Effects of Bariatric Surgery on Change of Brown Adipocyte Tissue and Energy Metabolism in Obese Mice

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Abstract

Background Bariatric surgery is an effective treatment for obesity causing changes in energy expenditure. Brown adipose tissue (BAT) is an energy-related organ, and the potential effects of bariatric surgery are yet to be investigated. We aimed to study the effects of different bariatric surgeries on growth hormone/insulin-like growth factor-1 (GH/IGF-1) axis, brown adipocyte differentiation, and energy metabolism in obese mice and explore the underlying mechanisms.

Methods Mice were fed a high-fat diet for 12 weeks and subjected to different bariatric procedures: adjustable gastric band (AGB), sleeve gastrectomy (SG), Roux-en-Y gastric bypass (RYGB), and sham operation (SO). Pre- and postoperative weight, a metabolic index, content, and metabolic activity of BAT was recorded by micro-positron emission tomography/computed tomography (micro-PET/CT). Altered energy metabolism was estimated by metabolic cage technology. Serum GH/IGF-1 level and the brown adipose cell differentiation-related gene expression were estimated.

Results By postoperative week 4, serum GH and IGF-1 levels, as well as the content and metabolic activity of BAT increased postoperatively. The differentiation factors of the brown adipose cell were significantly stronger, energy consumption increased, and respiratory exchange frequency decreased postoperative. The effect was predominant in RYGB; SG demonstrated superior result to ABG. With weight regain 8-week postoperation, these parameters deteriorated in the operation groups, significantly in the GB group; the RYGB group seemed superior to the SG group.

Conclusions Bariatric surgery elevated the GH/IGF-1 levels and increased BAT volume and activity, meanwhile decreasing the respiratory exchange frequency. This may help us better understand the mechanisms of bariatric surgery.

Keywords Obesity · Brown adipose · Bariatric surgery · Growth hormone · Insulin-like growth factor-1

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Introduction

Obesity has apparently become a global burden pertaining serious public health concerns [1]. Presently, the most effective treatment for morbid obesity and the comorbidities is bariatric surgery [2]. However, the underlying mechanisms responsible for the beneficial effects of bariatric surgery have not yet been elucidated [3].

Energy expenditure was speculated to be related to the mechanism of weight loss after bariatric surgery [4, 5]; however, a consensus has not been reached [6]. Brown adipose tissue (BAT) was found to play a major role in regulating body energy metabolism and triglyceride clearance [7, 8]. BAT changes after bariatric surgery has not been clearly studied. Only a few controversial conclusions were drawn from limited human literature [9], as well as some animal model studies [10–12]. Thus, whether the changes in BAT function are associated with bariatric surgery is yet to be substantiated.

Other studies showed that obesity is correlated to growth hormone/insulin-like growth factor-1 (GH/IGF-1). GH was noticeably suppressed in patients with obesity [13].

Moreover, the IGF-1 level was lower than the average and was inversely associated with visceral fat content [14, 15]. Bariatric surgery may increase GH and IGF-1 levels [16], and the GH/IGF-1 axis plays a vital role in adipocyte differentiation and metabolism effects. GH also functions in the recruitment of preadipocytes, whereas IGF-1 functions in the regulation of adipocyte differentiation and proliferation [17]. GH/IGF-1 might induce the BAT differentiation, which leads to the increase of BAT volume and activity. Since BAT is closely correlated to energy consumption via a non-shivering heat production way, body weight loss seems to be achieved. However, the effects of GH/IGF-1 on BAT are not well understood.

The present study aims to assess whether bariatric surgery in obese mice would induce an increased activity of BAT using the positron emission tomography (PET) sensitive metabolic tracer ^{18}F-fluorodeoxyglucose (^{18}F-FDG). We also studied the effects of different bariatric surgery procedures on GH/IGF-1 axis and energy metabolism.

Materials and Methods

Animals

Six-week-old male C57BL/6 mice were purchased from SLAC Laboratory Animal Co., Ltd. (Shanghai, China) and housed under a 12 h light/dark cycle with free access to food and water at the Animal Center, Shanghai Ninth People's Hospital. All animals were maintained on a high-fat butter-based diet (D12492 (60% fat, 5.24 kcal/g); Research Diets, New Brunswick, NJ, USA) for 12 weeks. All the procedures were performed in accordance with Shanghai Jiaotong University guidelines for the use of animals, with the approval of The Animal Care and Utilization Committee of Shanghai Jiaotong University.

Preoperative Care

Sixty C57BL/6 mice were equally divided into four groups: adjustable gastric band (AGB group, $n = 15$), sleeve gastrectomy (SG group, $n = 15$), Roux-en-Y gastric bypass (RYGB group, $n = 15$), and sham operation (SO group, $n = 15$). The mice fasted for 24 h before surgery, and the surgical procedures were performed under anesthesia with 10% chloral hydrate intraperitoneal injection (0.3 mL/100 g).

Surgery

We performed all the surgeries mainly according to the surgical model protocol[18]. In brief, for the AGB group, the gastric band obtained from a piece of silicon catheter (4 mm wide, 0.5 mm thick) was positioned around the upper stomach, closed, and sutured to the abdominal wall. In the case of the SG group, the greater curvature from the antrum to the fundus including the lower part of the stomach and the forestomach with ligation of the short gastric vessels was removed. Then, the incision line in the stomach was closed using 5–0 absorbable continuous sutures. After laparotomy, the transection of the stomach in the RYGB group was performed, and a small gastric pouch at 5% of the total gastric volume was generated. The Roux limb and the biliopancreatic limb were 4–5 and 6–7 cm in length, respectively. The jejunal section was achieved 15 cm from the ligament of Treitz. Interrupted stitches with 8–0 nylon sutures were utilized for the gastrojejunal and jejunojejunal anastomoses. In the SO group, the mice underwent laparotomy, and their stomachs were elevated and returned to the abdominal cavity.

Postoperative Care

All animals were administered 5 mL prewarmed saline and placed in a warm environment for revival. Mice were allowed access to water for 12 h and liquid diet 48 h postoperatively. On the third postoperative day, a high-fat diet and water were administered similarly to the preoperative method.

Blood Glucose, Body Weight, and Food Intake

Fasting blood was withdrawn from the tail vein, and blood glucose levels were measured with the OneTouch Ultra glucometer (Lifescan, Johnson & Johnson, CA, USA). The body weight and food intake were estimated weekly at 10:00 a.m.

Biochemical Tests

Blood samples were withdrawn from the tail vein into tubes containing EDTA. Subsequent to centrifugation ($3000 \times g$) at 4 °C for 10 min, the serum was immediately separated and stored at − 80 °C until further analysis. The blood total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels were evaluated using commercial test kits (YANJIN Life Science Inc., Shanghai, China). Immunosorbent assay (ELISA) kits were used to assess the plasma levels of insulin, GH, IGF-1 (USCN Life Science Inc., Wuhan, China). Furthermore, to evaluate insulin resistance, the homeostasis model assessment ratio (HOMA-R) was calculated based on the following formula: HOMA-R = fasting glucose (mmol/L) × fasting insulin (μU/mL) / 22.5.

IPGTT

The mice fasted for 4 h before the IPGTT. The blood glucose levels were measured at 0, 30, 60, 90, and 120 min after

intraperitoneal glucose injection (2 g/kg body weight) at 4 weeks postoperative.

Assessment of BAT

BAT was assessed at weeks 4 and 18, as well as, postoperative weeks 4 and 8, respectively, using the Siemens Inveon Micro-PET/CT (Inven MM Platform, Siemens Preclinical Solutions, Knoxville, TN, USA). The PET acquisition time was 60 min. The CT X-ray source was used at a power of 80 kVp and 500 μA with an exposure time of 1100 ms. Briefly, the overnight fasted mice were administered insulin injection intraperitoneally (0.75 IU/kg). Eight to 10 min later, we injected 7.3 MBq ^{18}F-FDG via the tail vein. Then, the mice were anesthetized and imaged by PET/CT. The images were analyzed by Inveon Research Workplace (IRW) 3.0, and the stand uptake value (SUV) of ^{18}F-FDG was quantified.

Detection of Energy Metabolic Parameters

O_2-consumption, CO_2-expiration, calorie consumption, activity, and respiratory exchange rate (RER) during day and night were recorded by Columbus Oxymax Lab Animal Monitoring System (Oxymax, Columbus Instruments, OH, USA). Each group of mice was monitored at week 18 and postoperative weeks 4 and 8, respectively.

Detection of BAT-Related Gene Expression

Total RNA from BAT samples was extracted using TRIzol reagent (Invitrogen, MA, USA) and subjected to reverse transcription by the Revert Aid First Strand cDNA Synthesis Kit (Thermo, MA, USA). The relative gene expression was quantified by real-time quantitative real-time polymerase chain reaction (qRT-PCR) using SYBR Green Real-Time PCR Master Mix (Toyobo, Osaka, Japan). The reactions were performed using an ABI Step one plus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The relative gene expression was analyzed using the $2^{(-\Delta\Delta CT)}$ method and normalized relative to glyceraldehyde 3-phosphate dehydrogenase ($GAPDH$). The primer sequences are listed in Supplementary Table 1.

Statistical Analysis

All the statistical analyses were performed according to standard methods using the Statistical Package for Social Sciences software (version 20.0; SPSS Inc., Chicago, IL, USA). The data were expressed as mean ± standard deviation. One-way ANOVA with SNK q test was employed for multiple comparisons. $P < 0.05$ was considered statistically significant. Power analysis and sample size were calculated by the Power Analysis and Sample Size Software (version 15.0; NCSS

Inc., Kaysville, UT, USA) based on the differences and standard deviation between each group in our observed data. We set the probability of committing a type I error at $\alpha = 0.05$, type II error at $\beta = 0.2$, and the power test over 0.8 $(1 - \beta)$ was considered acceptable. The calculated sample size was 9 in each group. However, due to the high mortality rate of the surgeries, we set 15 mice in each group considering the unexpected death.
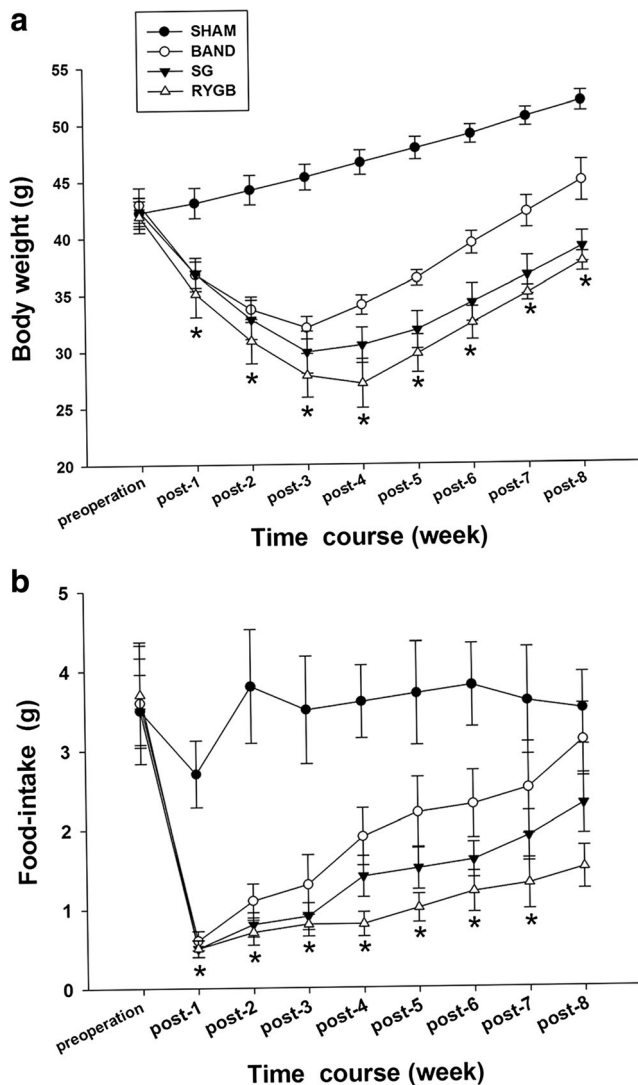
Results

General Conditions

The general surgery conditions are provided in Supplementary Table 2. In brief, two mice died in AGB group, four died in SG group, and five die in RYGB group due to different reasons, such as bleeding, gastric fistula, or stricture.

Changes in Body Weight and Food Intake

The AGB (BAND), SG, and RYGB groups reached their lowest level of body weight at postoperative week 2, week 4, and week 4, respectively. Eight weeks post operation, the mean body weights were 51.87 ± 0.9 g in the SO group, 44.86 ± 1.85 g in the AGB group, 39.02 ± 1.37 g in the SG group, and 37.70 ± 0.86 g in the RYGB group. Compared with the SO group, the mice of the BAND, SG, and RYGB groups showed a significant decrease in weight 8 weeks after the operation; however, a significant difference between the SG and RYGB groups was not observed (Fig. 1a). The food intake was measured weekly for 8 weeks post operation. The SG and RYGB groups showed significant decreases in weekly food intake as compared to the SO and BAND groups (Fig. 1b). However, both the body weight and the food intake in SG and RYGB groups remained statistically lower at week 8 postoperatively than the baseline.

Changes in Glucose Tolerance, Insulin Resistance, and Cholesterol Levels

Four weeks after the operation, the intraperitoneal glucose tolerance test results in the SG and RYGB groups were significantly lower than the SO and BAND groups (Fig. 2a), whereas no significant difference between the SG and RYGB groups was recorded. HOMA-IR data are shown in Fig. 2b. In comparison with the SO and BAND groups, HOMA-IR was significantly lower in the SG and RYGB groups. Similar results for the TC and LDL levels were observed in the operation groups, which were lower than the SO group. On the other hand, the HDL levels were remarkably higher in the operation groups (Fig. 3). At week 8 post operation, HOMA-IR in SG and RYGB was significantly lower

Fig. 1 Body weight and food intake. **a** Body weight of mice before and after surgery. Body weight in the surgery groups was lower than in the SHAM group; however, body weight of rats in the SG and RYGB groups was not significantly different. **b** Food intake of mice before and after surgery. The SG and RYGB groups showed significant decreases in weekly food intake as compared to the SHAM groups. *$P < 0.05$ vs. SHAM group

Fig. 2 IPGTT and HOMA-IR in mice. **a** IPGTT in mice 4 weeks after surgery. SG and RYGB groups were significantly lower than the SHAM and BAND groups. **b** HOMA-IR in mice before and after surgery. HOMA-IR was significantly lower in the SG and RYGB groups. *$P < 0.05$ vs. SHAM group

than the baseline, while the SO and BAND groups were significantly higher. Similar trends were seen in the TC and LDL-C groups, while the HDL-C showed a higher level in SG and RYGB groups.

Changes in BAT

The interscapular BAT was easily localized due to the butterfly-shaped nature of the organ (Fig. 4). The 3D image of BAT was captured and quantified by Inveon Research Workplace (IRW)3.0. The mean intensity of ^{18}F-FDG uptake was estimated, and the SUVs for each animal were calculated. Figure 5 shows that in the SO mice, both the volume of BAT
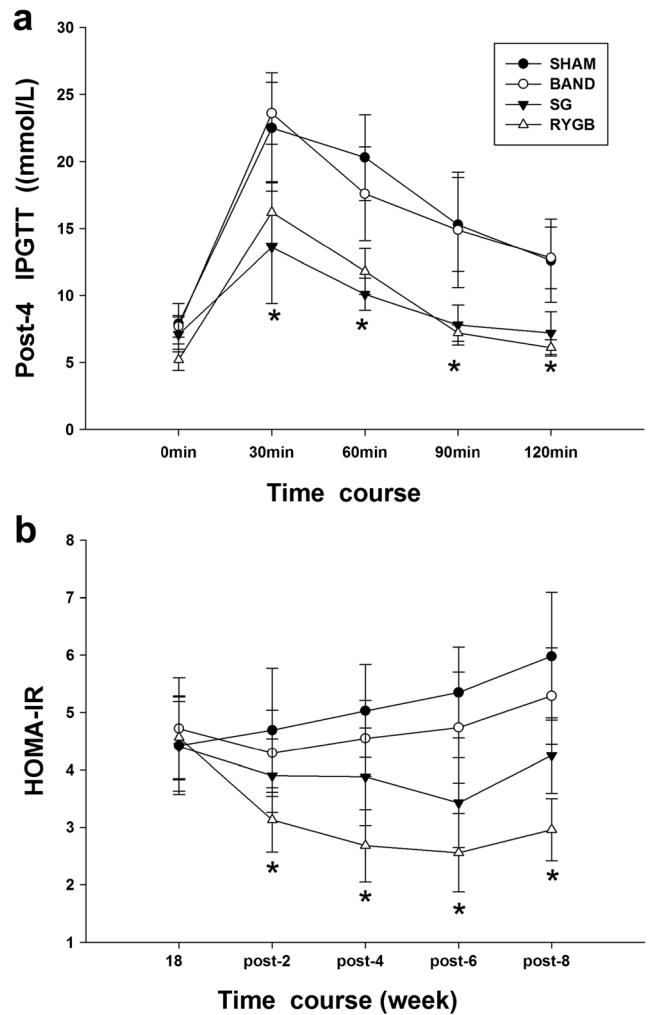
and the SUV continuously decreased 8 weeks postoperative while the operation groups showed an increase at week 4 postoperative; the RYGB was the most distinct. However, at postoperative week 8, the volume of BAT and SUV in all the groups showed a marked decrease. At week 8 post operation, both the BAT and the BAT SUV showed a remarkable increase in SG and RYGB groups compared to baseline, while the SO and BAND groups showed a further decrease.

Alterations in Energy Metabolism

Analysis of all the metabolic cage parameters was based on continuous high-frequency sampling after 7 days of adequate adaptation. The day and night data did not show any significant differences. The total calorie consumption, the volume of O_2 consumption, and CO_2 expiration for each animal in the SO group were significantly lower than the other groups.
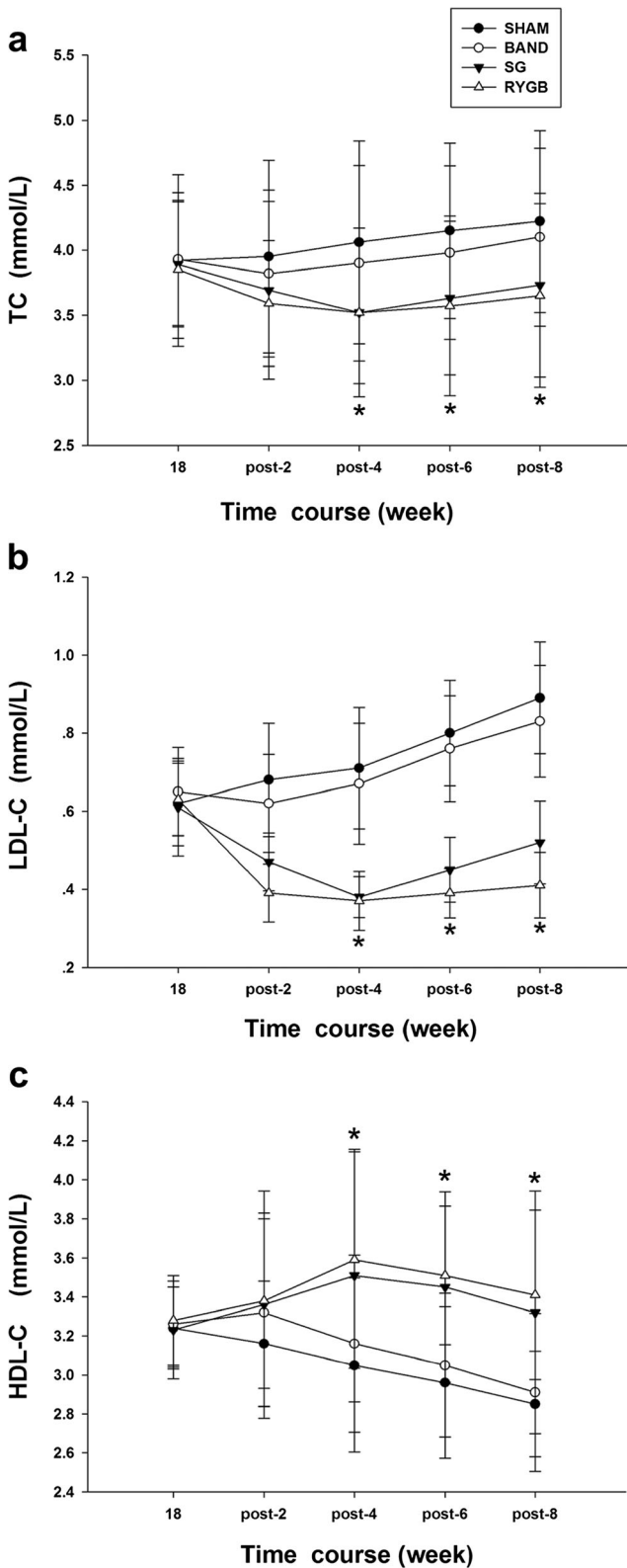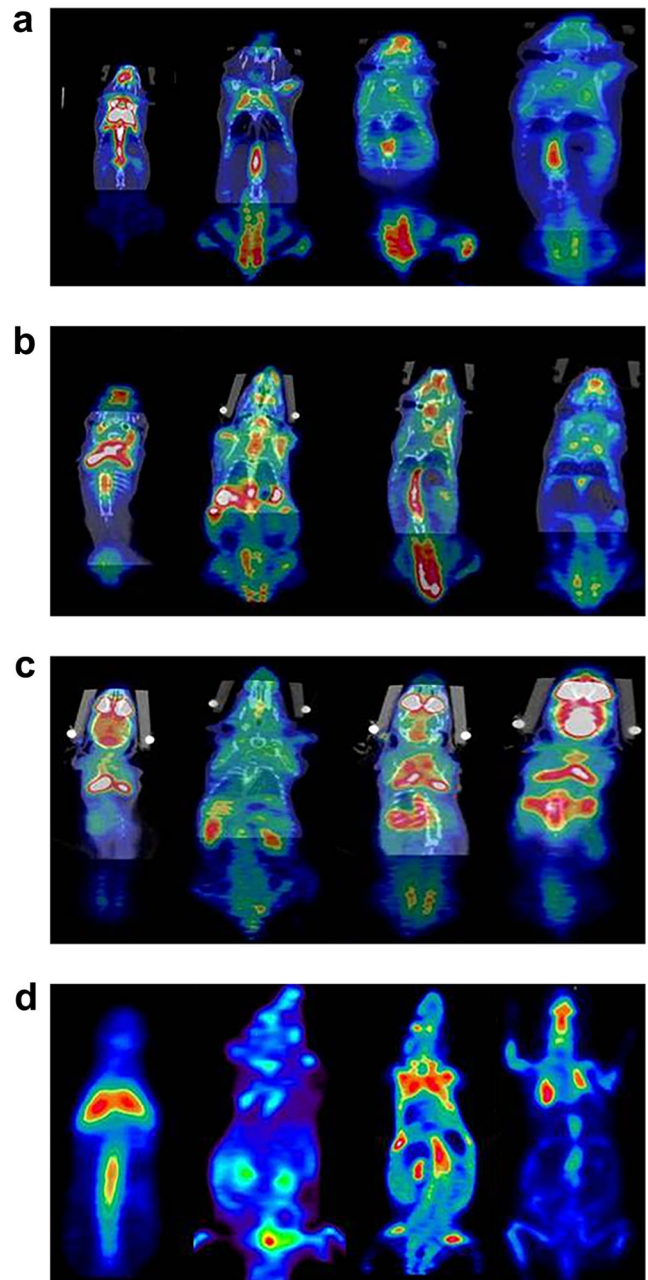
a

b

c

d

Fig. 4 BAT detected in mice before and after surgery. **a** SHAM group. **b** GB group. **c** SG group. **d** RYGB group. The red butterfly-shaped area in between the scapular indicated the location of BAT

Moreover, the results of the above parameters in the SG group were less than the RYGB group, but not significantly different. Although RER showed a decrease in the BAND, SG, and RYGB groups, the difference in the changes between the three groups was not remarkable. The activity in all groups displayed no statistical difference (Fig. 6). At week 8 post operation, SG and RYGB groups were higher in calorie and O_2 consumption comparing to the baseline, while SO and BAND groups stayed unchanged or lower. On the contrary, SG and RYGB groups were lower in RER and CO_2, while SO and BAND groups stayed unchanged or higher.

Fig. 3 Cholesterol levels in mice before and after surgery. **a** TC was significantly lower in the SG and RYGB groups. **b** LDL was also lower in SG and RYGB groups. **c** HDL was remarkably higher in the above two groups. *$P < 0.05$ vs. SHAM group
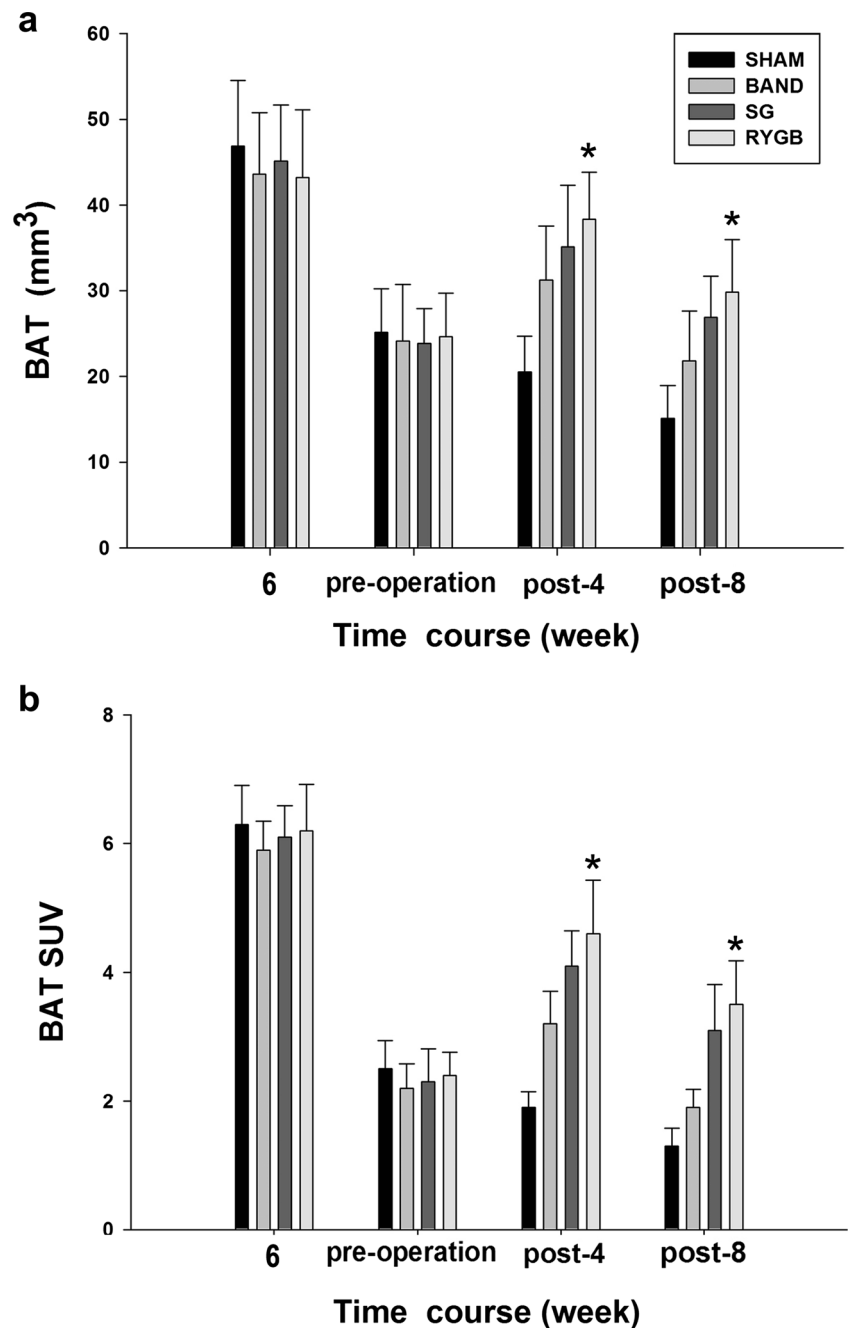
Fig. 5 BAT volume and SUV in mice before and after surgery. **a** BAT volume of the operation groups showed an increase at week 4 postoperative; the RYGB group was the most distinct. **b** Similar trends of BAT SUV were observed as BAT volume. *P < 0.05 vs. SHAM group

Changes in GH and IGF-1

With the increase of body weight, mice in all the groups showed a continuous decrease in GH and IGF-1 before surgery (Fig. 7). At postoperative week 8, GH in the SO group reached as low as 0.8 ng/mL, which was significantly lower than that at week 6 (6.3 ng/mL) and week 18 (2.2 ng/mL). A similar tendency was noted for IGF-1. In the operation groups, GH and IGF-1 showed a continuous increase in the first 4 weeks after surgery, as assessed from in-between the shoulder blades; however, from postoperative week 4 to week 8, the levels of GH and IGF-1 began to decrease with the body weight regain. However, all groups were statistically lower than the baseline in IGF-1 and GH at week 8 post operation.

Changes in BAT-Related Genes

In order to evaluate the differential influence of weight loss on changes in BAT-mediated thermogenesis, following various bariatric surgeries, the thermoregulatory genes in BAT were analyzed (Fig. 8). At postoperative week 4, PRD1-BF1-RIZ1 homologous domain-containing protein 16 (PRDM-16) and uncoupling protein-1 (UCP-1) messenger RNA (mRNA) expressions were higher in the surgery groups as compared to
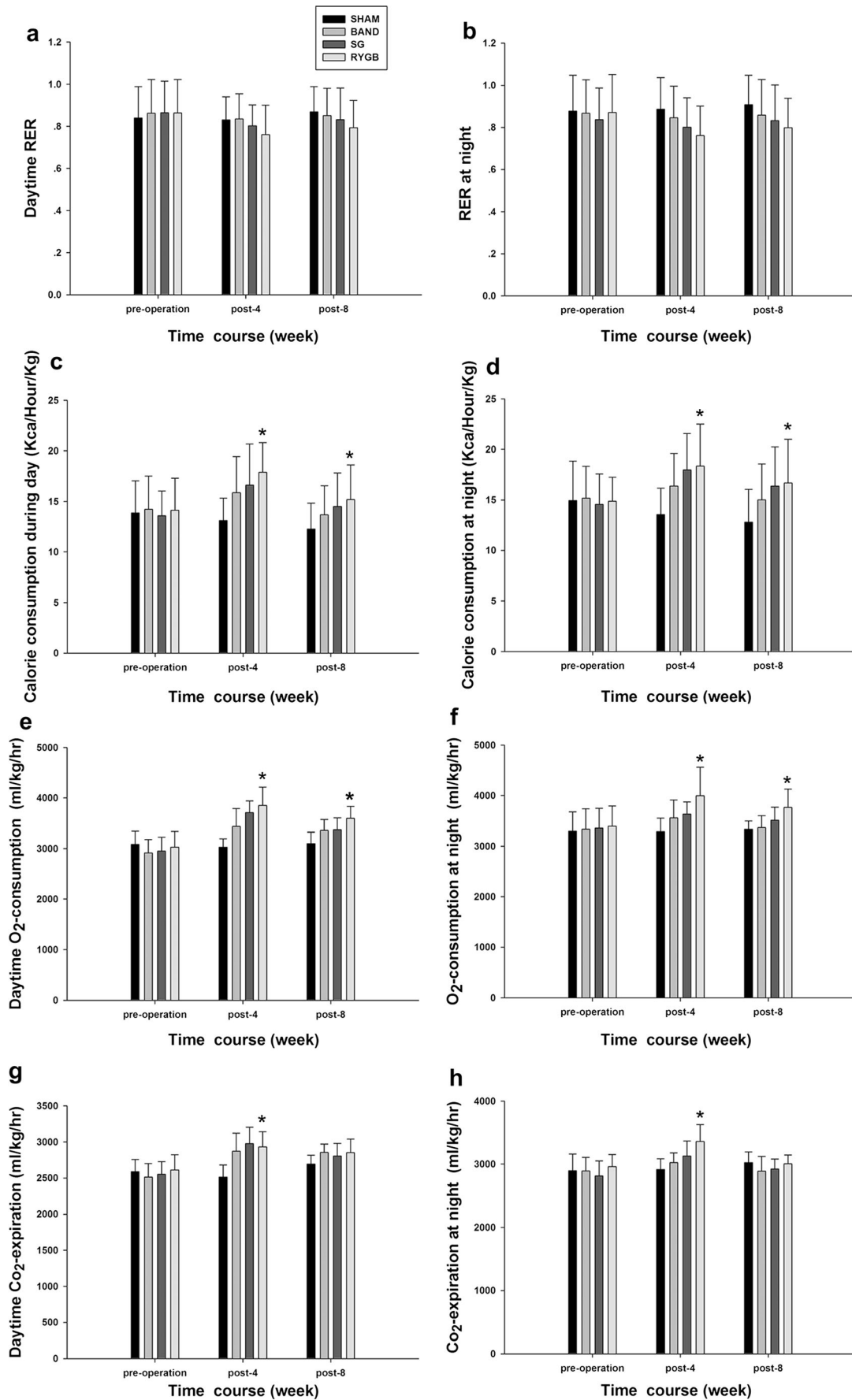
◀ **Fig. 6** Metabolic cage parameters before and after surgery. **a**, **b** RER during day and night, respectively. Although RER showed a decrease in the BAND, SG, and RYGB groups, the difference was not remarkable. **c**, **d** Calorie consumption during day and night, respectively. Calorie consumption in the SHAM group was significantly lower than the other groups. **e**, **f** O_2 consumption volume during day and night, respectively. Similar trends were observed as calorie consumption. **g**, **h** CO_2 expiration volume during day and night, respectively. Similar trends were observed as calorie consumption. $*P < 0.05$ vs. SHAM group

the SO group; the RYGB group exhibited the highest expression. However, at postoperative week 8, all groups showed a declining gene expression with the increase in the body weight. Besides, all groups except the SO group were significantly higher in PRDM-16 expression at week 8 post operation than the baseline; meanwhile, only the SG and RYGB groups were higher in the UCP-1 expression.
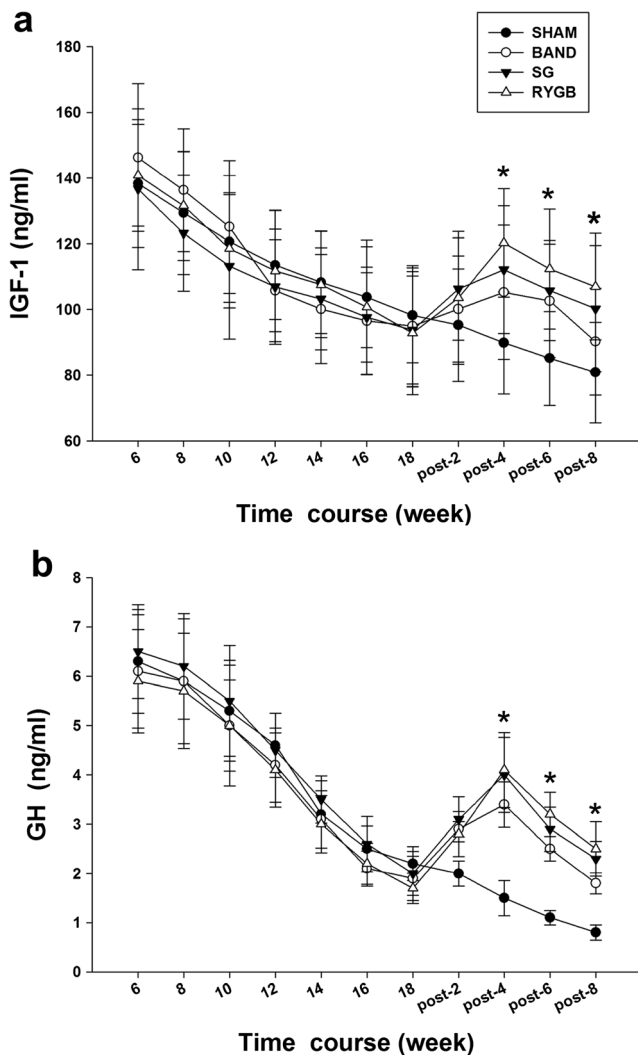

Fig. 8 Thermoregulatory gene expression in BAT before and after surgery. **a** PRDM-16 mRNA expressions were higher in the surgery groups as compared to the SO group; the RYGB group exhibited the highest expression. **b** UCP-1 mRNA expressions showed a similar trend as PRDM-16. $*P < 0.05$ vs. SHAM group

Fig. 7 IGF-1 and GH levels. **a** IGF-1 levels in mice before and after surgery. IGF-1 showed a continuous increase in the first 4 weeks after surgery. From postoperative week 4 to week 8, the levels began to decrease. **b** GH levels in mice before and after surgery. Similar trends were observed as IGF-1. $*P < 0.05$ vs. SHAM group
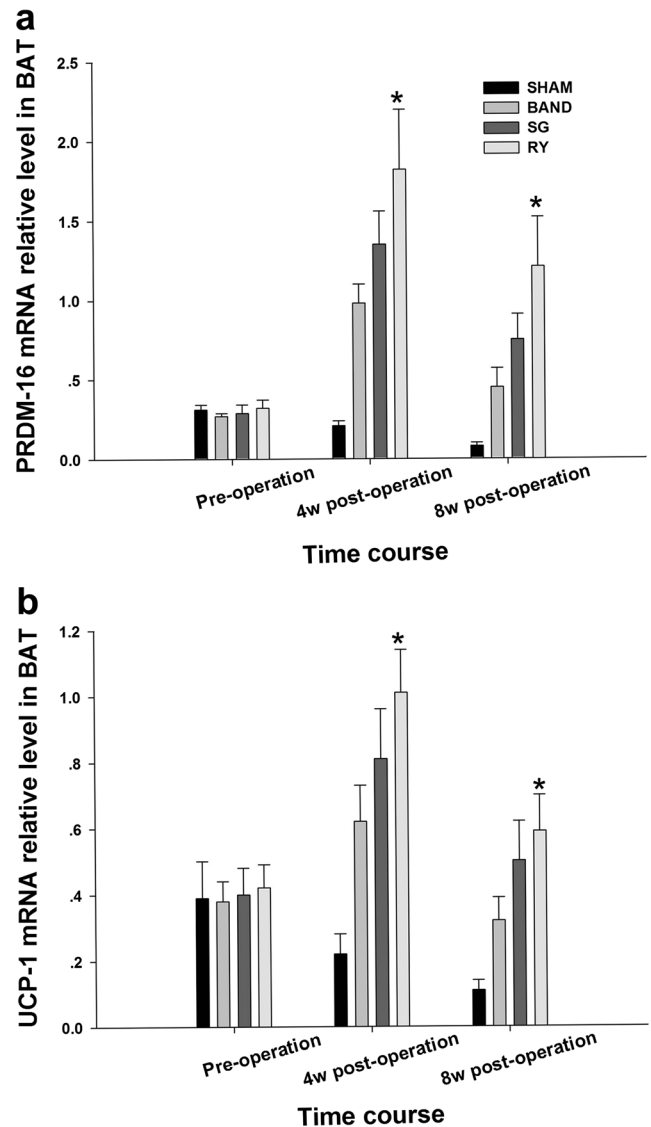
Discussion

Several animal studies have shown that bariatric surgery may increase energy expenditure [4, 5, 19], and BAT activation is speculated to be associated with energy metabolism. Therefore, we conducted PET/CT with the metabolic tracer ^{18}F-FDG to further assess whether BAT volume or activity is altered as a result of different bariatric surgeries. Our results showed that both ^{18}F-FDG uptake in BAT and energy consumption increased in the BAND, SG, and RYGB groups, as compared to the SO group. Interestingly, the bariatric surgery groups caused a significant elevation in GH and IGF-1 levels, which might regulate the adipocyte differentiation and functions [20, 21]; the lipid decline was observed with a similar

trend to that of BAT in our study. We further found significant differences in the UCP-1 and PRDM-16 mRNA expression in BAT between bariatric surgery groups and the SO group.

In addition, the bariatric surgery greatly reduced the food intake and body weight, which was in agreement with previous studies [5, 22]. However, in our experiment, this effect lasted only 2–4 weeks after surgery, when the BAND, SG, and RYGB groups reached their lowest levels. This phenomenon may be attributed to the inability of the mice to restrict their appetite as humans. Moreover, glucose, cholesterol, and HOMA-R were greatly improved after the bariatric surgery. At postoperative week 8, with the body weight regain, these metabolic parameters deteriorated again.

Both in vivo and in vitro studies have shown that GH and IGF-1 seemed to exert a marked influence in regulating the adipocyte proliferation and differentiation potentially via the PI-3K/AKT and MAPK/ERK signaling pathways [17, 21, 23, 24]. Also, IGF-1 may improve the UCP-1 expression in brown adipocytes [25]. GH and IGF-1 expressions were found to be suppressed in obese patients. GH has been demonstrated to play a crucial role in recruiting preadipocytes, whereas IGF-1 may induce proliferation and suppression of apoptosis in preadipocytes via the extracellular IGF-1 receptor α pathway [17, 21]. GH was also found to be directly involved in the energy balance regulation [24]. Other evidence has shown that the PI-3K/AKT signaling pathway was impaired in omental adipose tissue, and the IGF-1-stimulated DNA synthesis was significantly lower in omental when compared with the subcutaneous preadipocytes [23]. These limited number of studies provide some evidence that GH/IGF-1 may be involved in BAT differentiation. In the present study, GH and IGF-1 levels were suppressed before surgery and greatly improved after bariatric surgery, especially in the RYGB group, which is in line with the previous clinical reports [16]. Interestingly, we also observed a similar trend by intercapsular BAT volume and SUV. Therefore, we speculated that bariatric surgery might upregulate the GH/IGF-1 levels, which subsequently boosts the brown adipocyte differentiation, thereby raising the BAT volume. However, additional in vitro experiments are essential to substantiate these findings.

BAT is a novel target for anti-obesity treatments and is alleged to play an active role in clinical conditions of negative energy balance [26, 27]. In our study, a small volume of BAT was detected in mice before high-fat diet was initiated. Moreover, the relatively low UCP-1 expression was responsible for a non-shivering thermogenic effect [28]. After surgery, the SO group showed an unchanged energy expenditure per gram, most likely owing to the low UCP-1 expression in BAT. However, in the SG and RYGB groups, the micro-PET/CT revealed that the BAT activity and UCP-1 expression were both upregulated marginally, and the body energy expenditure increased correspondingly, which was recorded by the metabolic cage. Notably, the RYGB group displayed the best

effects among all the groups. Studies have shown that UCP-1 expression might be correlated to the upstream regulating gene *PRDM-16*, which was a BAT-activator [29]. We also found that the *UCP-1* and *PRDM-16* gene expressions are increased with weight loss after SG and RYGB. This may indicate that bariatric surgery induces BAT differentiation and increases its activity. However, between postoperative week 4 to week 8, both the UCP-1 and PRDM-16 gene expressions decreased with body weight regain. Furthermore, AKT and ERK signaling pathways were detected to be upregulated 4 weeks after RYGB. Hence, we postulated that IGF-1 and its downstream signaling pathways might be involved in the BAT differentiation.

Furthermore, although SO group showed a continuous increase in the body weight, the O_2 consumption, CO_2 expiration, energy expenditure, as well as RER and activity remained unaltered pre- and postoperatively, which might indicate that all the excessive energy was stored conserved as fat, thereby inducing obesity. In contrast, although the body weight in the bariatric surgery groups decreased at 4 weeks postoperative, the energy expenditure per gram increased, and the RER decreased. Owing to the respiratory quotient of fat 0.71, which is the lowest among the proteins, glucose, and fat, the fat metabolism ratio is presumed to greatly increase after bariatric surgery. This might cause an increased fat consumption and, thus, lead to weight loss. Moreover, since the mice activity in each group remained unchanged after surgery, we hypothesized that the weight loss was correlated with the increase in energy metabolism instead of increased activity. Since the thermogenic function of BAT allows high consumption of fatty acids because of the activity of UCP-1 in the internal mitochondrial membrane and dissipates energy in the form of heat rather than ATPs; this suggests that the more the BAT volume, the more heat it will create, leading to increased fat consumption [28, 30]. We found that mice in the RYGB group showed the highest BAT volume compared to the SG and BAND groups. Consecutively, the energy expenditure was also the highest, and the RER was the lowest; thus, the effects of RYGB may be correlated with the increase in BAT volume, which results in a high consumption of WAT oxidation. Cumulative evidence from both human studies and animal models strongly support that gastric bypass results in profound changes not only in the eating behavior but also in the energy expenditure [31–36]. However, only a few studies have demonstrated the relationship between the bariatric surgery and BAT; however, the contradictory results may be ascribed to various reasons, such as different animal models, feeding behaviors, and operations [4, 9–12]. In the current study, both the volume and activity of BAT increased after SG and RYGB, which might provide an insight into the mechanism underlying bariatric surgery.

Besides the GH/GH/IGF-1 way, the potential role of bile acids may also play a role in energy expenditure changes after

gastric bypass. Cumulative studies have shown that bile flow and plasma bile acids increased after gastric bypass and sleeve gastrectomy in human and animal models, which might lead to the novel treatments for obesity combined with type 2 diabetes [37, 38]. Further studies show that bile acid increases energy expenditure potentially through activation of brown adipose tissue [39, 40]. And this may provide a new insight into the study of the relationship between BAT and bariatric surgery.

Nevertheless, our study has some limitations. First, we did not establish the weight-matched group to explore the influence of body weight changes in the metabolic parameters in mice. On the other hand, since the bariatric surgeries are believed to alter the gut hormones, which might subsequently influence the central nervous system, the correlation of the gut hormones to our results is yet to be investigated. Besides, the fact that diet induced thermogenesis, which has been shown to increase after gastric bypass in rat models, has not been measured in our experiment [4], and theoretically, a diet-matched group should be established to exclude the influence of food intake on energy consumption. Moreover, blood glucose levels in our study were measured with the glucometer according to similar methods in other studies [41, 42], which might not be as precise as the whole blood glucose test, and we think it quite necessary to change this method in our later experiments.

In conclusion, we conducted an animal experiment to demonstrate that bariatric surgery may upregulate GH/IGF-1 levels and increase BAT volume and activity. Meanwhile, the frequency of the respiratory exchange was decreased, thus improving body energy consumption. Weight loss might be correlated to the above changes to some extent. This effect was most evident in the RYGB group. However, the effects were transient and not long lasting. Further in vitro experiments are essential in order to verify this effect.

Compliance with Ethical Standards

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

Statement of Animal Rights   All the procedures were performed in accordance with Shanghai Jiaotong University guidelines for the use of animals, with the approval of The Animal Care and Utilization Committee of Shanghai Jiaotong University. All animals were housed under standard conditions with free access to water and food.

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