease [8] and abnormal immune response [9]. Diabetic kidney disease (DKD) was considered a major factor causing diabetic complications, however the mechanism of treatment was still unclear.

Numerous researchers paid attention to obesity-related glomerulopathy [10]. Though obesity and hypertension were independent risk factors for DKD, clinical evidence showed obesity accelerating DKD course [11, 12]. Carla N. Holcomb and his colleagues [13] confirmed that bariatric surgery was beneficial to renal function recovery of patients with kidney disease. In addition, visceral fat accumulation might cause retinal neurodegenerative disorders [14]. However, mechanism of SG being a bariatric surgery treating kidney injury for recovering diabetes nephropathy was unclear. According to studies, diabetes nephropathy was associated with inflammation [15, 16], glucose metabolism and oxidative stress [17]. Moreover, visceral fat

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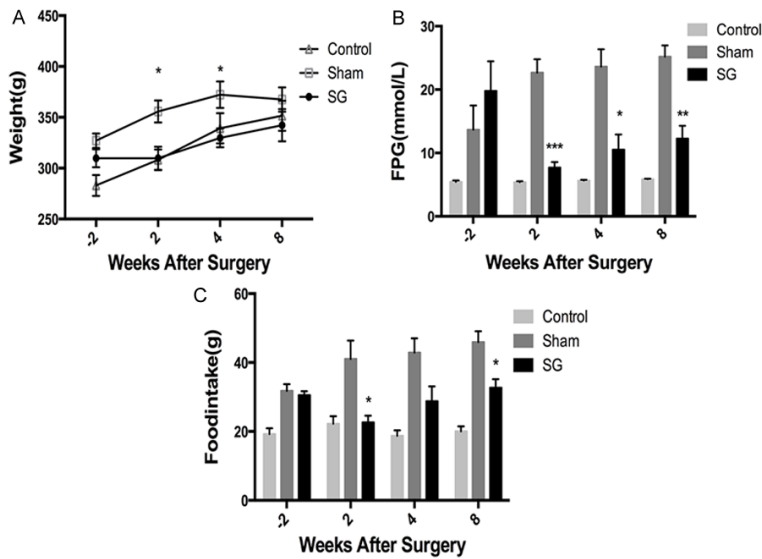


Figure 1. SG improved weight loss, decrease of fasting plasma glucose and food intake in ZDF rats (A), the body weight changes before and after surgery in all groups (B), fasting plasma glucose changes before and after surgery in all groups (C), food intake changes in all groups at 2 weeks before and 2,4 and 8 weeks after surgery. Data were expressed as mean \pm standard error of mean (SEM) with N as the number of experiments. The statistical significance of differences among groups was compared by analysis using one-way ANOVA and the paired t-test. Differences were considered significant at $P < 0.05$. Statistical analysis was conducted using GraphPad Prism version 6.0 (San Diego, CA, USA).

accumulation had greater risks for cardiovascular and renal function than subcutaneous fat, which was the precursor of the metabolic syndrome and insulin resistance. In this study, we performed SG on ZDF rats and demonstrated that the SG effectively improved the weight loss, food intake, fasting plasma glucose and glucose tolerance from histology and gene expression data. Furthermore, our data also showed that SG changed the metabolism performance through PI3K/Akt pathway. These evidences confirmed that SG is an effective method for losing weight and improving glucose metabolism in type 2 diabetes patients.

Materials and methods

Animals

16 male Zucker diabetic fatty rats and 6 male lean Zucker rats with 300 ± 15 g mean body weight at 8-10 weeks were purchased from Shanghai Slyke Laboratory Animal Corp. All animals were housed in individual caging in specific pathogen free (SPF) condition with 12 h

light/dark cycle, relative humidity of 40-70% and 21-25°C. After adaptation 2 weeks, the rats were divided into 3 groups according to the surgical procedure performed, including (1) SG (n=8), (2) sham surgery (n=8), (3) control (n=6). All operations were performed according to the Guide for the Care and Use of Laboratory Animals. The study was approved by the Institutional Animal Care and Utilization Committee of Fudan University Pudong Medical Centre.

Surgical procedures

In brief, preoperative fasting of solid food took place for at least 14 h, while water was allowed. Before the surgery, the operation area was sterilized with 70% alcohol meanwhile the rat was anaesthetized using 3% pentobarbital sodium. The abdomen was

opened through a 3 cm midline epigastric incision. About 80% of the glandular stomach was removed by resecting the organ from both sides using haemostatic forceps, the gastric remnant was sutured with 5/0 swaged needle. After abdominal flush using 0.9% NaCl at least three times, which is necessary, the open abdomen was subsequently closed by continuous running suture. Sham-operated animals underwent the same abdominal incisions and transections of the small bowel followed by resuturing. As a result, the anatomic structure was not changed.

Postsurgical manipulations

At the end of the surgery, all rats received 1 ml 0.9% NaCl with subcutaneous injection in order to maintain hydration during keeping warm. The vital postoperative care was injections of penicillin during the next three days. The rats were allowed to drink purified water for 24 h after surgery, and food was provided for 48 h after surgery. Thereafter, the rats received HFD until 8 weeks after surgery.

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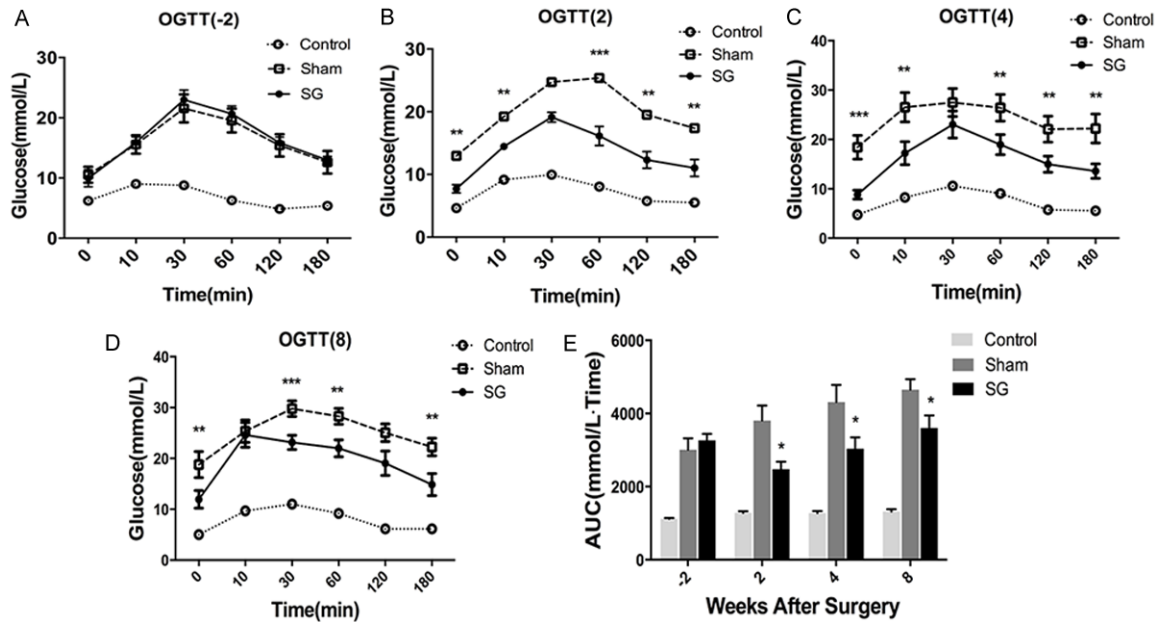


Figure 2. OGTT was measured at 10, 30, 60, 120 and 180 time points after surgery. The OGTT of ZDF rats in all groups (n=8). (A) 2 weeks preoperative and (B) 2, (C) 4, (D) 8 weeks postoperative. (E) The area under the curve (AUC) of OGTT glucose excursion. Data were expressed as mean \pm standard error of mean (SEM) with N as the number of experiments. The statistical significance of differences among groups was compared by analysis using one-way ANOVA and the paired t-test. Differences were considered significant at $P < 0.05$. Statistical analysis was conducted using GraphPad Prism version 6.0 (San Diego, CA, USA).

Weight, food intake, fasting plasma glucose, oral glucose tolerance test (OGTT)

All animals were fasted for 6 h and then weight was measured by an electronic scale, and fasting plasma glucose was detected by handheld glucometer (Accu-Chek Performa, Roche Diagnostics, Indianapolis, Indiana, USA) 0, 2, 4 and 8 weeks after the surgery. Food intake was measured at pre-operation (2 weeks) and post-operation (2, 4 and 8 weeks). An average of 3 days' food intake was employed in our study, and litter was removed during this time frame. At 2, 4 and 8 weeks after the surgery, 12-h-fasted rats received a bolus of D-glucose (2 g/kg) and plasma glucose levels were measured at 0, 10, 30, 60, 120 and 180 minutes after by tail bleeding.

Serum lipid and renal function

Serum lipid including cholesterol (CHOL), triglycerides (TG), high density lipoprotein-cholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL-C) and renal function including blood urea nitrogen (BUN) and creatinine (CREA) were measured using rats serum after

fasting. An approximately 800 μ L blood sample was collected from all the rats tail veins into the tubes containing EDTA. Then the samples were separated by centrifugation (3000 rpm) at 4°C for 10 min and stored at -80°C for ready.

Body metabolic parameters

Free cumulative activities, respiratory exchange ratio (RER = VCO_2 / VO_2) and energy expenditure were measured towards ZDF rats. RER was the ratio of VCO_2 to VO_2 and RER reading of 0.7 indicated that fat was a predominant fuel source, 0.85 suggested a mix of fat and carbohydrates, and value of 1.00 or above demonstrating that carbohydrate was predominant fuel source. All animals were monitored indirect calorimetry, food intake, and free activity using system (TSE LabMaster, TSE Systems, Bad Homburg, Germany). Rats were accommodated in the metabolic cages for 24 hours and then monitored for an additional 24 hours. Data was collected from the last 24 hours in order to calculate free activity, average RER, and energy expenditure during a daily cycle of 12 hours light/darkness.

Sleeve gastrectomy improves lipid and energy metabolism in rats

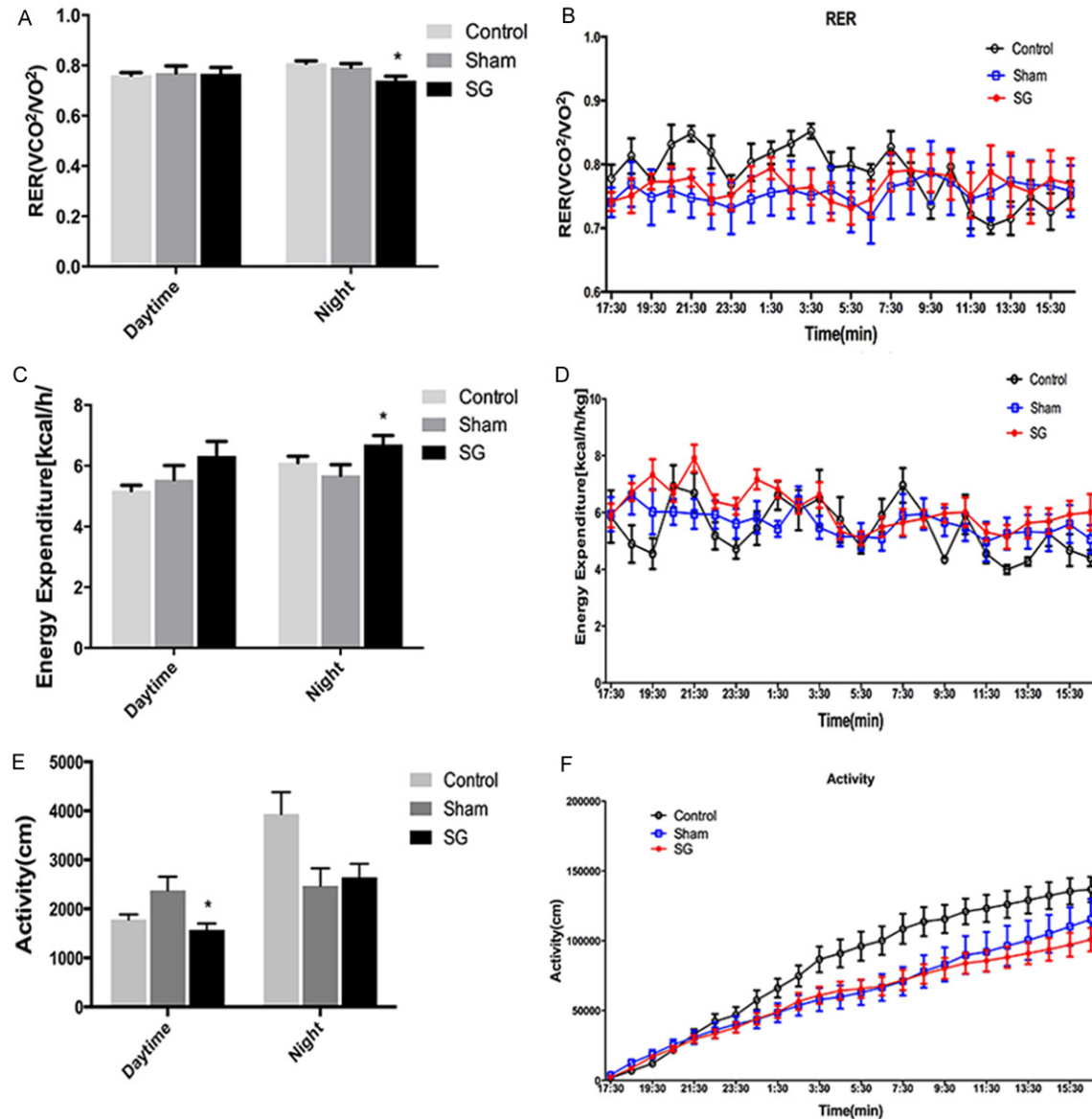


Figure 3. The body metabolic parameters measured by TSE LabMaster after SG surgery in all groups (n=8). (A) Daytime RER compared with that of at night. (B) RER change followed by time (C). Daytime energy expenditure compared with that of at night (D). Energy expenditure change (E), daytime free activity compared with that of at night. (F) Activity change followed by time. Data were expressed as mean \pm standard error of mean (SEM) with N as the number of experiments. The statistical significance of differences among groups was compared by analysis using one-way ANOVA and the paired t-test. Differences were considered significant at $P < 0.05$. Statistical analysis was conducted using GraphPad Prism version 6.0 (San Diego, CA, USA).

RNA extraction and expression analysis by real-time RT-PCR

Total RNAs were extracted from kidney tissue using TRIzol (Invitrogen), and synthesis of cDNA was by reverse-transcribed the RNA using reverse-transcribed kit (QIAGEN). Real-Time PCR amplification was performed using ViiA 7 Software (Applied Biosystems) with the follow-

ing conditions: 95°C for 30 sec and 40 cycles of amplification (95°C for 5 secs, 60°C for 30 sec).

The primer sequences were as follows:

ATGL, 5'-AGTTCAACCTTCGCAATCTC-3' and 5'-GTCACCCAATTTCTCTTGG-3'; PPAR γ , 5'-ACTGCCTATGAGCACTTAC-3' and 5'-GATGGCATTG-

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Table 1. Sleeve Gastrectomy changed CHOL, TG, BUN and CREA

CHOL	Control (n=6)	Sham (n=8)	SG (n=8)
Week -2	2.19±0.04	3.75±0.15	3.72±0.15
Week 2	2.45±0.05	4.39±0.20	3.44±0.21**
Week 4	2.49±0.04	4.53±0.25	3.63±0.14**
Week 8	2.52±0.05	6.25±0.36	3.60±0.20***
TG			
Week -2	0.63±0.01	1.02±0.03	1.06±0.03
Week 2	0.77±0.03	1.14±0.06	0.74±0.04****
Week 4	0.71±0.01	1.36±0.14	0.86±0.04*
Week 8	0.63±0.01	1.32±0.08	0.84±0.04
BUN			
Week -2	6.16±0.15	6.91±0.23	6.44±0.33
Week 2	5.72±0.21	5.86±0.17	6.10±0.23
Week 4	5.54±0.11	6.53±0.26	7.63±0.40*
Week 8	6.34±0.19	10.39±0.32	9.06±0.44
CREA			
Week -2	26.33±1.12	16.38±1.60	15.43±0.92
Week 2	29.17±1.30	14.50±1.10	18.50±0.82*
Week 4	27.67±0.67	15.38±0.96	18.29±0.57*
Week 8	38.00±2.60	19.00±1.05	24.50±1.94*

*P<0.05, **P<0.01, ***P < 0.001, ****P<0.0001 between the Sham group and SG group.

TGAGACATCC-3'; HSL, 5'-GCGCCTATTCAGGGAC-AGAG-3' and 5'-CCAGGAAGGAGTTGAG-CCAT-3'; PGC-1a, 5'-GTGAGATTGAGTGCACC-3' and 5'-GACCTTGATCTTGACCTGG-3'; SREBP-1C, 5'-TGCCCTAAGGGTCAAAACCA-3' and 5'-TGGCGGGCACTACTTAGGAA-3'; ACC, 5'-ACAGAGATGGTGGCTGATGT-3' and 5'-GATCCCCATGGCAATCTGGA-3'.

The 2^{-ΔΔCT} method was used to calculate the relative gene expression level.

Western blotting

Kidney tissues were homogenized and total proteins were extracted using RIPA buffer with phosphatase inhibitors cocktail. The concentration of proteins was measured by BCA assay, equivalent amount of protein from each sample was loaded onto 8% and 10% SDS-PAGE. The gels were transferred to PVDF membranes, and incubated with primary antibodies against anti-phospho-AKT (1:1000), anti-AKT (1:1000), anti-Phospho-PI3K (1:1000), anti-PI3K (1:1000) and anti-β-actin (1:4000) at 4°C overnight, then incubated with secondary anti-rabbit or anti-mouse HRP-conjugated IgG antibodies

(1:4000) for 2 h at room temperature. Finally, the membranes were detected through ECL plus Western blotting detection system. To quantify the protein signal, background was subtracted and normalized according to β-actin value. As for the phospho-specific protein, normalized signals were total target protein and β-actin amount.

Histology

After extraction, epididymal white adipose tissue (eWAT) and kidney white adipose tissue (kWAT) were fixed in 4% paraformaldehyde at 4°C overnight. All tissues were subsequently dehydrated, embedded in paraffin and 4 μm thick sections were cut using a microtome. For general histology, HE staining was performed referring to the standard methods. Adipose cells were collected randomly from photos and the area were calculated by Image J (Image J 1.51e).

Statistical analysis

Data were expressed as mean ± standard error of mean (SEM) with N as the number of experiments. The statistical significance of differences among groups was compared by analysis using one-way ANOVA and the paired t-test. Differences were considered significant at P<0.05. Statistical analysis was conducted using GraphPad Prism version 6.0 (San Diego, CA, USA).

Results

Sleeve gastrectomy improves ZDF rats weight, fasting plasma glucose and food intake

After sleeve gastrectomy surgery, the rats weight significantly decreased during 2, 4 and 8 weeks compared to sham group (**Figure 1A**, P<0.05 at 2 and 4 weeks, 8 weeks with no statistical significance but trends comparable to the controls). After 6 hours fasting, FPG in Sham group was obviously higher than that in SG group at postoperative measuring time (P<0.001 at 2 weeks, P<0.05 at 4 weeks and P<0.01 at 8 weeks). After SG, food consumption of ZFD rats significantly decreased compare to sham and control groups at 2 and 8 weeks (P<0.05 at 2 and 4 weeks), and there was no difference between sham and control groups. These data demonstrated that SG can efficiently improve weight, food intake and decreased fasting plasma glucose in ZDF rats.

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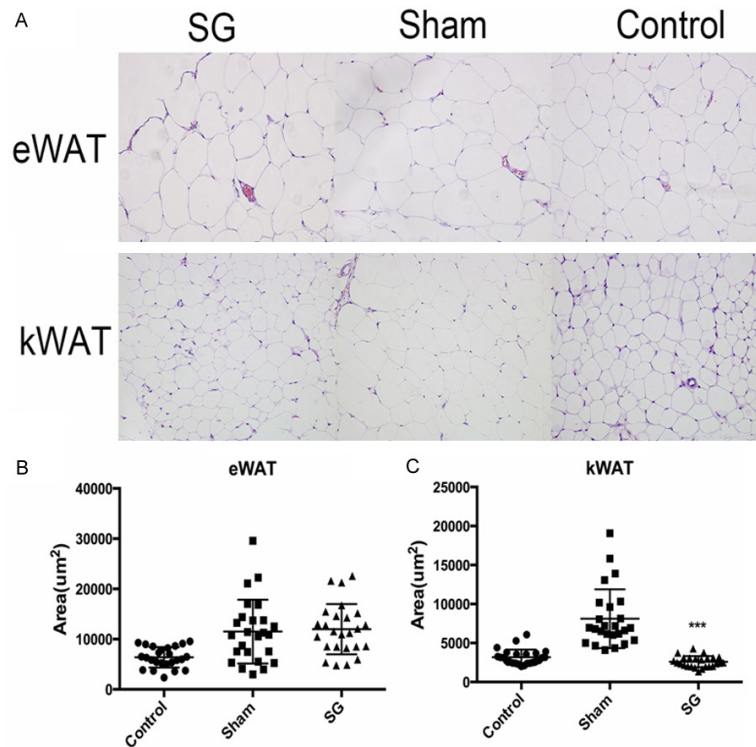


Figure 4. The adipocytes of eWAT and kWAT measured by HE staining in ZDF rats. (A), Representative images of HE staining of epididymis and kidney adipose tissue in SG group, Sham group and Control group. Representative images were presented. Scale bar:100 μm (B, C) the mean adipocytes area of random 25 adipose cell in SG group, Sham group and Control group. Data were expressed as mean \pm standard error of mean (SEM) with N as the number of experiments. The statistical significance of differences among groups was compared by analysis using one-way ANOVA and the paired t-test. Differences were considered significant at $P < 0.05$. Statistical analysis was conducted using GraphPad Prism version 6.0 (San Diego, CA, USA).

Sleeve gastrectomy improved glucose metabolism through oral glucose tolerance test (OGTT)

All data were measured after rats with 12 hours fasted. The absorption of glucose improved after surgery, but glucose homeostasis was significantly improved at 2, 4 and 8 weeks after SG compared to sham groups according to AUC in **Figure 2E**. $P < 0.05$ at 2, 4 and 8 weeks. These data demonstrated that SG can improve glucose metabolism through oral glucose tolerance test in ZDF rats.

Sleeve gastrectomy changed fat and calories consumption according to body metabolic parameters

Body metabolic parameters including respiratory exchange ratio (RER), energy expenditure (EE) and free activity were measured by TSE

LabMaster. Rats were monitored continuously during one week. RER in SG group was significantly lower than that in sham group at night (**Figure 3A, 3B**, $P < 0.05$). Energy expenditure at night was different between SG group and sham group, with statistical significance (**Figure 3C, 3D**, $P < 0.05$). Moreover, ZDF rats after SG was more active at day time than that in sham group (**Figure 3E, 3F**, $P < 0.05$), and night activity distance of rats was longer than daytime activity. These data demonstrated that SG can change metabolic parameters including RER, EE and free activity and effect fat and calories consumption in ZDF rats.

Sleeve gastrectomy changed the level of serum lipid and renal function

CHOL and TG are main indexes of serum lipid. As shown in **Table 1**, CHOL and TG in serum lipid (SL) of ZDF rats after SG significantly decreased. BUN and CREA are main indexes of renal function, CREA in SG group

enhanced compared to sham group in postoperation, which was possibly due to less muscle metabolism while BUN had no significantly difference for two groups. These data demonstrated that SG can decrease the level of serum lipid and improve renal function in ZDF rats.

Sleeve gastrectomy changed adipocyte size of eWAT, kWAT

Previous study proved that SG can improve renal function. We explored the volume change of visceral fat including Epididymis (eWAT) and kidney adipose tissue (kWAT) of ZDF rats through HE staining. **Figure 4B** showed that there was obvious difference between SG and sham group in kWAT, however with no obvious difference in eWAT. These data demonstrated that SG can decrease the adipocyte size of eWAT and kWAT of ZDF rats.

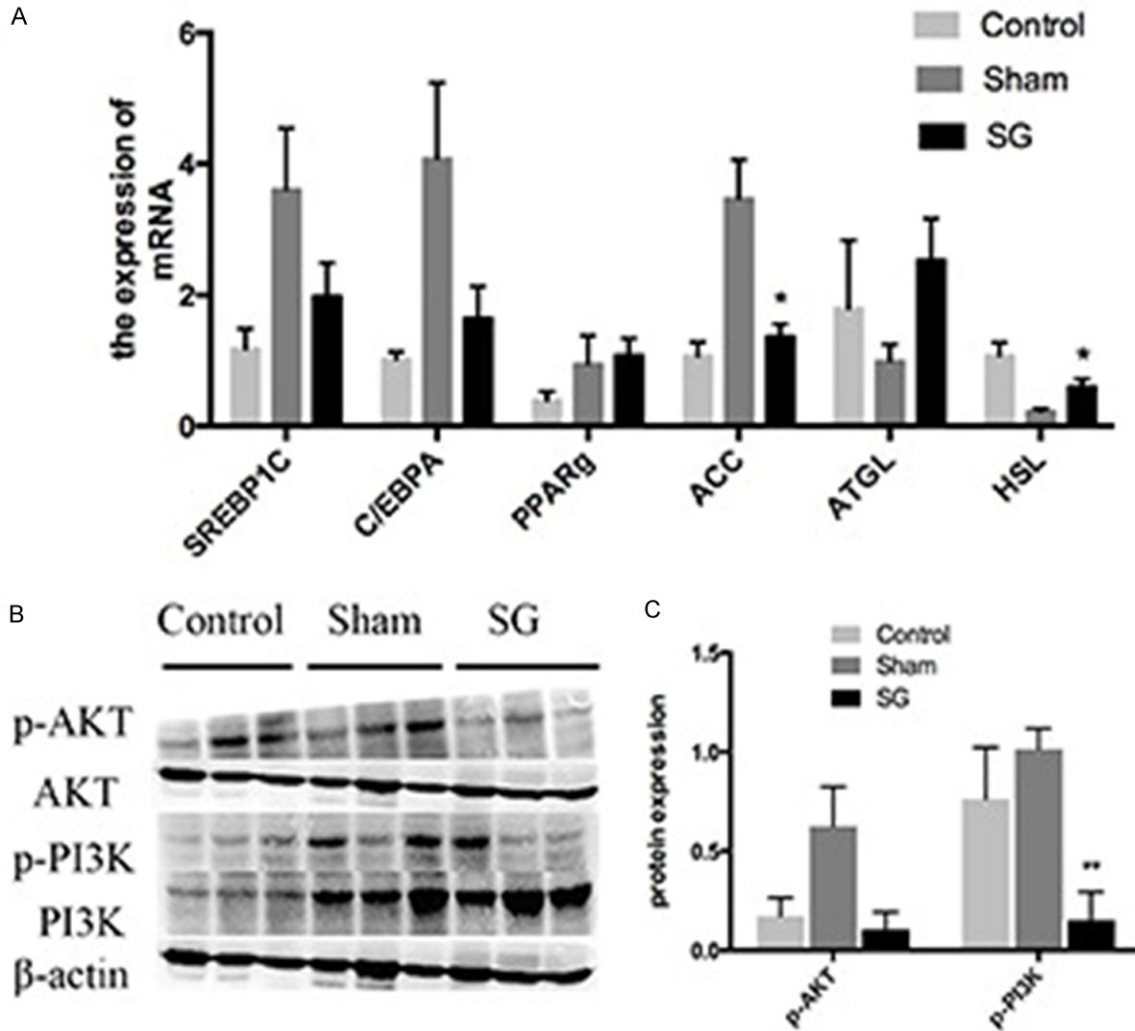


Figure 5. PI3K/AKT levels after SG surgery in rat kidneys. Western blot quantitative analysis of (A) Real-time RT-PCR quantitative analysis of lipogenesis and adipogenesis about SREBP1C, C/EBPA, PPAR γ , ACC, ATGL and HSL mRNA levels in SG group, Sham group and Control group (n=8). (B, C) Western blot quantification of AKT and PI3K kidney protein expression in SG group, Sham group and Control group. Data were obtained by densitometry and were normalized using β -actin as loading control. Data were expressed as mean \pm standard error of mean (SEM) with N as the number of experiments. The statistical significance of differences among groups was compared by analysis using one-way ANOVA and the paired t-test. Differences were considered significant at $P < 0.05$. Statistical analysis was conducted using GraphPad Prism version 6.0 (San Diego, CA, USA).

Sleeve gastrectomy changed lipogenesis and adipogenesis by PI3K/AKT signaling pathway in renal

Sleeve Gastrectomy changed renal function and kWAT in ZDF rats. Further, lipogenesis and adipogenesis markers expression in kidney were detected. The results showed that ACC expression significantly decreased, HSL significantly increased in SG group compared to sham group, but lipogenesis genes, including SREBP1C, C/EBPA, PPAR γ and ATGL were no dif-

ference in **Figure 5A**. Western blot analysis revealed that p-PI3K and p-AKT were down-regulated in **Figure 5B** and **5C**. These data demonstrated that SG can change the mRNA expression associated with lipogenesis and adipogenesis by PI3K/AKT signaling pathway in renal.

Discussion

Sleeve gastrectomy, which was contributed to lose weight through decreasing the stomach

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cubage, consists in a partial gastrectomy of the fundus to create a tubular gastric conduit constructed along the lesser curve of stomach [18-20]. SG, a popular and effective bariatric procedure, has widely applied on clinics compared to other therapy methods, such as diet and medication [21]. It was speculated that weight loss after gastric bypass was associated with mechanical restriction and malabsorption [20] including intake glucose and calorie [22]. In order to eliminate its mechanism for fat loss after surgery, metabolic parameters of SG, sham and control rats were monitored using TSE LabMaster. Analysis of RER showed that ZDF rats food intake and calories normally with less activity distance after SG and taking the same high-fat diet. These results revealed that energy restriction after SG was not the main reason for greatly improve body weight and glucose homeostasis. Therefore these results confirmed that bariatric surgery is an effective mean to sustain long-term weight loss.

DKD was etiology and pathogenesis due to lack of insulin with causing metabolism disorder of glucose, protein and fat [23-25]. Several metabolic factors such as advanced glycation, increased polyols synthesis, and enzyme activation including protein kinase C, and haemodynamic factors include systemic hypertension, intraglomerular hypertension mainly resulted in obesity-induced nephropathy [26, 27]. However, lipid accumulation and metabolism have rarely taken into consideration. Recent studies have showed that obesity was strongly associated with diabetes complications, however fat accumulation site was an evident risk factor for DKD [28, 29]. Moreover, excess visceral adipose tissue led to greater obesity-related metabolic disturbances, including adipocytokine-related inflammation and insulin resistance compared to subcutaneous adipose tissue [30]. In addition, lipid accumulation obviously destroyed renal function, therefore it being one indication for predicting renal dysfunction [31, 32]. Hence, the recovery of diabetes nephropathy is closely related to lipid degradation. In this study, the adipocytes in perinephric fat tissue after SG were smaller than that in the epididymis fat tissue, and lipogenic genes expression was up-regulated, while adipogenic genes expression was down-regulated. It suggested that SG enhanced ZDF rats fat degradation [33-36].

taas activa-PI3K/PI3K,Ctes, and t studies on To investigate furtherly how SG surgery changed perirenal adipose size, we explored that SG surgery increased ratios of p-PI3K/PI3K and p-Akt/Akt, indicating that PI3K/Akt signaling pathway was activated in this process. Therefore, our data provided novel proof that SG surgery could decrease perirenal fat or visceral fat tissue accumulation through activating AKT/PI3K pathway, remitting DKD. This provided new therapeutic approaches for preventing and curing diabetes nephropathy. Compared with conventional therapy such as medication, complementary therapies, physical therapy and inserting a catheter into the bladder to release urine [37], SG surgery is valid for people in body mass index (BMI) ≥ 35 (kg/m^2) with diabetic nephropathy, which can effectively relieve the pain of patients.

In conclusion, our study demonstrated that DKD was associated with lipid metabolism and sleeve gastrectomy could relieve renal function owing to increasing lipid degradation.

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Disclosure of conflict of interest

None.

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References

- [1] Eid GM, Brethauer S, Mattar SG, Titchner RL, Gourash W and Schauer PR. Laparoscopic sleeve gastrectomy for super obese patients: forty-eight percent excess weight loss after 6 to 8 years with 93% follow-up. *Ann Surg* 2012; 256: 262-265.
- [2] Rosenthal RJ; International Sleeve Gastrectomy Expert Panel, Diaz AA, Arvidsson D, Baker

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- RS, Basso N, Bellanger D, Boza C, El Mourad H, France M, Gagner M, Galvao-Neto M, Higa KD, Himpens J, Hutchinson CM, Jacobs M, Jorgensen JO, Jossart G, Lakdawala M, Nguyen NT, Nocca D, Prager G, Pomp A, Ramos AC, Rosenthal RJ, Shah S, Vix M, Wittgrove A, Zundel N. International sleeve gastrectomy expert panel consensus statement: best practice guidelines based on experience of >12,000 cases. *Surg Obes Relat Dis* 2012; 8: 8-19.
- [3] Barzin M, Motamedi MA, Serahati S, Khalaj A, Arian P, Valizadeh M, Khalili D, Azizi F and Hosseinpanah F. Comparison of the effect of gastric bypass and sleeve gastrectomy on metabolic syndrome and its components in a cohort: Tehran obesity treatment study (TOTS). *Obes Surg* 2017; 27: 1697-1704.
- [4] Wang X, Chang XS, Gao L, Zheng CZ, Zhao X, Yin K and Fang GE. Effectiveness of laparoscopic sleeve gastrectomy for weight loss and obesity-associated co-morbidities: a 3-year outcome from Mainland Chinese patients. *Surg Obes Relat Dis* 2016; 12: 1305-1311.
- [5] Mason MC and Pournaras DJ. Sleeve gastrectomy: Managing the morbidity of obesity. *Angiology* 2017; 3319717724941.
- [6] Sheng B, Truong K, Spitler H, Zhang L, Tong X and Chen L. The long-term effects of bariatric surgery on type 2 diabetes remission, microvascular and macrovascular complications, and mortality: a systematic review and meta-analysis. *Obes Surg* 2017; 27: 2724-2732.
- [7] da Silva AV, Gouvea SA, da Silva AP, Bortolon S, Rodrigues AN, Abreu GR and Herkenhoff FL. Changes in retinal microvascular diameter in patients with diabetes. *Int J Gen Med* 2015; 8: 267-273.
- [8] Bourdel-Marchasson I, Mouries A and Helmer C. Hyperglycaemia, microangiopathy, diabetes and dementia risk. *Diabetes Metab* 2010; 36 Suppl 3: S112-118.
- [9] Svane MS and Madsbad S. Bariatric surgery - effects on obesity and related co-morbidities. *Curr Diabetes Rev* 2014; 10: 208-214.
- [10] Serra A, Esteve A, Navarro-Diaz M, Lopez D, Bancu I and Romero R. Long-term normal renal function after drastic weight reduction in patients with obesity-related glomerulopathy. *Obes Facts* 2015; 8: 188-199.
- [11] Navaneethan SD and Yehnert H. Bariatric surgery and progression of chronic kidney disease. *Surg Obes Relat Dis* 2009; 5: 662-665.
- [12] Li K, Zou J, Ye Z, Di J, Han X, Zhang H, Liu W, Ren Q and Zhang P. Effects of bariatric surgery on renal function in obese patients: a systematic review and meta analysis. *PLoS One* 2016; 11: e0163907.
- [13] Holcomb CN, Goss LE, Almehtmi A, Grams JM and Corey BL. Bariatric surgery is associated with renal function improvement. *Surg Endosc* 2018; 32: 276-281.
- [14] Shiba C, Shiba T, Takahashi M, Hori Y and Maeno T. Relationships among serum lipoprotein lipase mass, visceral fat, and retinal nerve fiber layer thickness. *Graefes Arch Clin Exp Ophthalmol* 2015; 253: 1883-1888.
- [15] Yang X, Huo F, Liu B, Liu J, Chen T, Li J, Zhu Z and Lv B. Crocin inhibits oxidative stress and pro-inflammatory response of microglial cells associated with diabetic retinopathy through the activation of PI3K/Akt signaling pathway. *J Mol Neurosci* 2017; 61: 581-589.
- [16] Schneck AS, Iannelli A, Patouraux S, Rousseau D, Bonnafous S, Bailly-Maitre B, Le Thuc O, Rovere C, Panaia-Ferrari P, Anty R, Tran A, Gual P and Gugenheim J. Effects of sleeve gastrectomy in high fat diet-induced obese mice: respective role of reduced caloric intake, white adipose tissue inflammation and changes in adipose tissue and ectopic fat depots. *Surg Endosc* 2014; 28: 592-602.
- [17] Behl T, Kaur I and Kotwani A. Implication of oxidative stress in progression of diabetic retinopathy. *Surv Ophthalmol* 2016; 61: 187-196.
- [18] Schroeder R, Harrison TD and McGraw SL. Treatment of adult obesity with bariatric surgery. *Am Fam Physician* 2016; 93: 31-37.
- [19] Moncada R, Becerril S, Rodriguez A, Mendez-Gimenez L, Ramirez B, Catalan V, Gomez-Ambrosi J, Gil MJ, Fernandez S, Cienfuegos JA, Valenti V and Fruhbeck G. Sleeve gastrectomy reduces body weight and improves metabolic profile also in obesity-prone rats. *Obes Surg* 2016; 26: 1537-1548.
- [20] Barbiero G, Romanucci G, Ortu V, Zuliani M, Miotto D, Pomerri F, Albanese A, Verdi D, Prevedello L and Foletto M. Relationship between gastric pouch and weight loss after laparoscopic sleeve gastrectomy. *Surg Endosc* 2016; 30: 1559-1563.
- [21] Bredella MA, Greenblatt LB, Eajazi A, Torriani M and Yu EW. Effects of Roux-en-Y gastric bypass and sleeve gastrectomy on bone mineral density and marrow adipose tissue. *Bone* 2017; 95: 85-90.
- [22] Bueter M, Lowenstein C, Olbers T, Wang M, Cluny NL, Bloom SR, Sharkey KA, Lutz TA and le Roux CW. Gastric bypass increases energy expenditure in rats. *Gastroenterology* 2010; 138: 1845-1853.
- [23] Carney EF. Diabetic nephropathy: the genetic architecture of DKD in T1DM. *Nat Rev Nephrol* 2016; 12: 714.
- [24] Li L, Wang C, Yang H, Liu S, Lu Y, Fu P and Liu J. Metabolomics reveal mitochondrial and fatty acid metabolism disorders that contribute to the development of DKD in T2DM patients. *Mol Biosyst* 2017; 13: 2392-2400.

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- [25] Altemtam N, Russell J and El Nahas M. A study of the natural history of diabetic kidney disease (DKD). *Nephrol Dial Transplant* 2012; 27: 1847-1854.
- [26] Cooper ME. Pathogenesis, prevention, and treatment of diabetic nephropathy. *Lancet* 1998; 352: 213-219.
- [27] Chang AR, Grams ME and Navaneethan SD. Bariatric surgery and kidney-related outcomes. *Kidney Int Rep* 2017; 2: 261-270.
- [28] Borgeson E, Johnson AM, Lee YS, Till A, Syed GH, Ali-Shah ST, Guiry PJ, Dalli J, Colas RA, Serhan CN, Sharma K and Godson C. Lipoxin A4 attenuates obesity-induced adipose inflammation and associated liver and kidney disease. *Cell Metab* 2015; 22: 125-137.
- [29] Marvyn PM, Bradley RM, Button EB, Mardian EB and Duncan RE. Fasting upregulates adipose triglyceride lipase and hormone-sensitive lipase levels and phosphorylation in mouse kidney. *Biochem Cell Biol* 2015; 93: 262-267.
- [30] Lee HH, Yoon YE, Kang SK, Bravo RC, Alabandi AM, Huh KH, Kim MS, Kim SI, Kim YS and Han WK. Clinical investigation into plasma neutrophil gelatinase-associated lipocalin and body adipose tissue associated with remaining renal function in living kidney donor. *Transplant Proc* 2017; 49: 935-939.
- [31] Zhang K, Li Q, Chen Y, Wang N and Lu Y. Visceral adiposity and renal function: an observational study from SPECT-China. *Lipids Health Dis* 2017; 16: 205.
- [32] Cobo G, Cordeiro AC, Amparo FC, Amodeo C, Lindholm B and Carrero JJ. Visceral adipose tissue and leptin hyperproduction are associated with hypogonadism in men with chronic kidney disease. *J Ren Nutr* 2017; 27: 243-248.
- [33] Pereira VH, Marques F, Lages V, Pereira FG, Patchev A, Almeida OF, Almeida-Palha J, Sousa N and Cerqueira JJ. Glucose intolerance after chronic stress is related with downregulated PPAR-gamma in adipose tissue. *Cardiovasc Diabetol* 2016; 15: 114.
- [34] Janevski M, Ratnayake S, Siljanovski S, McGlynn MA, Cameron-Smith D and Lewandowski P. Fructose containing sugars modulate mRNA of lipogenic genes ACC and FAS and protein levels of transcription factors ChREBP and SREBP1c with no effect on body weight or liver fat. *Food Funct* 2012; 3: 141-149.
- [35] Karbowska J and Kochan Z. Fat-reducing effects of dehydroepiandrosterone involve up-regulation of ATGL and HSL expression, and stimulation of lipolysis in adipose tissue. *Steroids* 2012; 77: 1359-1365.
- [36] Morak M, Schmidinger H, Riesenhuber G, Reichberger GN, Kollroser M, Haemmerle G, Zechner R, Kronenberg F and Hermetter A. Adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) deficiencies affect expression of lipolytic activities in mouse adipose tissues. *Mol Cell Proteomics* 2012; 11: 1777-1789.
- [37] Thomas MC and Groop PH. New approaches to the treatment of nephropathy in diabetes. *Expert Opin Investig Drugs* 2011; 20: 1057-1071.