Increased Energy Expenditure and Energy Loss through Feces Contribute to the Long-term Outcome of Roux-en-Y Gastric Bypass on Diet Induced Obese Mouse Model

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Research

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Abstract

Background Roux-en-Y gastric bypass (RYGB) has been proved to be more effective than other bariatric procedures in long-term on body weight loss and remission of diabetes. However, the mechanism remains poorly understood. Long-term change of energy metabolism after RYGB has rarely been reported. Objectives To investigate the long-term outcome of RYGB on mouse model and its mechanism from the perspective of energy metabolism.

Methods High-fat diet induced obesity (DIO) mice were assigned to two groups receiving RYGB(n=8) and sham operation(n=7), followed by high-fat diet feeding until 12 weeks after surgery. Body weight and food intake were recorded weekly, measurement of body composition and energy metabolism by metabolic chamber were conducted on week 4, 8 and 12 after surgery. Fecal energy measurement, Intraperitoneal Glucose Tolerance Test (IPGTT) and Insulin Tolerance Test (ITT) were conducted on week 12 after surgery.

Results Food intake was reduced in RYGB group within the first 3 weeks after surgery and increased to be the same withSham group from postoperative week 4. At 12 weeks after surgery, body weight reduced by 36±3.2% in RYGB group comparing to 16±2% body weight gain in Sham group, while fat mass was significantly reduced in RYGB group than in Sham group (9.2±1.5% versus 30.1±0.7%). Energy expenditure was significantly higher on postoperative week 8 in RYGB group than in Sham group. In comparison with Sham group, respiratory exchange ratio (RER) was unchanged, decreased and increased in RYGB group at postoperative week 4, 8 and 12, respectively. Fecal energy measurementshowed that feces from mice in RYGB group contained higher energy level than Sham group. Glucose metabolism was significantly improved in RYGB group in contrast to Sham group, demonstrated by the result of Intraperitoneal Glucose Tolerance Test (IPGTT) (AUC: 1502± 104 versus 2277±198, respectively) and the Insulin Tolerance Test (ITT) (AUC: 524 ±50 versus 838±63, respectively).

Conclusions Increased energy expenditure and energy loss through feces contribute to the long-term body weight control after RYGB. Enhanced glucose utilization might play a role in the long-term improvement of glucose metabolism.

Introduction

Bariatric surgery has been demonstrated to be effective not only for body weight loss, but also for the improvement of obesity related comorbidities, such as type 2 diabetes, by a number of retrospective studies in last decades[1, 2]. Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG) were proved to be superior to medical treatment for type 2 diabetes mellitus(T2DM) according to a 5-year perspective randomized clinical trail [3] which lead to the consensus that bariatric surgery was listed in the treatment algorithm of T2DM [4]. Moreover, RYGB showed better long-term effect than SG [3] which raised the interests about the physiological change in the long-term period after RYGB. However, the mechanism underlying the remission of T2DM after RYGB remains poorly understood. Different animal models of bariatric surgery have been used [5–7], but creating mouse model of RYGB is challengeable due to the technical difficulty. Meanwhile, the long-term result of metabolic effect of RYGB on mouse model has rarely been reported. Based on our previous experience on mouse model of duodenojejunal bypass(DJB) [8] and other reports [9, 10], we successfully established a reproducible RYGB mouse model with optimal long-term outcome. In this report, the long-term effect of RYGB was presented with analysis on the mechanism from the perspective of energy metabolism.

Materials And Methods

Animal and diet

Male C56BL/6J mice, aged 7 weeks (Hunan SJA Laboratory Animal Co.), were housed in groups under 12-hour light/dark cycles (lights on at 7:00 am) and a room temperature of 21-23^{o}C, provided food and water ad libitum before surgery. Upon arrival at The Second Xiangya Hospital of Center South University Animal Research Center, mice were introduced to the high fat diet (60% kcal% high-fat diet; Research Diets, No. D12492) for 12 weeks, until they reached a body weight of 40-50 g (we identified the obesity as body weight more than 25% compared to age-matched chow-fed counterpart[11]). Mice were assigned to two groups according to body weight match: RYGB group (n=8) and Sham group (n=7). Mice were feed with ENSURE as liquid diet at 24 hours before the surgery.The body weight of mice had no significantly different between two groups before surgery. The Institutional Animal Care and Use Committee of The Second Xiangya Hospital of Central South University proved all experiments.

Anesthetization

General anesthesia was induced in the induction chamber with 5% isoflurane (0.4L/min) and O_2 (dioxygen; 0.4 L/min). After loss consciousness, the mouse was transfer to operating table and continuously anesthetized with 2% isoflurane (0.4L/min) and O_2(dioxygen; 0.4L/min)through a nozzle of the anesthesia system. The mouse is placed on heating pad in dorsal recumbency. The abdominal skin was molted by depilatory cream and disinfected with povidone-iodine swabs. The entire body was covered with sterile surgical drape. During the surgery, the body core temperature was maintained within 30-32^{o}C. A respiration rate and toe-pinch test were used to confirm the depth of anesthesia.

Surgical procedure of Roux-en-Y Gastric Bypass

Before this study, we performed RYGB on 17 DIO mice, but the postoperative mortality was very high and some mice died within 1 month due to malnutrition. Then based on our previous experience on DJB and other reports[8-10], we made some technical modification and established an effective, reproducible model of RYGB on DIO mouse with no mortality. The surgical procedure and technical details were described stepwise as following.

1. A 2cm long midline incision was made right below xiphoid, and then placed two stay stitches on each side of incision for the retraction to expose abdomen.
2. Expose the mesocolon and identify the ligament of Treitz by moving small bowel with moistened cotton bud, then the jejunum was ligated with 8/0 suture at 4cm distal to ligament of Treitz, normally there is a nonvascular gap on the mesentery of this site. The jejunum was transected right proximal to the ligation; as a result, the proximal part of jejunum will be the biliopancreatic limb (BP limb) (Figure 1a).
3. Measurement was conducted again start from the point of ligation on distal part of jejunum for 4cm in length as the designated alimentary limb (AL limb), followed by making a 2mm longitudinal incision on the bowel at the end of AL limb as preparation for the subsequent anastomosis. Then an end-to-side jejunojejunostomy was performed between BP limb and AL limb in an interrupted fashion with 11/0 suture (Figure 1b).
4. Small soaked gauze were placed around liver, stomach and spleen to expose epigastric area, then ligaments between liver and distal part of esophagus were divided to expose the esophagogastric junction. The esophagogastric junction was ligated by a 8/0 suture ligation, the suture was then stitched to a proper sized soaked gauze ball and placeed over mouse body and hanged in the air out of operating table as a retraction to well expose the distal part of esophagus. A small cut on muscular layer of esophagus was performed with tiny blade, the incision was extended to about 2mm by scissor then the underneath whitish mucosal layer will be exposed. The mucosa was cut open longitudinally with scissor for 2mm in length right superior to the suture ligation (Figure 1c).
5. A 2mm incision was made on proximal end of AL limb after it was placed near esophagogastric junction, then a side-to-side anastomosis was performed between distal part of esophagus and AL limb in a fashion of running suture with 11/0 suture (Figure 1d).
6. Abdominal incision was closed with 4/0 absorbable suture after realignment of the small bowel, followed by subcutaneous injection of 1ml saline (0.9%) for fluid compensation and 1 mg/kg buprenorphine.

Surgical procedure of Sham group

The sham procedure consists of laparotomy, jejunal transection at 4cm distal to ligament of Treitz and re-anastomosis in situ. The Sham group mice undergo the same operating time and received the same perioperative care.

Postoperative care

1. Mice were placed on electric blanket for regaining consciousness and continuously monitor until normal behavior (walk and stand without falling) before the mice were transferred to cage and given water and liquid diet al libitum. The temperature of incubator set at 30^{o}C. Solid high-fat diet was resumed once defecation was observed.
   2.Because surgical stress inhibite mice groom habit which may lead to hair wetting, we used dry

swab to groom it twice a day. Mice exhibited loose and shapeless stool for about two weeks after RYGB, which may lead to rectocele. We nursed anus with normal saline daily.

Measurement of Body weight and body composition

Body weight was measured in every Thursday between 10:00-12:00am throughout the duration of the study. Body composition including fat mass, lean mass and fluid mass was measured by DEXA (GE Medical Systems, Madison, WI) at 0, 4, 8, and 12 weeks after surgery. The adiposity index was calculated by dividing fat mass by lean mass.

Measurement of Food intake

Food intake was measured weekly by housing the mice individually for 24 hours with elevated grid floor without bedding. The quantity of food intake was calculated by subtract the amount of food remaining plus spillage found under the grid floor (distinguishable by the blue color of high-fat crumbs) from the previous day`s measurement.

Measurement of Fecal energy analysis

Feces were collected over 24 hours on the postoperative week 12. Feces were weighted and reserved in -80°C refrigerator. The fecal gross energy was measured using a ballistic bomb calorimeter C6000 (IKA, Staufen, Germany) after the feces were dried in oven. The result of was presented by thermal energy in per gram feces.

Measurement of Metabolic Chamber

The oxygen consumption and the carbon dioxide output were measured in metabolic chambers at 4 weeks, 8weeks and 12 weeks after surgery. Mice were transferred to the Comprehensive Laboratory Animal Monitoring System (CLAMS; Columbus Instruments, OH, USA) under a 12-hour/12-hour day/night cycle (lights on at 7:00 am). Mice were adapted to the cage for 24 hours before each trial. Data was analyzed by the suited Oxymax software package (Columbus Instruments). The equations used were RER = VCO_2 / VO_2 And EE = (3.815 + 1.232 × RER) × VO_2 × 0.001 (kcal/ [kg × h]). Due to the stress brought by metabolic chamber that might lead to great weight loss and even death as we observed in our pilot study, Mice from RYGB group were evenly divided into two groups, one group underwent metabolic chamber on 4 and 8 weeks after surgery, the other group underwent metabolic chamber at 12 weeks after surgery only.

Measurement of Glucose metabolism

Mice were pre-stimulated for 1 week and fasting overnight before test at 11 weeks after surgery. Intraperitoneal Glucose Tolerance Test (IPGTT) was performed after administrating glucose solution(1.5 g/Kg body weight) by intraperitoneal injection, while the Insulin Tolerance Test (ITT) was conducted with intraperitoneal injection of insulin at 0.75 U/Kg body weight after 6 hours fasting. In both experiment, blood glucose level was measure from tail cut at 0 min, 15 min, 30min, 60 min, 120 min after injection.

The glucose tolerance and the insulin tolerance were assessed by calculating the area under the glucose curves (AUC).

Statistical analysis

Statistical analysis was performed using SPSS 19.0 software (SPSS Inc., Chicago, IL) and Prism 6 software. Statistical analysis of the data was performed using ANOVA or the Student t test. Data was expressed as the mean ± SEM. A P value of <0.05 was considered to be statistically differences.

Results

Mice were euthanized and underwent laparotomy at the end of 12 weeks after surgery; the abdominal viscera and entire alimentary tract were examined to ensure the complete exclusion of stomach and patency of anastomosis and no surgical complication(Figure 1e).

Modified technique improved surgical outcome after RYGB

After technical improvement, the operating time was reduced from 103±2 min in the group using old technique (Unmodified Group, n=17) to 59±3 min in the group using modified technique (Modified Group, n=8), short-term survival rate (within 1 week after surgery) increased from 53% in Unmodified Group to 100% in Modified Group. Long-term survival rate at 12 weeks after surgery increased from 41% to 100%(Figure1f).

RYGB result in more body weight loss without reducing food intake

Body weight of the mice in RYGB group dropped sigin the first 2 weeks after surgery and continued to decline for 2 more weeks until approximately 36±3.7% of origin body weight was lost, then kept stable for the rest 8 weeks. While in Sham group, mice lost about 10±1.3% of body weight in the first week after surgery due to the surgical stress and then start to regain weight gradually. At 4 weeks after surgery, body weight has already exceeded the preoperative level in Sham group (Fig.2 a, b). Food intake measurement demonstrated that mice in RYGB group eat less in the first 3 weeks than Sham group, but the difference kept decreasing from postoperative week 1 to week 3. From postoperative week 4, food intake remained no different between two groups (Fig.2 c).

Fecal energy analysis revealed that more energy was lost by defecation after RYGB
The feces collected at 11 weeks after surgery was weighted and measured for its energy level, which showed that there was significantly higher level of energy in the feces from RYGB group than Sham group (Fig.2 d), indicating energy from food were lost remarkably through feces in the long-term after RYGB (12 weeks in mouse lifespan equal 7 years in human lifespan[12]).

RYGB reduced fat mass and changed body composition in long-term

Body composition measurement revealed that percentage of fat mass were substantially reduced and the percentage of lean mass were accordingly increased in RYGB group comparing with Sham group at 4 weeks after RYGB. Moreover, the alteration of body composition was proved to be long-term effect as it remained unchanged at 12 weeks after RYGB (Fig.3).

Energy expenditure was significantly increased at 8 weeks after surgery, preferential substrate utilization for energy production was switched from fat to glucose at 12 weeks postoperatively

Date from metabolic chamber showed that energy expenditure in RYGB group started to increase at 4 weeks after surgery and became significant higher during night than that in Sham group at 8 weeks postoperatively. However, at 12 weeks after surgery, there was no difference between two groups (Fig.4 a).

In contrast with Sham group, the value of RER was found to be no different, significantly decreased and significantly increased in RYGB group at 4, 8 and 12 weeks after surgery respectively (Fig.4 b), suggesting that the preferential substrate utilization for energy production was changed in the long-term postoperative course, specifically from fat at week 8 to glucose at week 12 after surgery.

RYGB improved glucose tolerance and insulin sensitivity in the long-term

Mice in RYGB group showed significant lower level of blood glucose than mice in Sham group according to IPGTT test (Fig.5 a, b). ITT also revealed that level of blood glucose was remarkably lower in RYGB group (Fig.5 c, d), suggesting mice in RYGB group responded better to insulin than Sham group at 12 weeks postoperatively.

Discussion

Although mouse model has been used for investigating the mechanism of RYGB in many studies, the surgical technique described in those reports was either lacking of detail or non-reproducible due to the technical difficulty. According to our study, some problems need to be addressed before a successful RYGB model could be produced on DIO mouse, which includes determination of the length of the bypassed small bowel to avoid postoperative malnutrition while keeping the bariatric and metabolic benefits of RYGB at meantime; the method of bypassing stomach to minimize technical complexity; the refining of technique to reduce surgical complications such as anastomotic obstruction and leakage, and the improvement of perioperative care to facilitate mouse recover from surgical stress.

Bypassing small bowel is a key component to the bariatric and metabolic benefit of RYGB, but it could also lead to malnutrition if too much small bowel was excluded from nutrients digestion. In our pilot study, the length of BP limb and AL limb were first set to be 6 cm each[13] which lead to death within 5 weeks due to malnutrition. Thus in this study we shorten the length of both limb to be 4cm each, which resulted in 36±3.7% average body weight loss without death from malnutrition, as comparable to other report[14]. Although creating a small gastric pouch with volume of 30ml (using a balloon gastric tube for measurement) is a standard step of RYGB in human[15], it is debatable to use such gastric pouch on mouse model[10, 13, 16]. Firstly, dissecting the vessels around EGJ and dividing stomach to create the gastric pouch posing great risk of bleeding and postoperative death[10, 13, 16]; Secondly, considering the gastric pouch is very small, bypassing whole stomach would not make significant difference on the outcome of RYGB while remarkably reduce the difficulty and risk of the procedure[18]. Therefore, we ligated the EGJ to bypass the whole stomach in our study, leaving distal part of esophagus for the anastomosis to AL limb. These modifications helped us to establish a standardized and step-wised surgical procedure that succeeded in replicating RYGB on DIO mouse with no mortality.

Similar to other reports[12, 19, 20], body weight and food intake were both declined within first 2 weeks in Sham group, then turned to increasing thereafter, indicating the impact of surgical stress lasted for 2 weeks in this study. Mice in RYGB group, however, continue to loss weight until 5 weeks after surgery. Unlike the mice in Sham group regained all the lost weight and became 16±2% heavier at 12 weeks after surgery, body weight of the mice in RYGB group were kept at its lowest level in the whole postoperative period despite the food intake increased to be the same as Sham group. These findings demonstrated that RYGB is effective on long-term body weight loss which is consistent with clinical studies[12, 19, 20].

Excessive fat accumulation was the key factor for the development of insulin resistance and metabolic syndrome, thus reduction of fat mass would ameliorate metabolic disorders [21-23]. Clinical studies proved that RYGB could lead to higher remission rate of type 2 diabetes than SG and medical treatment in long-term[4, 24]. In this study, body composition was found to be altered after RYGB as fat mass was significantly reduced, and this alteration was kept unchanged at 8 and 12 weeks after surgery. Thus it could be concluded that fat mass reduction is the major contributor for body weight loss after RYGB and the body composition change could last in long-term. As a result, the insulin sensitivity was significantly improved as reflected by ITT test in our study. In addition, prompt insulin response to glucose uptake was enhanced by RYGB. IPGTT test in this study found that the time point of blood glucose peak level was at 15min in RYGB group, which is earlier than that in Sham group. Notably, these measurements were conducted at 12 weeks after surgery in our study, proving that the bariatric and metabolic effects of RYGB were maintained in the long-term course after surgery.

Excessive energy transformed into lipids that stored within fat cells was considered the origin cause of obesity[25, 26], thus the balance of energy input and output is the key factor that affect the body weight. Metabolic cage measurement in our study revealed that energy expenditure increased at 4 weeks after RYGB although the difference was not statistically significant. The food intake was significantly less in RYGB group compared with Sham group within first 3 weeks after surgery, which might be the main cause to the body weight loss at that time of period. Since week 4 after surgery, the food intake in RYGB group increased to be the same with Sham group, but the body weight still remained unchanged, which is in consistent with other reports[27, 28]. Thus the significantly increased energy expenditure played an important role in maintaining low body weight in this period as energy expenditure became significant higher in RYGB group at postoperative week 8. At 12 weeks after surgery, however, energy expenditure in

RYGB group dropped back to be the same with Sham group despite the body weight gap between two groups became even wider. Taking the same food intake into consideration, some other factor might contribute to the maintenance of the low body weight in RYGB group. Alimentary tract was re-aligned after RYGB which altered the digestion and absorption of nutrients [29-31]. Fecal energy analysis in this study proved that there was more energy contained in the feces from mice underwent RYGB than sham surgery, suggesting RYGB lead to more energy loss through defecation. Therefore, increased energy loss through defecation might contribute to maintain low body weight in the long-term period after surgery.

RER value represents the preferential substrate utilization for producing mitochondrial ATP[32], which usually used for determining the ratio of fat and carbohydrate as energy resource[32]. On week 4 after surgery, RER was not different between two groups meaning the preferential resource of energy producing has not been changed by RYGB yet. On postoperative week 8, RER was decreased suggesting there was more fat utilized to produce energy by mice in RYGB group then Sham group. On week 12 after surgery, RYGB group showed an increased RER, suggesting that mice used more glucose for energy production. Therefore, although the effect of raising energy expenditure by RYGB was diminished in the long term, glucose utilization was enhanced which could be beneficial for maintaining normal blood glucose level. These findings might help to explain that some patients still keep normal glucose metabolism despite body weight regain many years after RYGB.

Conclusions

For the first time, this study revealed the long-term energy metabolism profile after RYGB with regard to energy expenditure, preferential substrate utilization and fecal energy loss, implicating multiple mechanisms might be involved in the long-term effects of RYGB. These findings will provide new insights for the future study of metabolic surgery and obesity.

Abbreviations

RYGB: Roux-en-Y Gastric Bypass

SG: Sleeve Gastrectomy

DJB: Duodenojejunal Bypass

IPGTT: Intraperitoneal Glucose Tolerance Test

ITT: Insulin Tolerance Test

RER: Respiratory Exchange Ratio

EGJ: Esophagogastric Junction

DIO: Diet Induced Obesity

BP limb: Biliopancreatic limb

AL limb: Alimentary limb

Declarations

Ethics approval and consent to participate: The Institutional Animal Care and Use Committee of The Second Xiangya Hospital of Central South University proved all animal use in this study.

Consent for publication: not applicable

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions:

CK contribute to the design of the study, animal experiment and metabolic chamber measurements. BX contribute to the design of the study, animal experiment and glucose metabolism measurements. ZZ contribute to the postoperative animal care and measurement of body weight, food intake. WP contribute to the postoperative animal care and measurement of fecal energy. WL contribute to the conception and design of the work, analysis of the data, and draft of the work. All authors read and approved the final manuscript.

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Figures

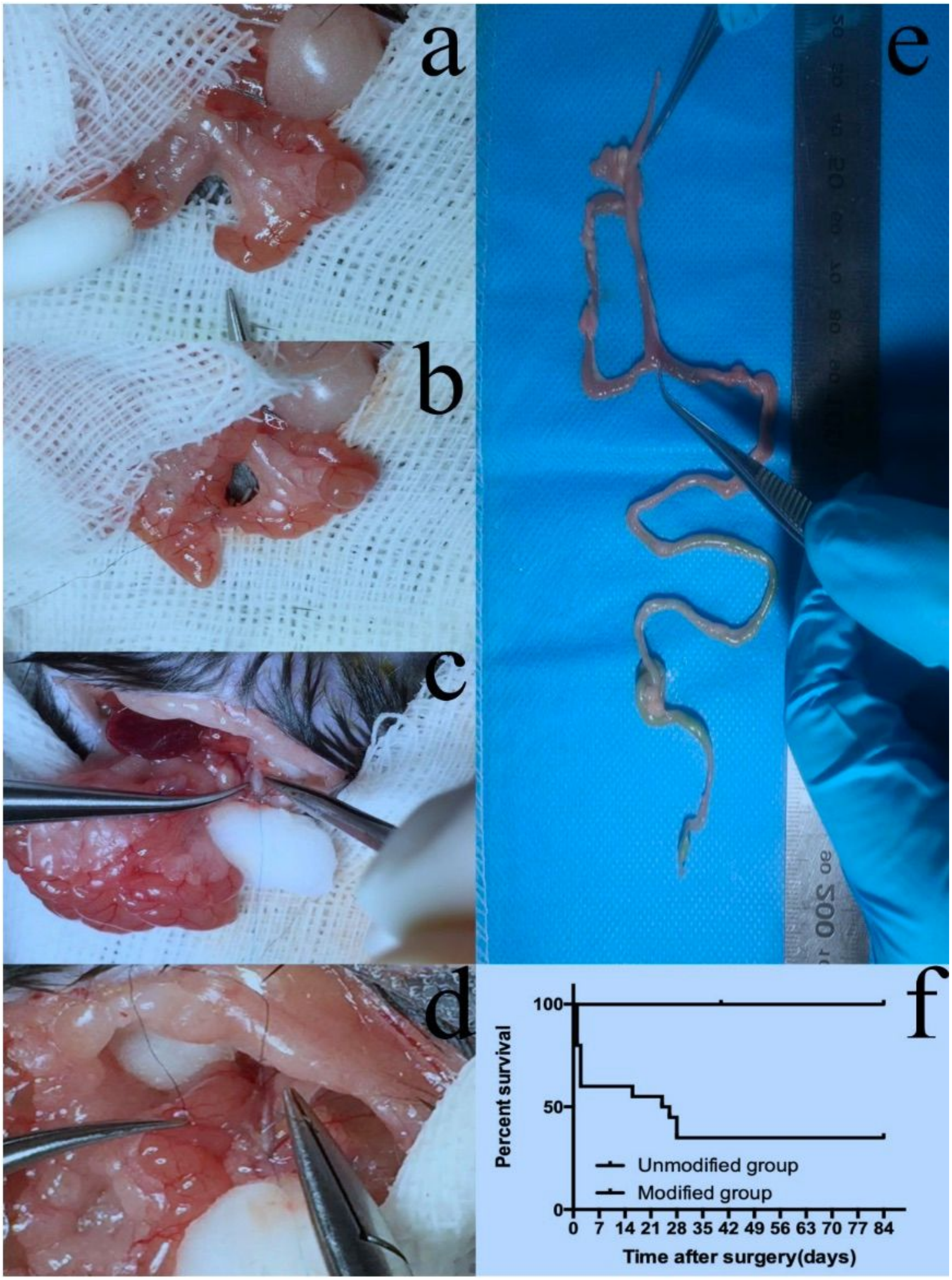

Figure 1

. Image of Roux-en-Y gastric bypass surgery and survival rate. a. The jejunum was transected and the proximal part of jejunum will be the BP limb; b. The end-to-side jejunojejunostomy was performed between BP limb and AL limb; c. The mucosa was cut open longitudinally for 2mm right superior to the suture ligation on EGJ; d. A side-to-side anastomosis was performed between distal part of esophagus and AL limb; e. Entire alimentary tract showed complete excluded stomach and patent anastomosis after RYGB; f. The long-term survival rate between the Unmodified group and the Modified group.

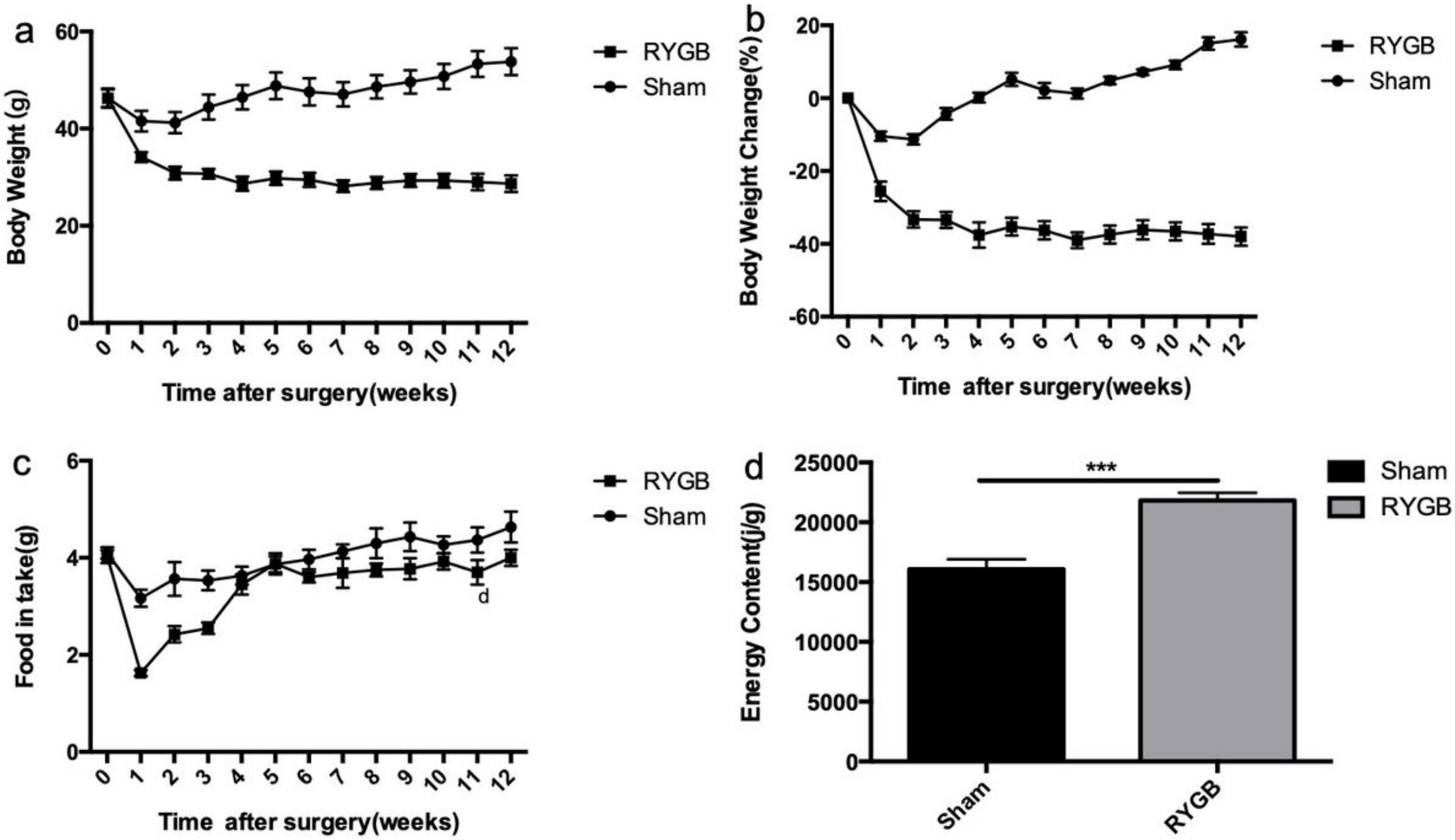

Figure 2

RYGB produces the significant weight loss independent of food intake. a. Absolute body weight was significantly lower in RYGB group after surgery. b. The percentage change in body weight was significantly higher in RYGB group after surgery. c. Food intake was significantly reduced in RYGB group on postoperative week 1, 2 and 3, and increased to same level with Sham group on week 4 and thereafter. d. Fecal energy measurement was significantly higher in RYGB group at postoperative week 12. Values are the mean ± SEM. Statistical differences were analyzed by one-way ANOVA followed by the student`s t-test. *** P<0.001 vs Sham.

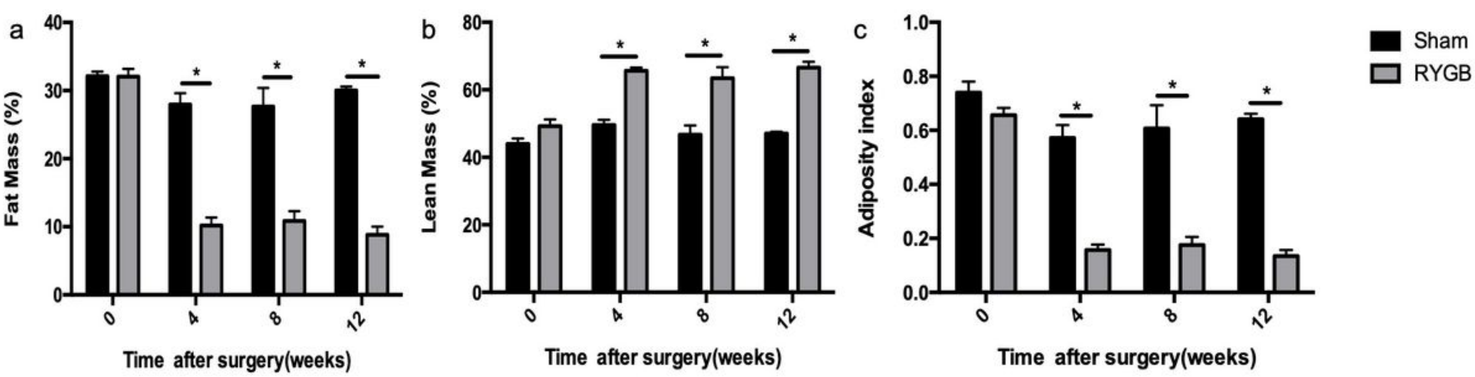

Figure 3

RYGB result in a significant change in body composition. a. The percentage of fat mass calculated as the radio of fat mass to body weight. b. The percentage of lean mass calculated as the radio of lean mass to body weight. c. The adiposity index calculated as the radio of fat mass to lean mass. Statistical differences were analyzed by one-way ANOVA followed by the student`s t-test. *** P<0.001 vs Sham.

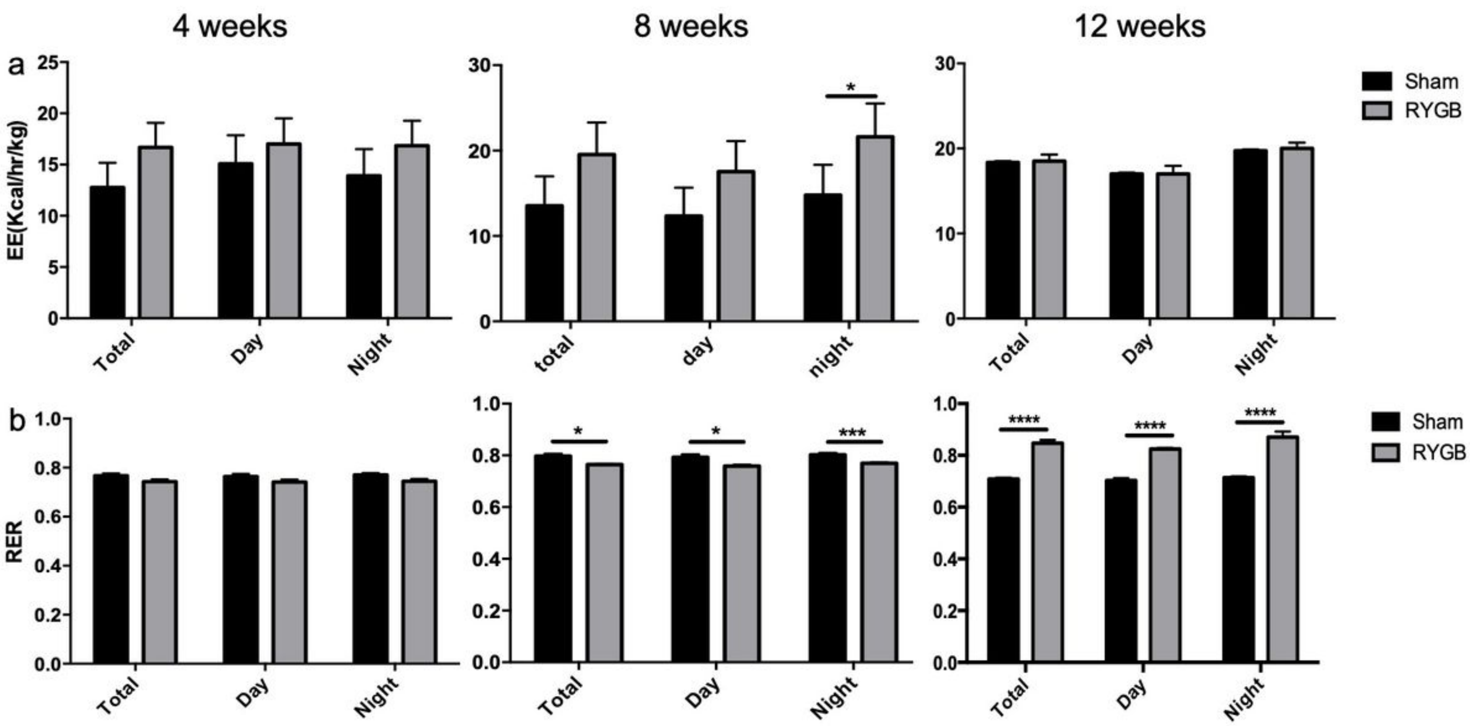

Figure 4

RYGB affect the energy expenditure, respiratory exchange radio at 4 weeks, 8 weeks and 12 weeks postoperative. a. Energy expenditure per day normalized to total body weight. b. Respiratory exchange radio. Statistical differences were analyzed by one-way ANOVA followed by the student`s t-test. * P<0.05 vs Sham, *** P<0.001 vs Sham, **** P<0.0001 vs Sham.

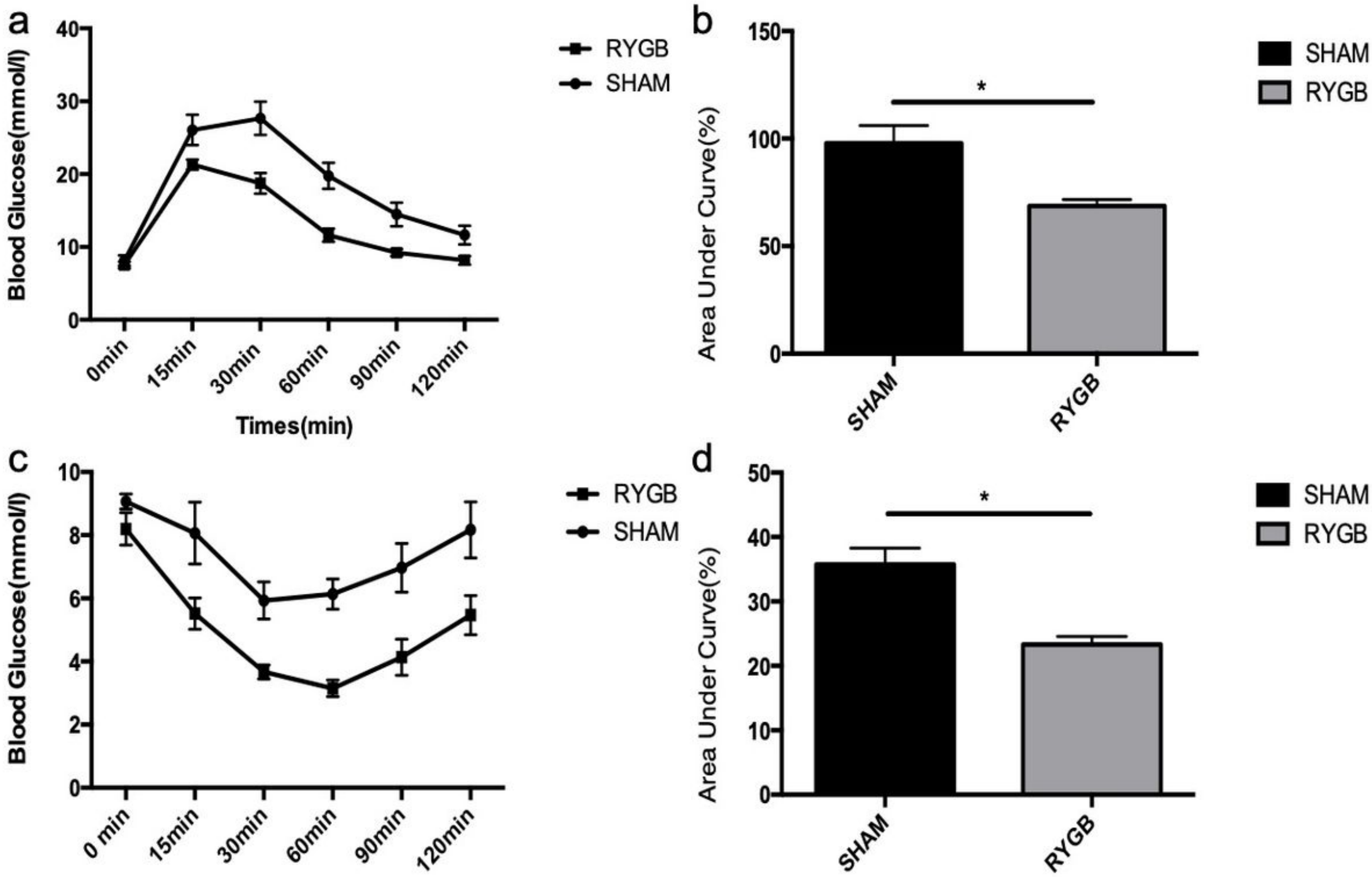

Figure 5

RYGB improves glucose metabolism compared to the Sham. a. Glucose excursion curves after IPGTT between the Sham and RYGB at postoperative week 12. b. The AUC for IPGTT between the Sham and RYGB at postoperative week 12. c. Glucose excursion curves after ITT between the Sham and RYGB at postoperative week 12. d. The AUC for ITT between the Sham and RYGB at postoperative week 12.Statistical differences were analyzed by one-way ANOVA followed by the student`s t-test. * $P<0.05$ vs Sham.