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Body composition, food intake, and energy expenditure in a murine model of Roux-en-Y gastric bypass surgery

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Abstract

Background—The mechanisms by which Roux-en-Y gastric bypass surgery (RYGB) so effectively lowers body weight and improves glycemic control are not well understood and murine models are essential for identifying the crucial signaling pathways involved.

Aims—To characterize the time course of RYGB on body weight, body composition, food intake, and energy expenditure in diet-induced obese mice, and establish a tissue bank for global “omics” or targeted biochemical and structural analyses.

Methods—High-fat diet-induced obese mice were subjected to RYGB using an improved surgical technique with a small gastric pouch. The effects on body weight, body composition, food intake, and energy expenditure were compared to sham surgery, high-fat diet-restricted weight-matched controls, and never obese chow-fed controls.

Results—Without mortality or complications, RYGB surgery in high-fat diet-induced obese mice gradually decreased body weight to a plateau that was more or less sustained for up to 12 weeks (33 g, −18%, $p<0.01$) and significantly lower compared with sham-operated mice (51 g, +25%, $p<0.01$), but higher (+18%, $p<0.01$) than age-matched, chow-fed control mice (27 g). Energy intake after RYGB was significantly suppressed compared to sham only for the first 10 days, but significantly higher compared to weight-matched mice. Energy expenditure after RYGB was higher throughout the study compared with weight-matched, but not sham animals.

Conclusions—RYGB surgery in diet-induced obese mice results in similar body weight and body composition changes as observed in humans, but in contrast to humans, this is achieved mainly through increased energy expenditure rather than decreased food intake.

Keywords

Bariatric surgery; obesity; high-fat diet; calorie restriction; energy balance; mouse

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Introduction

Rodent models for bariatric surgery are important tools to reveal the molecular mechanisms driving the remarkably beneficial effects of these surgeries on obesity and its comorbid conditions. A number of rat models [1-6] have successfully characterized many changes in structure, physiology and behavior that point to potential mechanisms, without unequivocally proving their sufficiency and necessity. It became clear that mouse models with the ability to selectively manipulate specific signaling pathways would be indispensable for such rigorous hypothesis testing. Therefore, mouse models for Roux-en-Y gastric bypass (RYGB) [7-12] and vertical sleeve gastrectomy (VSG) [13] have been developed just in the last few years. However, progress with mouse models for RYGB has been somewhat hindered by high mortality and complication rates in some of these models. Because weight loss is one of the major outcomes, it is essential to minimize non-specific weight loss induced by complications of the surgery. This is particularly important in the mouse, which has relatively little stored energy.

We have developed a mouse model with a surgical technique that largely prevents excessive non-specific early weight loss [10, 14]. Initial weight loss is more moderate but is nonetheless sustained over many weeks, which is in contrast to the more extreme weight loss in other models, where it is typically followed by accelerated or “catchup” weight regain later [7, 15]. Although weight regain does occur in up to 30% of human RYGB patients, body weight stays relatively stable at the lower level over many years in most patients [16-18].

The purpose of the present study was twofold. First, to better characterize changes in body weight, body composition, energy intake, and energy expenditure in our mouse RYGB model with high-fat diet-induced obese mice, and to contrast it with two non-surgical control groups with either equal weight loss induced by high-fat diet caloric restriction or with normal never obese chow-fed animals. Second, we aimed to harvest a variety of tissues at termination to create a tissue bank that will be useful in future studies investigating molecular and structural changes. In this study, we intentionally avoided any pharmacological interventions and invasive techniques that could have non-specifically affected body weight and composition, energy intake and expenditure, as well as the tissues harvested.

Materials and Methods

Animals

Male diet-induced obese C57BL6J mice were purchased from Jackson Laboratories at the age of 18 weeks. Upon arrival at the Pennington Biomedical Research Center, they were continued on the same high-fat diet (60% fat, Research Diets D12492) and also given access to regular chow (13% fat; Purina 5001) in shoebox group cages. Ten days before surgery, mice were single housed on grid floors for the measurement of food intake. Animals were kept under standard laboratory conditions with a room temperature of 21-23°C and a 12 h light-dark cycle (lights on at 07:00), with exceptions detailed below.

Animal Care

Animal care and experimentation was approved by the Pennington Biomedical Research Center Institutional Animal Care and Use Committee and strictly followed rules and guidelines provided by the American Physiological Society and NIH.

Experimental Overview

The study included 42 mice and was conducted in 2 separate cohorts to accommodate all animals in the 24 available metabolic chambers. After 12-14 weeks on high-fat diet, at an age of 18-20 weeks, DIO mice were stratified into 3 treatment groups each with matching average body weight and fat mass [RYGB, n = 14; sham-surgery (sham), n = 13; no-surgery weight-matched controls (WM), n = 9]. All 3 groups were fed a two-choice diet consisting of high-fat and regular chow pellets for the duration of the study, except for the time in the metabolic chambers (days 14-25 and 55-70 after surgery) during which they received only high-fat diet. The two-choice high-fat/regular chow diet was chosen because we found that RYGB increases preference for low-fat regular chow in both rats and mice [5], which seems to contribute to faster recovery after surgery. An additional, age-matched control group (n = 6) was fed only regular chow and was not subjected to any surgery. Except for the time in the metabolic chambers, no other tests and invasive procedures were conducted that could perturb normal body weight regulation. At the end of the observation period, blood plasma and a large number of tissues were harvested for biochemical and histological analyses to be reported in future communications.

Surgery

A detailed description of our mouse RYGB surgery can be found elsewhere [10, 14]. Briefly, overnight fasted mice were anesthetized with inhalation anesthesia (1-4% isoflurane) and midline laparotomy was performed. Then, 5-6 cm long Roux, 6-7 cm long biliopancreatic, and 15-18 cm long common limbs were formed by transecting the jejunum near the ligament of Treitz and the stomach near the cardia, and anastomosing the proximal end of the cut jejunum to the small (< 5% of stomach volume) gastric pouch, and the distal end to the lower jejunum. Great care was taken to preserve gastric vessels and nerves near the cardia. Non-continuous sutures with 11-0 nylon sutures were used for the gastro-jejunal and jejuno-jejunal anastomoses. Animals promptly emerged from anesthesia and were returned to their home cage, which was placed on a regulated heating blanket for the first night. Additional analgesia was given as necessary, and all animals were given access to unlimited amounts of two-choice diet the next morning (day 1). Sham surgery consisted of midline laparotomy and gentle mobilization of stomach and intestines.

Measurement of food intake and choice

Food intake was measured to the nearest 0.1 g daily between 10:00 and 12:00 h, throughout the duration of the study, by subtracting the amount of high-fat and chow diet recovered from the amount weighed in, corrected for spillage found under the grid floor (distinguishable by the blue color of high-fat crumbs). Two- or three-day averages were measured over weekends after the first two weeks post-surgery. Metabolizable energy was calculated as 5.4 kcal/g for high-fat and 3.02 kcal/g for chow. Chow preference was

calculated as the percentage of total caloric intake obtained from chow diet. Animals of the WM group were given ~93% of total restricted calories as high-fat and ~7% as regular chow, based on the observed preference of RYGB mice.

Measurement of body weight and body composition

Body weight was measured daily between 10:00 and 12:00 h to the nearest 0.1 g throughout the duration of the study, except for some weekends. Body composition was measured to the nearest 0.1 g with whole body MRI (Minispec LF 50, Brucker, The Woodlands, TX) on average 1 week before, and 2, 4, 8, and 12 weeks (±5 days) after surgery. The adiposity index was calculated by dividing fat mass by lean mass.

Measurement of energy expenditure in the metabolic cages

VO_2, VCO_2, RER, locomotor activity, food and water intake were measured in metabolic chambers (Phenomaster/Lab Master, TSE Systems, Germany) at the end of the rapid weight loss period (12-25 days after surgery) and during the stable weight/weight regain phase (56-70 days after surgery). After 2-4 days with training lids on their home cages to facilitate learning to eat from hanging wire baskets, mice were transferred to special metabolic chambers. For both measurement periods, mice were kept at 23°C, and after an adaptation period of 3 days, all parameters were sampled at a frequency of 40 min and averaged over 4 consecutive days and nights. For the 8-9 week period, after collecting data at 23°C, chamber temperature was increased to 29°C (near thermoneutrality) to minimize the potential masking effect of cold-induced thermogenesis.

Energy expenditure in kcal was calculated on the basis of VO_2 and VCO_2 by company supplied software and expressed a) per mouse, b) per kg body weight, and c) per kg lean body mass. For lean mass the average of measurements before and after the metabolic chamber period was used. Resting energy expenditure was defined as the 10% time spent with the lowest VO_2 consumption. In addition, linear regression analysis was performed between energy expenditure per mouse and lean body mass.

Measurement of plasma hormones and glucose

Trunk blood obtained at euthanasia in overnight food deprived mice was collected in tubes containing 83.5 μl EDTA (Sigma, St. Louis, MO) and 15 μl of a protease inhibitor cocktail (5 μl of each of the following: Protease inhibitor, Sigma, St. Louis, MO; DPP-IV inhibitor, EMD Millipore, St. Charles, MO; Pefabloc SC, Roche, Indianapolis, IN) and immediately centrifuged at 4° C and 3000 RPM for 10 minutes to separate the plasma from the whole blood. Plasma aliquots were frozen in liquid nitrogen, and stored at −80° C prior to processing. Plasma was subjected to ELISA for measurement of insulin and leptin concentrations (MMHMAG-44K Milliplex map mouse metabolic hormone magnetic bead panel – metabolism multiplex assay, EMD Millipore, St. Charles, MO). One drop of trunk blood was used for glucose measurement (Onetouch Ultra Glucometer, LifeScan INC, Milpitas, CA; Onetouch Ultra Strips, LifeScan INC, Milpitas, CA).

Statistical analysis

One-way ANOVA was used to analyze body weight change, cumulative food intake, feed efficiency, chow preference, energy expenditure, RER, locomotor activity, and plasma hormone assays. Two-way repeated measures ANOVA was used to analyze body weight, body composition, and food intake. All analyses included Bonferroni-corrected multiple comparison follow-ups. Significance was accepted at the $p < 0.05$ level. All data was expressed as mean ± SEM.

Results

Mortality and complications

Most importantly, there was zero mortality and no complications in this cohort of 14 RYGB mice. Mice started to eat solid high-fat and chow diet one day after surgery and returned to pre-surgical food intake levels after only about 10 days. All mice appeared completely healthy and required no medical treatment or special diets throughout the duration of the study.

Body weight

The 14-week exposure to high-fat diet increased body weight to about 41 g, which is almost 60% higher than the 26 g of chow-fed, age-matched control mice (Fig. 1A). RYGB resulted in rapid weight loss which reached a nadir at ~32 g (−22%) around 3 weeks after surgery (Fig. 1A,B). For the remainder of the 12 week observation period RYGB mice regained a moderate amount of ~4% of body weight (Fig. 1C). Body weight of RYGB mice remained significantly above the chow-fed controls throughout the observation period. Although calorie restriction-induced weight loss in the WM group was initially slower than weight loss in RYGB mice, the body weight curves were almost identical after 3 weeks. Sham surgery resulted in a transient loss of body weight, followed by a continued increase to ~50 g at 12 weeks.

Body composition

Similar to total body mass, fat mass rapidly and significantly decreased after RYGB and remained relatively stable at about 5-6 g throughout the remainder of the observation period. However, fat mass remained almost twice as high as in chow-fed mice (Fig. 2A). In contrast, after an initial non-significant drop at 2 weeks, fat mass continued to rise significantly in sham mice to reach ~20 g at the end of the observation period.

Lean mass fell only transiently and non-significantly after RYGB and completely recovered at the end of the observation period (Fig. 2B). In WM mice, lean mass decreased more substantially than in RYGB mice and was no longer significantly different from chow-fed mice at 8 and 12 weeks.

The adiposity index largely reflected changes in fat mass. However adiposity index decreased less in WM compared with RYGB mice (Fig. 2C).

Food intake, feeding efficiency and food choice

As expected, energy intake of high-fat fed mice before surgery was significantly higher than in chow-fed controls (Fig. 3A). This increase of +36% when calculated on a per animal basis, shrunk to +21% when calculated on a per gram lean body weight basis and reversed to a decrease of −12% when calculated on a per gram body weight basis (data not shown).

After an initially significant suppression of total food intake, RYGB mice returned to the intake of sham animals and even slightly (+5%), but not significantly exceeded intake of sham animals during days 26-76, in spite of their marked body weight loss (Fig. 3B). Importantly, food restricted WM mice required significantly less food (~35% less compared with RYGB mice) to achieve weight-matching.

Calculation of feeding efficiency over 50 days (days 26-76) of relative weight stability or slight weight regain in RYGB and WM mice showed that high-fat fed sham mice where about 5-fold more efficient than chow controls, and that both RYGB and WM completely reversed this increased efficiency despite eating mostly high-fat diet (Fig. 3C). In fact, RYGB further reduced feed efficiency significantly below chow and WM mice.

As expected, if given a choice of nutritionally complete high and low fat diets, mice overwhelmingly preferred the high-fat, with a preference for chow of less than 5% before treatment (Fig. 3D). As we have seen earlier in rats [5], RYGB slightly but significantly increased preference for chow to about 8% for the period before the second metabolic chamber experiment, while sham mice continued with a lower chow preference of around 5%.

Energy expenditure, RER, and locomotor activity

Analysis of all metabolic chamber parameters was based on 4 days of continuous high-frequency sampling after adequate adaptation. Total energy expenditure per animal was significantly lower after RYGB compared with sham surgery at both time points and at both chamber temperatures (Fig. 4A) and this pattern was largely preserved when normalized to lean body mass (Fig. 4C). However, this decrease in energy expenditure of RYGB mice turned into a significant increase compared to sham mice when normalized to total body mass (Fig. 4B). Importantly, compared to WM mice at 8 weeks with identical body weight and very similar body composition, RYGB still significantly increased energy expenditure, but only when normalized to total body weight (Fig. 4B). However, the increase was relatively small amounting to ~11%.

To further unmask potential surgery-induced changes in energy expenditure from cold-induced thermogenesis that occurs at 23°C, we measured energy expenditure at 29°C, which is near thermoneutrality. Under these conditions, compared with WM mice, increased energy expenditure of RYGB mice was more pronounced and highly significant, no matter how it was normalized. Separate analysis of the 12 h light and dark periods showed that the increased energy expenditure effects of RYGB occurred mainly during the dark period, with RYGB mice expending up to 35% more energy compared with WM mice (data not shown). For additional direct comparisons of energy expenditure between RYGB and WM mice normalized differently, percent differences are listed in Table 1.

The absence of any significant differences in locomotor activity between the 3 high-fat fed groups of mice (Fig. 5B) prompted us to analyze resting energy expenditure, operationally defined here as the 10% time spent with the lowest VO_2 consumption. Similar to total energy expenditure, resting energy expenditure of RYGB mice was significantly higher compared to weight-matched controls at 2 and 8 weeks after surgery (Fig. 4D, Table 1). Eight weeks after surgery, at thermoneutrality, RYGB mice spent 20% more energy than WM while resting and 21% more than chow-fed controls.

Covariate analysis of lean body mass and energy expenditure at thermoneutrality showed significantly different slopes for RYGB, weight-matched, and sham-operated mice, confirming the higher energy expenditure of RYGB vs. weight-matched controls (Fig. 4E). Covariate regression analysis using total body mass instead of lean mass showed a similar outcome (not shown).

Finally, there was a small but significant effect of RYGB on the respiratory exchange rate at 8, but not 2 weeks after surgery (Fig. 5A). RYGB significantly increased RER compared with both WM and Sham surgery at 8 weeks and with both chamber temperatures. As expected, the RER of chow-fed controls was significantly higher compared with all high-fat fed groups at both time points and temperature conditions.

Fasting plasma leptin, insulin, glucose, and HOMA-IR

At termination and as expected, fasting plasma levels of leptin and insulin were roughly 10-fold higher in sham-operated, high-fat fed, obese mice compared to chow controls (Fig. 6). In addition, fasting plasma glucose levels and HOMA-IR were significantly increased. Both RYGB and WM almost completely normalized all these parameters, which were no longer significantly different from chow controls, with the exception of fasting plasma glucose, 2 weeks after RYGB.

Discussion

Only a handful of studies have been reported on RYGB in the mouse, with varying designs and outcomes regarding body weight, food intake and energy expenditure [7-12]. Therefore, we wanted to better characterize the effects of our own model of murine RYGB and also use these mice for a comprehensive tissue harvest for future biochemical and histological analyses. For this latter objective, we refrained from any pharmacological treatments and invasive procedures such as frequent blood sampling, which could influence both food intake and body weight as well as biochemistry and structure of sampled tissues.

Most importantly, we demonstrate here that RYGB in the mouse does not necessarily have to cause mortality or serious complications that resulted in the use of special diets and large initial weight loss in other models. Therefore, our model better reflects the net effects of RYGB surgery, rather than the non-specific effects of surgical trauma and complications that can result in abnormally low energy intake, exaggerated initial weight loss, and even death.

In these “healthy” RYGB mice, food intake reduction only lasts for about 10 days and body weight loss is gradual to a nadir of about −22% at 2-3 weeks and then stabilizes or

transitions into a moderate weight regain. This is unlike the body weight curves of some mouse RYGB and VSG models, in which there is excessive early body weight loss due to abnormally low food intake up to about 10 days and then a recovery of lost body weight during the next 5-8 weeks, when typically most of the critical measures of energy expenditure and glucose homeostasis are made [7, 15, 19]. Our findings in terms of weight loss, body composition changes, and food intake are very similar to those reported by Liou et al. [9], which also reported a relatively low mortality of 20%. With our special surgical technique, we were able to reduce mortality even further, with all of the 14 RYGB mice surviving without any complications, in spite of eating solid high-fat food starting on the day after surgery.

Also, in our model, body weight and fat mass stay significantly lower compared to pre-surgical levels for at least 12 weeks, in contrast to some mouse models of VSG, where body weight typically reaches or even surpasses pre-surgical levels after 4-12 weeks [19, 20]. Our chow-fed control group clearly demonstrates that this is not an issue of continued growth, as they maintained a relatively stable body weight throughout the study under the single housing conditions employed. It is likely that in models with exaggerated initial weight loss, significant amounts of lean mass are lost, which could be the trigger for rebound weight gain. The mouse is very different from humans in this respect, as it has only a relatively small amount of stored energy to shed - a 24 h fast in a mouse is equivalent to a much longer fast in humans.

Another important finding confirms earlier reports that in spite of the rapid return of energy intake to pre-surgical levels, RYGB mice remain at a significantly lower body weight and fat mass level [7, 21]. This is very different from RYGB in humans, where energy intake remains suppressed up to 10 years after surgery [22-24] and where reduced energy intake appears to be the main driver of sustained weight loss [25]. In assessing where energy is lost in the mouse after RYGB, increased energy expenditure, increased fecal energy loss, or a combination thereof are the candidate mechanisms. Unfortunately, we did not assess fecal energy loss in the present study, but based on the findings by Nestoridi et al. [7] and Liou et al. [9] using the same high-fat diet, the fecal energy loss is about 1-2 kcal/day higher in RYGB vs. sham mice. Similarly, in humans, fecal energy loss accounted for no more than about 10% of ingested energy [26].

The present results confirm earlier reports of increased metabolism and energy expenditure after RYGB in mice [7]. However, interpretation of energy expenditure data is complicated by lack of consistency regarding normalization of raw data to body mass and composition. Correct normalization is particularly important when body mass and composition is vastly different between the groups compared, as is the case with bariatric surgery cohorts. Here we show that at both 2-3 weeks and 8-9 weeks after RYGB surgery, energy expenditure is significantly higher compared to mice weight matched through caloric restriction. This relative increase is highest at 8-9 weeks after surgery under conditions of thermoneutrality, and because body weight and composition are similar, little affected by normalization. Thus, the 15.3% higher energy expenditure per gram of lean body mass of RYGB mice compared with WM mice is both statistically and biologically significant and is likely responsible for the sustained lower body weight in spite of normal or slightly increased food intake.

However, in contrast to earlier reports [7, 8, 21], energy expenditure of RYGB mice was significantly lower compared with sham-operated mice at either time point or temperature if expressed on a per animal basis or normalized to lean body mass. Only if normalized to total body mass, which is incorrect given the highly significant differences in body composition, is energy expenditure greater in RYGB vs. sham mice.

Increased energy expenditure rather than decreased food intake after RYGB in mice appears to be almost opposite what is found in human RYGB patients. Energy expenditure more often decreased rather than increased, and energy intake clearly decreased in clinical studies [25, 27, 28]. Part of this inconsistency in the human literature likely has to do with inadequate measuring and normalization techniques for both parameters, but there may also be some underlying species difference. Again, the large differences in fuel storage capacity may be crucial for such different responses.

Unexpectedly, the respiratory exchange rate of RYGB mice was also slightly, but consistently higher compared with WM mice at both 2-3 and 8-9 weeks and compared with sham mice at 8-9 weeks after surgery, suggesting a slight shift towards lower fat oxidation. Because only high-fat diet was available in the metabolic cages, this difference cannot be explained by differences in the diet. Given the continued fat mass loss of RYGB mice at this time, one would have predicted a lower, not higher RER, particularly compared with sham mice that are in a lipid storage mode. It will be interesting to find the origin of these unexpected changes in substrate metabolism.

Finally, fasting plasma levels of glucose, insulin, and leptin at the end of the study clearly demonstrate the detrimental effects of high-fat diet and the almost complete reversal by both RYGB and caloric restriction-induced weight loss. Similar effects have been demonstrated in obese humans after RYGB or after a short-term very low calorie diet matching intake of RYGB patients [29, 30]. Therefore, our mouse model will be useful for future mechanistic studies in which specific signaling pathways are genetically altered.

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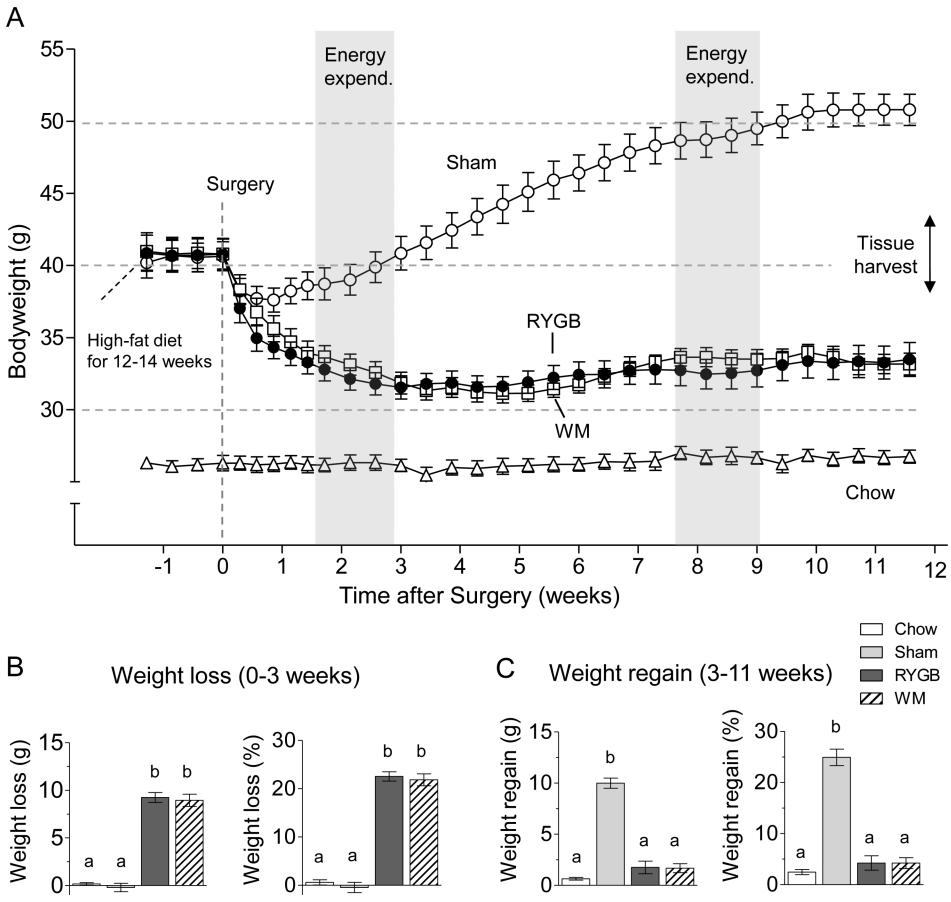

Fig. 1.

Effect of Roux-en-Y gastric bypass surgery (RYGB, n = 14) on body weight in high-fat diet-induced obese mice. Controls included sham surgery (Sham, n = 13), high-fat caloric restriction to match the body weight of RYGB (WM, n = 9), and never obese, regular chow-fed mice (Chow, n = 6). **A:** Absolute body weight of the 4 groups over the course of the study, with periods of measurements in metabolic chambers indicated by gray bars. **B:** Weight loss at 3 weeks after surgery expressed in grams and percent. **C:** Weight regain from week 3-11 expressed in grams and percent. Bars that do not share the same letters are significantly ($p < 0.05$) different from each other (based on ANOVA, followed by Bonferroni-corrected multiple comparisons).

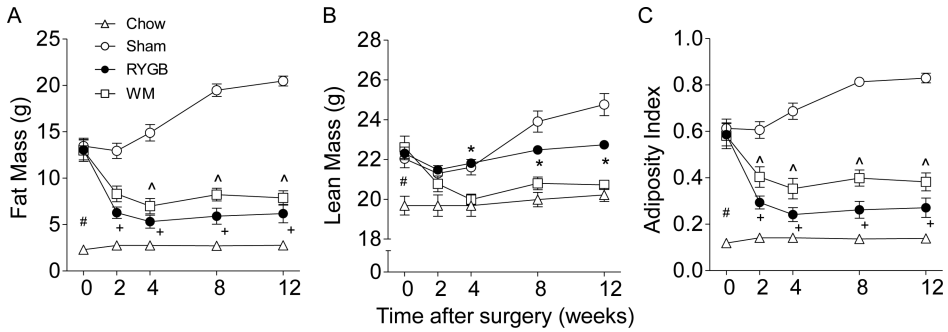

Fig. 2.

Effect of Roux-en-Y gastric bypass surgery (RYGB, filled circles, n = 14) on body composition in high-fat diet-induced obese mice before and 2, 4, 8, and 12 weeks after surgery. Controls included sham surgery (Sham, open circles, n = 13), high-fat caloric restriction to match the body weight of RYGB (WM, open squares, n = 9), and never obese, regular chow-fed mice (Chow, open triangles, n = 6). **A, B:** Fat mass and lean mass as determined by whole body NMR spectroscopy. **C:** Adiposity index calculated as the ratio of fat mass to lean mass. # $p<0.05$, Chow vs. all other groups; * $p<0.05$, RYGB vs. WM and Chow; + $p<0.05$, RYGB vs. Sham; ^ $p< 0.05$, WM vs. Chow and Sham (based on repeated measures ANOVA followed by Bonferroni-corrected multiple comparisons).

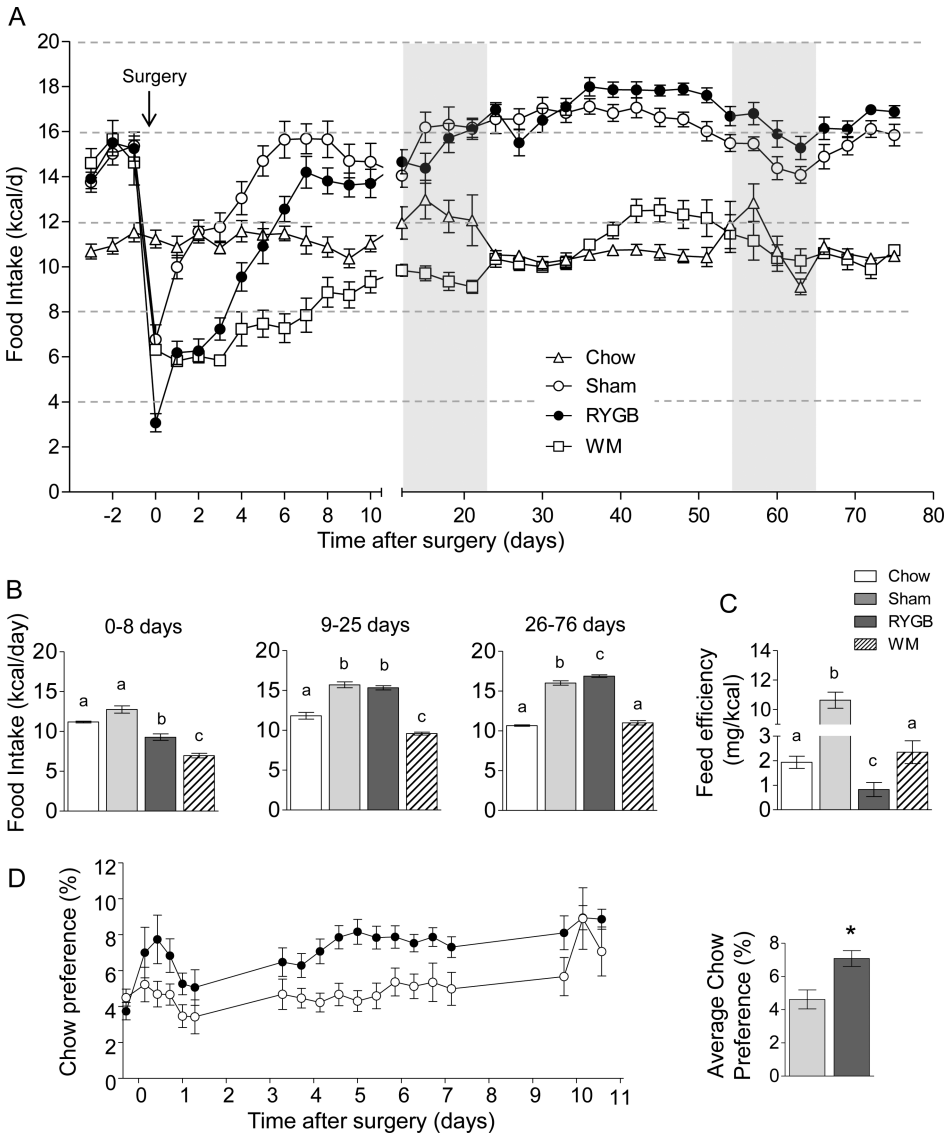

Fig. 3.

Effect of Roux-en-Y gastric bypass surgery (RYGB, n = 14) on food intake, feed efficiency, and food choice in high-fat diet-induced obese mice. Controls included sham surgery (Sham, n = 13), high-fat caloric restriction to match the body weight of RYGB (WM, n = 9), and never obese, regular chow-fed mice (Chow, n = 6). **A:** Total food intake (kcal/day) over the course of the study, with periods of measurements in metabolic chambers indicated by gray bars. **B:** Average total food intake (kcal/day) for three phases of the study. **C:** Feed efficiency for days 26-76 after surgery. Bars that do not share the same letters are significantly ($p < 0.05$) different from each other (based on ANOVA, followed by Bonferroni-corrected multiple comparisons). **D:** Time course and average preference for regular chow over high-fat diet for RYGB and sham-operated mice. * $p < 0.05$ (based on t-test).

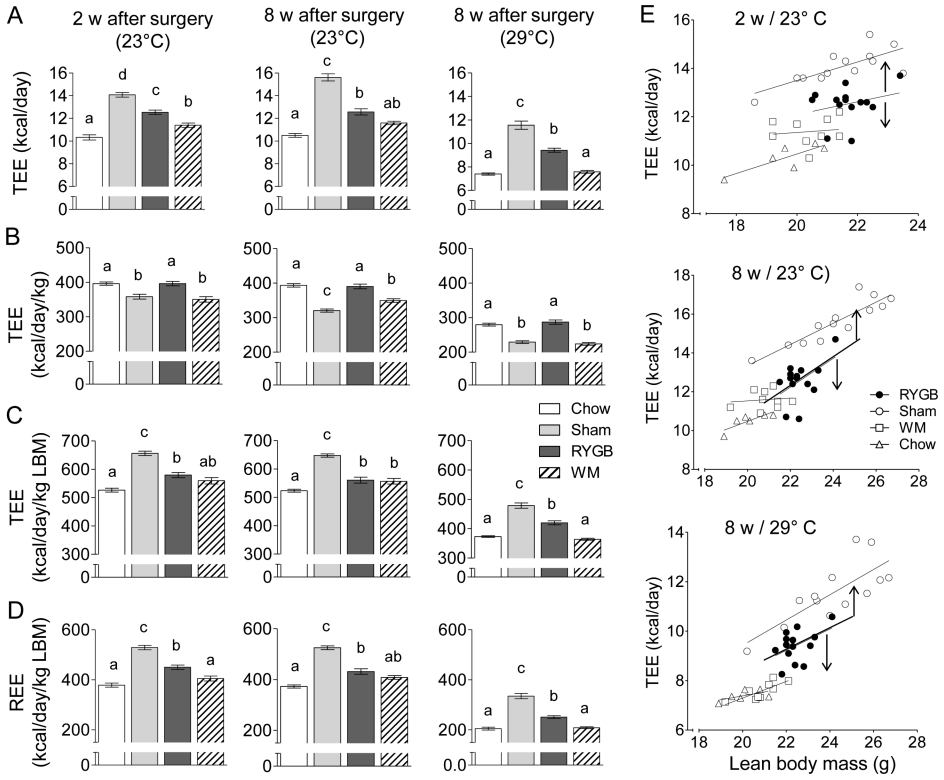

Fig. 4.

Effect of Roux-en-Y gastric bypass surgery (RYGB, n = 14) on energy expenditure in high-fat diet-induced obese mice at 2 and 8 weeks after surgery and at 23°C and 29°C (near thermoneutrality) as determined from VO_2 and VCO_2 in metabolic chambers. Controls include sham surgery (Sham, n = 13), high-fat caloric restriction to match the body weight of RYGB (WM, n = 9), and never obese, regular chow-fed mice (Chow, n = 6). **A:** Total energy expenditure per day and mouse. **B:** Total energy expenditure per day normalized by total body mass. **C:** Total energy expenditure per day normalized by lean body mass. **D:** Resting energy expenditure per day, normalized to lean body mass. **E:** Covariate regression analysis of total energy expenditure per day and animal vs. lean body mass. Bars that do not share the same letters are significantly ($p < 0.05$) different from each other (based on ANOVA, followed by Bonferroni-corrected multiple comparisons).

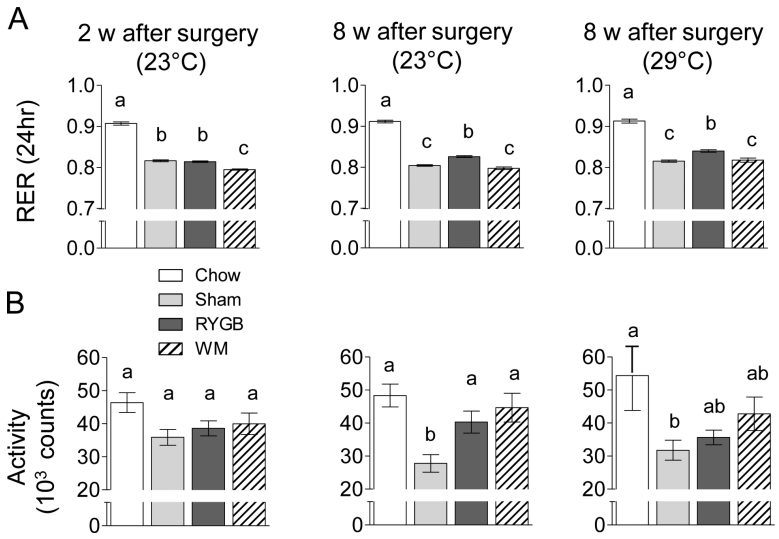

Fig. 5.

Effect of Roux-en-Y gastric bypass surgery (RYGB, n = 14) on respiratory exchange rate (RER) and locomotor activity in high-fat diet-induced obese mice at 2 and 8 weeks after surgery and at 23°C and 29°C (near thermoneutrality) as determined in metabolic chambers. Controls include sham surgery (Sham, n = 13), high-fat caloric restriction to match the body weight of RYGB (WM, n = 9), and never obese, regular chow-fed mice (Chow, n = 6). **A:** RER averaged over 24 hours. **B:** Locomotor activity as measured by average beam breaks per 24 hours. Bars that do not share the same letters are significantly ($p < 0.05$) different from each other (based on ANOVA, followed by Bonferroni-corrected multiple comparisons).

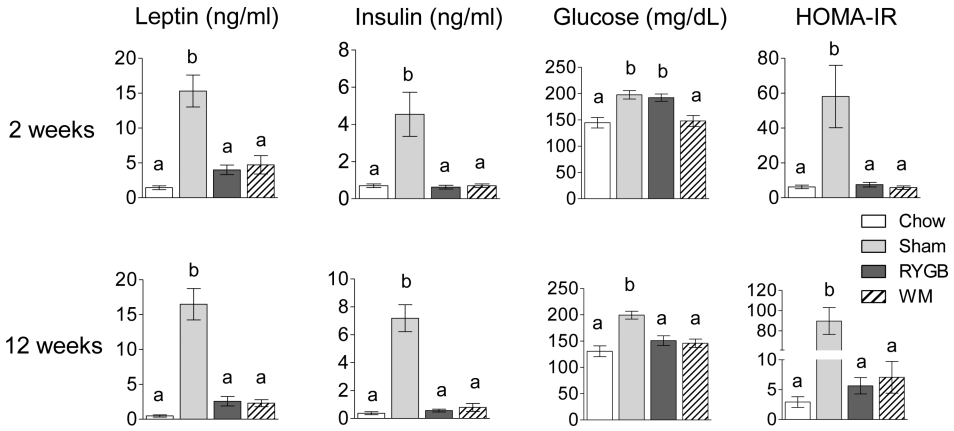

Fig. 6.

Effect of Roux-en-Y gastric bypass surgery (RYGB, n = 14) on fasting plasma levels of leptin, insulin, and blood glucose and on HOMA-IR at 12 weeks after surgery in high-fat diet-induced obese mice. Controls include sham surgery (Sham, n = 13), high-fat caloric restriction to match the body weight of RYGB (WM, n = 9), and never obese, regular chow-fed mice (Chow, n = 6). Bars that do not share the same letters are significantly ($p < 0.05$) different from each other (based on ANOVA, followed by Bonferroni-corrected multiple comparisons).

Table 1

Relative energy expenditure differences (Δ% of group means) between RYGB mice and mice weight-matched to RYGB by calorie restriction.

24 h EE	Correction	2 weeks/ 23 °C	8 weeks/23 °C	8 weeks/29 °C
Total	No correction	+ 9.6 *	+ 8.6	+ 24.3 *
	Total body mass	+ 13.1 *	+ 11.7 *	+ 28.2 *
	Lean mass	+ 3.6	+ 0.7	+ 15.3 *
	Total body mass $^{0.66}$	+ 17.3 *	+10.5	+26.7 *
Resting	No correction	+ 16.9 *	+ 14.1 *	+ 30.3 *
	Total body mass	+ 20.9 *	+ 17.2 *	+ 33.6 *
	Lean mass	+ 11.1 *	+ 5.9	+ 20.1 *
	Total body mass $^{0.66}$	+ 20.0 *	+ 15.9 *	+ 31.8 *

* $p < 0.05$, based on ANOVA, followed by Bonferroni-corrected multiple comparison as shown in Fig. 4.