Effect of bariatric surgery combined with medical therapy versus intensive medical therapy or calorie restriction and weight loss on glycemic control in Zucker diabetic fatty rats

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[1]*Institute of Veterinary Physiology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland;* [2]*Department of Digestive Surgery, School of Medicine Santiago, Pontificia Universidad Católica, Santiago, Chile;* [3]*Diabetes Complications Research Centre, Conway Institute, School of Medicine and Medical Sciences, University College Dublin, Dublin, Ireland;* [4]*Zurich Center for Integrative Human Physiology, University of Zurich, Zurich, Switzerland;* [5]*Institute of Laboratory Animal Science, University of Zurich, Zurich, Switzerland;* [6]*Gastrosurgical Laboratory, University of Gothenburg, Gothenburg, Sweden; and* [7]*Investigative Science, Imperial College London, London, United Kingdom*

Abegg K, Corteville C, Docherty NG, Boza C, Lutz TA, Muñoz R, le Roux CW. Effect of bariatric surgery combined with medical therapy versus intensive medical therapy or calorie restriction and weight loss on glycemic control in Zucker diabetic fatty rats. *Am J Physiol Regul Integr Comp Physiol* 308: R321–R329, 2015. First published December 24, 2014; doi:10.1152/ajpregu.00331.2014.— Bariatric surgery rapidly improves Type 2 diabetes mellitus (T2DM). Our objective was to profile and compare the extent and duration of improved glycemic control following Roux-en-Y gastric (RYGB) bypass surgery and vertical sleeve gastrectomy (SG) and compare against calorie restriction/weight loss and medical combination therapy-based approaches using the Zucker diabetic fatty rat (ZDF) rodent model of advanced T2DM. Male ZDF rats underwent RYGB ($n = 15$) or SG surgery ($n = 10$) at 18 wk of age and received postsurgical insulin treatment, as required to maintain mid-light-phase glycemia within a predefined range (10–15 mmol/l). In parallel, other groups of animals underwent sham surgery with ad libitum feeding ($n = 6$), with body weight ($n = 8$), or glycemic matching ($n = 8$) to the RYGB group, using food restriction or a combination of insulin, metformin, and liraglutide, respectively. Both bariatric procedures decreased the daily insulin dose required to maintain mid-light-phase blood glucose levels below 15 mmol/l, compared with those required by body weight or glycemia-matched rats ($P < 0.001$). No difference was noted between RYGB and SG with regard to initial efficacy. SG was, however, associated with higher food intake, weight regain, and higher insulin requirements vs. RYGB at study end ($P < 0.05$). Severe hypoglycemia occurred in several rats after RYGB. RYGB and SG significantly improved glycemic control in a rodent model of advanced T2DM. While short-term outcomes are similar, long-term efficacy appears marginally better after RYGB, although this is tempered by the increased risk of hypoglycemia.

bariatric surgery; Roux-en-Y gastric bypass; sleeve gastrectomy; glycemic control; medical therapy; caloric restriction; Zucker diabetic fatty rat

ROUX-EN-Y GASTRIC BYPASS (RYGB) and vertical sleeve gastrectomy (SG) surgery can be used as a treatment alternative to intensive management in obese patients (body mass index >30 kg/m^2) with Type 2 diabetes mellitus (T2DM) (5, 29, 39).

After these operations, most patients have improved glycemic control, and $\sim$40% of patients achieve a HbA1c <42 mmol/mol (6%) after one year. It remains controversial whether RYGB and SG lead to comparable rates of metabolic improvement and T2DM remission. Some studies report a higher T2DM remission rate or that glycemic targets can be achieved with fewer medications after RYGB compared with SG surgery (3, 22, 23, 32), while others show a similar glycemic outcome for both procedures (12, 16, 28, 46). Data on long-term remission and relapse rates are scarce, but at 3 years, 38% of those after RYGB and 24% of those after SG had a HbA1c <42 mmol/l (6%) (39). The use of glucose-lowering medications, including insulin, was lower after RYGB than SG, while weight loss was 25% and 21%, respectively (39). Others have suggested that a significant proportion of patients with T2DM may relapse within a few years after initial remission (2, 3, 16, 40). Major predictors of relapse of diabetes seem to be longer disease duration (2, 3), preoperative insulin use (2, 6, 16), poor preoperative glycemic control (2), and weight regain (3, 6, 16), which is of relevance because bariatric surgery is currently considered an important treatment option, particularly for patients with uncontrolled diabetes (9, 39).

For the purposes of this study, we used male Zucker diabetic fatty (ZDF) rats as a model of obesity and advanced T2DM. The ZDF rat is a commonly used rodent model for the study of obesity-related insulin resistance and advanced T2DM. ZDF rats have an autosomal recessive (*fa/fa*) mutation that abolishes leptin receptor function (14, 48), and similar to many patients with T2DM, this leads to progressive obesity, insulin resistance, glucose intolerance, hyperlipidemia, and hypertension (11). Plasma insulin levels are elevated in male ZDF rats from $\sim$5 wk of age and progressively rise to levels that are 8–12 times higher compared with those of control rats by 8–10 wk of age, when mild hyperglycemia starts to become noticeable (10, 11). Older ZDF rats display deficient β-cell function, low insulin levels, and hyperglycemia after 20 wk of age (13, 44).

The specific purpose of the current investigation was to investigate and compare the effects of RYGB and SG surgery on glycemic control in the ZDF model and to contrast results with those obtained using separate body weight-matching and combination medical therapy-based approaches to glycemic control. Toward this end, 18-wk-old ZDF rats underwent RYGB or SG surgery and were observed over a 12-wk period,

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during which time postsurgical insulin treatment was provided as required to maintain blood glucose within a predefined range of 10–15 mmol/l; the target level of glycemia was slightly above normal physiological levels to avoid the risk of hypoglycemia. Two sham-operated groups of animals were included in the study; the first group underwent sham surgery and was body weight-matched to the RYGB group, while a second group was glycemia-matched to the RYGB-operated rats using a combined treatment of insulin, metformin, and liraglutide.

The long-term control of blood glucose in older ZDF rats confronted us with several technical difficulties, especially in the RYGB and SG rats, and, therefore, we first describe the challenges of such a study and how we addressed them. Understanding the challenges will allow refinement of approaches in order to optimally harness the model for future studies focusing on the impacts of RYGB and SG surgeries on long-term glycemic control, advanced T2DM, and diabetic end-organ damage.

MATERIALS AND METHODS

Animals

Ten-week-old male ZDF rats (*fa/fa*) (*n* = 47) (Charles River Laboratories, Germany) were group-housed in a temperature- and humidity-controlled room with a 12:12-h light-dark cycle (lights on from 0200 to 1400). Rats had free access to tap water and a Purina Lab diet no. 5008 (Purina Mills; 16.7% of calories provided by fat) during the entire study, except where specifically noted otherwise. All experimental procedures were approved by the Veterinary Office of the Canton Zurich, Zurich, Switzerland. At the age of 16 wk, rats were housed individually in wire-mesh cages. Body weight progression and food intake were measured twice weekly. From the age of 17 wk, blood glucose (BG) was measured in all rats four times weekly with a point-of-care glucometer (Breeze2; Bayer). Blood was taken from the tail between 0900 and 1100 (which represented the middle of the light phase) in a nonfasted state.

Surgical Procedures

At 18 wk of age, ZDF rats were randomly assigned to either SG (*n* = 10), RYGB (*n* = 15), ad libitum-fed sham surgery (ALS; *n* = 6), or glycemia-matched sham surgery (GMS) to achieve similar glycemic control to RYGB-operated rats (*n* = 8) and body weight-matched shams (BMS) to achieve similar body weight to RYGB-operated rats (*n* = 8). The increased numbers in the RYGB and SG group were to compensate for the expected increased mortality after microsurgery. For 3 days prior to surgery, all rats were treated with subcutaneous insulin injections (10–32 IU/day; Levemir, Novo Nordisk) twice daily (0900–1100 and 1700–1900) to achieve mid-light-phase glucose levels between 15 and 20 mmol/l in an attempt to reduce postsurgical complications, such as infections (7). The night before the surgery, rats were food-deprived, having access to one Purina Lab pellet (~5 g). Body weight and blood glucose were measured in the morning before surgery. To mimic clinical practice in humans after bariatric surgery, insulin was subcutaneously injected according to blood glucose levels to achieve plasma levels <20 mmol/l, which required administration of approximately half of the insulin dose of the previous morning (2.5–10 U). If the blood glucose remained above 20 mmol/l in the evening after surgery, additional insulin was given. Immediately before surgery, a subcutaneous prophylactic dose of the antibiotic enrofloxacin (10 mg/kg) and carprofen (5 mg/kg) for analgesia was given. Anesthesia was provided with inhaled isoflurane (2–3%). For every procedure, a midline laparotomy was used to open the abdomen, and at the end, the abdominal wall and the skin were closed in two layers using continuous sutures. All SG and sham

procedures were performed by one surgeon (R. Muñoz), and all RYGB surgeries were performed by another surgeon (C. Corteville).

Vertical sleeve gastrectomy. After mobilization of the stomach and transection of the connecting ligaments to the liver and spleen, the stomach was placed on saline-moistened gauze outside the abdominal cavity. A 5-mm gastrotomy was performed lateral to the gastroesophageal junction and another one close to the pylorus at the greater curvature. A 6-French silicone tube was inserted through these gastrostomies, serving as a calibration buggie used to guide the stapler when constructing the sleeve. Approximately 70% of the stomach was removed using an ETS 45-mm staple gun (Ethicon Endosurgery). After withdrawal of the silicone tube, both gastrostomies were closed, and the tubular gastric remnant was placed back into the abdominal cavity.

Roux-en-Y gastric bypass. The jejunum was dissected 60 mm distal from the ligament that attaches the jejunum to the colon transversum. A 7-mm side-to side small bowel anastomosis was performed between the biliopancreatic limb and the lower jejunum 250–300-mm proximal to the cecum to create the common channel. After exposure and careful mobilization of the gastro-esophageal junction, the stomach was transected just below the gastro-esophageal junction to create a small gastric pouch about 2% of the original stomach size. The stomach remnant was subsequently closed, and the small gastric pouch was anastomosed end-to-side to the alimentary limb, which was ~500 mm in length (4).

Sham surgery. After mobilization of the entire gastrointestinal tract, a 10-mm gastrotomy was performed on the anterior wall of the stomach with subsequent closure in two layers; further, sham rats had a 7-mm jejunotomy with subsequent closure.

Postoperative care. Immediately postoperatively, all rats received wet mash (Purina powder mixed with water), and solid food was gradually reintroduced over 3–5 days. The sham-operated rats were back to normal food on the 3rd day after surgery; for the rats that received the vertical sleeve gastrectomy (SG) and RYGB, normal food was provided on the 5th day after surgery. For the first 2 days after surgery, all rats were treated with enrofloxacin (10 mg/kg once daily). The SG and RYGB rats additionally received carprofen (5 mg/kg once daily) for analgesia.

Measurement of Body Weight Progression and Food Intake After Surgery

Body weight and food intake were measured three times weekly. Food restriction in BMS rats was started 5 days after surgery. Initially, BMS rats received about 50% of the amount of food consumed by ALS rats; this amount was reevaluated three times weekly on the basis of body weight development, such as to allow for body weight matching to the RYGB group.

Glucose Control

Phase 1 (aged 18–24 wk, i.e., weeks 1–6 after surgery). After surgery, the insulin doses were reduced by 50% every day in all rats except for the GMS. Insulin treatment was completely stopped in ALS and BMS rats at *day 6* after surgery at the latest, and blood glucose was measured twice weekly thereafter. In RYGB and SG rats, twice-daily insulin treatment was continued if necessary to maintain mid-light-phase blood glucose levels below 15 mmol/l; the target level of glycemia was slightly above normal physiological levels to avoid the risk of hypoglycemia. Blood glucose was measured three times weekly before insulin injections in the middle of the light phase (0900–1100), and the insulin doses were adjusted accordingly.

GMS rats received twice-daily insulin treatment in combination with metformin (300 mg·kg^{-1}·day^{-1} po; Metformin-Mepha, Mepha) and liraglutide (1 mg·kg^{-1}·day^{-1}; Victoza, Novo Nordisk) to maintain blood glucose levels below 15 mmol/l. After the morning insulin injections (0900–1100), the food hoppers of GMS rats were removed and they received powdered metformin mixed with 5 g of Purina

chow. Liraglutide was administered before the evening insulin injection (1700–1900), after which the food hoppers were returned. To avoid adipsia-induced dehydration (45) and aversive effects, liraglutide was dose-titrated daily over a 12-day period with doses of 0.05, 0.1, 0.15, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 mg/kg.

Phase 2 (aged 25–30 wk, i.e., weeks 7–12 after surgery). After one ALS rat (aged 24 wk) died due to suspected hyperosmolar nonketotic coma (HONC) or diabetic ketoacidosis (DKA), insulin treatment consisting of 2 U in the morning (0900–1100) and 3 U in the evening (1700–1900) was reintroduced for the ALS and BMS groups at the age of 25 wk to avoid mortality due to HONC or DKA in these severely diabetic rats (43). Because of increasing difficulties in maintaining blood glucose within target range (10–15 mmol/l) in GMS, RYGB, and SG rats, blood glucose was measured before every insulin injection, i.e., twice daily, in these groups from the age of 25 wk. All rats were killed 13 wk after surgery aged 31 wk. Urine was collected to determine the level of glucosuria.

Statistical Analysis

Data are presented as means ± SE. Blood glucose area under the curve (AUC) between surgery and study termination was calculated for each rat. One-way ANOVA, followed by Bonferroni's multiple-comparison tests, was used to compare body weight, food intake, blood glucose, and glucose AUC between groups because the primary readout in this study was the comparison of parameters across experimental groups rather than over time. Weight regain for RYGB and SG rats was calculated as the difference between the lowest body weight after surgery and the final body weight. Statistical significance was set at $P < 0.05$.

RESULTS

Mortality

Four RYGB and five SG rats had to be euthanized as a result of early postsurgical complications, i.e., insufficiency of the small bowel anastomosis and the gastric staple line, respectively. One RYGB and one SG rat were found at death to have significant intra-abdominal adhesions and abscesses. Four RYGB rats died at 24–26 wk as a result of severe hypoglycemia. One ALS rat was found dead at 24 wk with suspected HONC or DKA. Because of increased difficulties maintaining blood glucose levels in the target range and because of the complications leading to the death of several RYGB animals from the age of 24 wk onward, the results are presented in two phases.

Body Weight Progression

Phase 1 (aged 18–24 wk). In the first 6 wk after surgery, GMS rats weighed significantly more than RYGB and SG rats within the first week ($P < 0.05$), but there was no significant difference in body weight between RYGB, SG, BMS, and ALS rats during any stage of phase 1. The progressive weight gain of GMS rats led to a significantly higher body weight compared with all other groups from *week 22* onward ($P < 0.001$; Fig. 1*A*).

Phase 2 (aged 25–30 wk). The introduction of low-dose insulin treatment in *week 25* resulted in an increase in body weight in ALS rats, with higher body weight compared with RYGB and BMS rats in *week 30* ($P < 0.01$ vs. RYGB and $P < 0.05$ vs. BMS). SG rats also showed an increase in body weight and weighed significantly more than RYGB rats from *week 28* ($P < 0.05$) and weighed more than BMS rats from *week 29* ($P < 0.05$). Weight regain was significantly higher in

SG than in RYGB rats (114.0 ± 31.9 vs. 23.5 ± 13.0 g, respectively; $P < 0.05$). GMS rats maintained a significantly higher body weight compared with all other groups until the end of the study ($P < 0.05$; Fig. 1, *A* and *B*, Table 1*A*).

Food Intake

Phase 1 (aged 18–24 wk). Food intake of ALS rats was significantly higher compared with all other groups ($P < 0.05$). GMS rats ate significantly more than RYGB rats from *week 20* ($P < 0.05$). The amount of food that was given to BMS rats had to be increased progressively from *week 22* to allow them to regain weight and catch up with RYGB rats, as the BMS rats' body weight started to fall slightly below the target. This led to a significant difference in food intake between BMS and RYGB rats in *weeks 23* and *24* ($P < 0.001$). There was no difference in food intake between RYGB and SG rats in the first 6 wk after surgery (Fig. 1*C*).

Phase 2 (aged 25–30 wk). Food intake of BMS rats reached similar levels as in ALS rats in *weeks 25* to *27*. Apart from that, ALS continued to eat significantly more than all of the other groups ($P < 0.01$). The low-dose insulin treatment that started in *week 25* led to increased food efficiency in BMS rats (i.e., with a reduction in urine calorie loss, more of the calories consumed were stored), as a result of which, calorie restriction had to be reintensified from *week 28*. Food intake in GMS rats remained significantly higher compared with RYGB rats ($P < 0.05$; Fig. 1*C*). Consistently, cumulative food intake over the 12 wk after surgery was significantly higher in ALS rats than in all of the other groups ($P < 0.001$) and in both BMS and GMS rats compared with RYGB rats ($P < 0.001$). In addition, RYGB rats had a significantly lower cumulative food intake than SG rats ($P < 0.01$; Fig. 1*D*, Table 1). The difference between RYGB and SG rats disappeared, however, when food intake was plotted as relative intake compared with the respective presurgical baseline (Fig. 1*E*).

Glucose Control

Phase 1 (aged 18–24 wk). During the first 6 wk after surgery, blood glucose of GMS, RYGB, and SG rats could be kept in the desired target range of 10 to 15 mmol/l by adjusting the insulin doses three times per week based on mid-light-phase (0900–1100) blood glucose measurements. When blood glucose was above 15 mmol/l, the daily insulin dose was increased by 1–3 units for GMS rats and by 0.5–1 unit for RYGB and SG rats. As a guideline, rats received 40% of their daily insulin dose in the morning (0900–1100) and 60% in the evening (1700–1900). This distribution was chosen for two reasons: *1*) because the time interval between injections was shorter after the morning injection and *2*) because we expected rats to eat more during the interval between evening and morning injection since a larger proportion of the dark phase coincided with that interval. As expected, blood glucose was significantly higher in ALS rats but was also higher in the BMS rats than in the insulin-treated GMS, RYGB, and SG rats ($P < 0.001$; Fig. 2*A*). Despite the combined hypoglycemic treatments of GMS rats with insulin, metformin, and liraglutide, the average daily insulin dose required to maintain blood glucose below 15 mmol/l was more than 10-fold higher in GMS compared with RYGB and SG rats (17.3 ± 1.3 vs. 0.8 ± 0.3 and 1.7 ± 0.6 U/day, respectively; $P < 0.001$). There was no
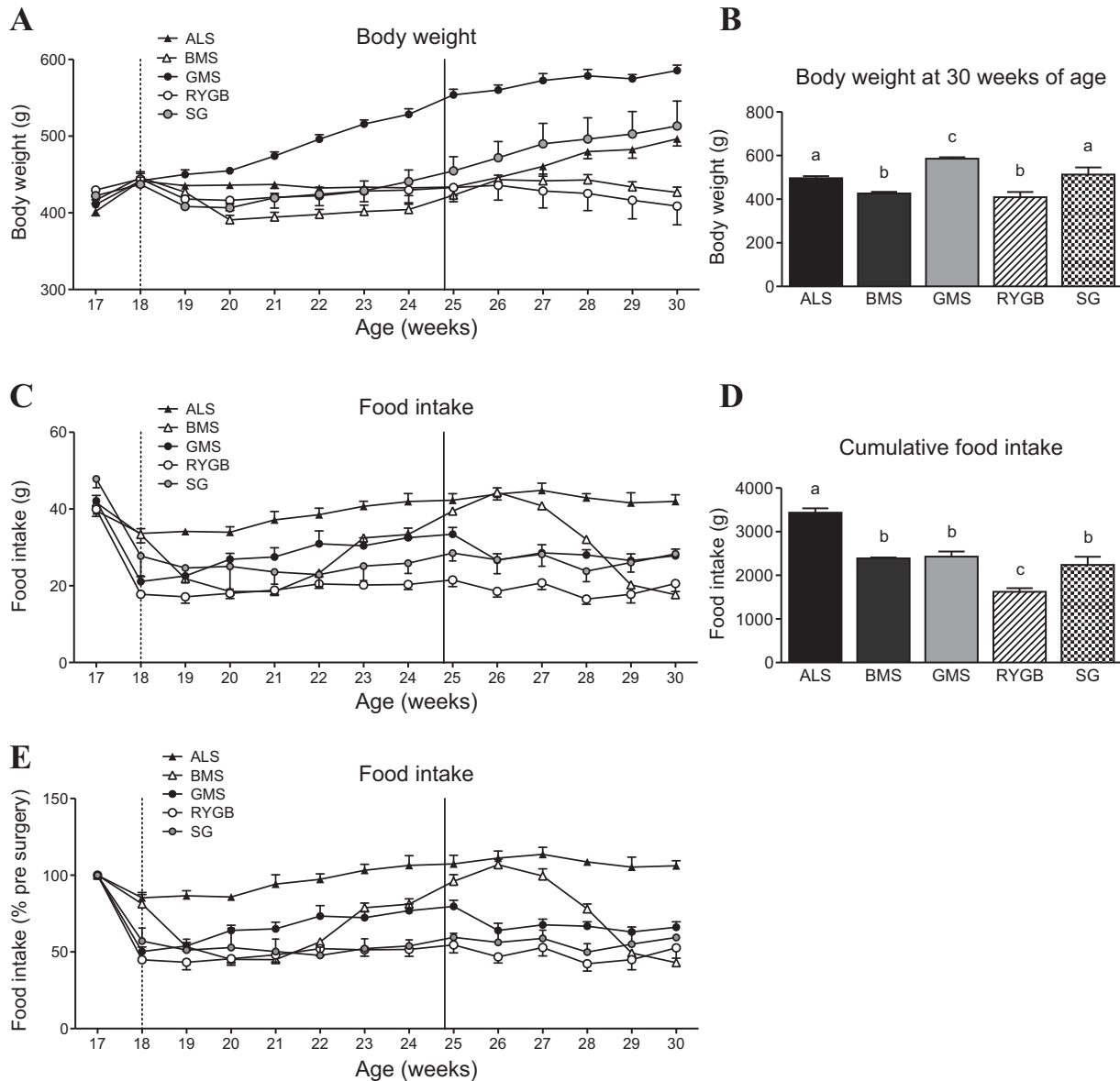
Fig. 1. Body weight gain or loss (*A*) and final body weight (*B*) at study termination, average weekly food intake (*C*), cumulative postsurgical food intake (*D*), and average weekly food intake as a percentage of presurgical baseline (100%) (*E*). ALS, ad libitum-fed shams ($n = 6$ from 18–24 wk; $n = 5$ from 25 to 30 wk); BMS, body weight-matched shams ($n = 8/8$); GMS, glycemically matched shams ($n = 8/8$); RYGB, Roux-en-Y gastric bypass ($n = 11/7$); SG, vertical sleeve gastrectomy ($n = 5/5$). For *A* and *C*, see text for significant differences. For *B* and *D*, different letters (a, b, c) indicate statistical significance (*$P < 0.05$).

significant difference in insulin requirement between RYGB and SG rats ($P = 0.21$; Fig. 2*B*), the insulin dose range was similar in both groups (0–9 and 0–6.5 U/day, respectively), and the total number of units received by RYGB vs. SG rats were not different (172.8 ± 60.5 vs. 395.3 ± 118.2, respectively; $P = 0.10$). However, there were more RYGB (5/6) than SG (1/4) animals that did not require any insulin treatment for a week or longer, although the average amount of days without insulin treatment did not reach statistical significance between RYGB and SG (24.2 ± 5.6 vs. 8.8 ± 5.1, respectively; $P = 0.09$).

Phase 2 (aged 25–30 wk). The introduction of low-dose insulin treatment in *week 25* led to a higher food efficiency in BMS rats and, consequently, to a substantial reduction in the allocated daily amount of food. As a result of combined insulin

treatment and food restriction, blood glucose declined to levels comparable to GMS, RYGB, and SG rats, but only in *week 29*. Because insulin treatment of ALS and BMS rats was only introduced to prevent HONC or DKA and because this development interfered with the aim of the study to investigate the effects of body weight loss and glycemic control independently, the insulin dose for ALS and BMS rats was reduced to 3 units per day, which led to a rapid reversion of blood glucose in BMS rats to levels similar to ALS rats in *week 30*.

In *week 24*, one RYGB rat was found severely hypoglycemic (blood glucose <0.6 mmol/l) and died as a consequence a few minutes later. After this incidence and because RYGB has been reported to lead to hyperinsulinemic hypoglycemia (26–29), the schedule for blood glucose measurement was intensified from three times per week to once daily (0900–1100) in

Table 1. *Bonferroni post hoc values for final body weight at study termination, cumulative postsurgical food intake, and postsurgical AUC of blood glucose levels*

Bonferroni Comparison	Mean Difference	t	Significance
Final Body Weight at Study Termination			
ALS vs. BMS	69.77	3.211	*
ALS vs. GMS	−89.1	4.1	**
ALS vs. RYGB	87.57	3.794	**
ALS vs. SG	−16.85	0.659	ns
BMS vs. GMS	−158.9	8.336	***
BMS vs. RYGB	17.79	0.8643	ns
BMS vs. SG	−86.63	3.711	**
GMS vs. RYGB	176.7	8.582	***
GMS vs. SG	72.25	3.095	*
RYGB vs. SG	−104.4	4.244	**
Cumulative Postsurgical Food Intake			
ALS vs. BMS	1046	7.384	***
ALS vs. GMS	1006	7.099	***
ALS vs. RYGB	1812	12.04	***
ALS vs. SG	1194	7.164	***
BMS vs. GMS	−40.32	0.3245	ns
BMS vs. RYGB	765.5	5.704	***
BMS vs. SG	148.2	0.9736	ns
GMS vs. RYGB	805.8	6.004	***
GMS vs. SG	188.5	1.239	ns
RYGB vs. SG	−617.3	3.848	**
Postsurgical AUC of Blood Glucose Levels			
ALS vs. BMS	273.6	2.624	ns
ALS vs. GMS	1099	10.54	***
ALS vs. RYGB	1327	11.97	***
ALS vs. SG	1226	9.986	***
BMS vs. GMS	825.1	9.02	***
BMS vs. RYGB	1053	10.66	***
BMS vs. SG	951.9	8.497	***
GMS vs. RYGB	227.8	2.305	ns
GMS vs. SG	126.8	1.132	ns
RYGB vs. SG	−101	0.8551	ns

ALS, ad libitum fed shams; BMS, body weight-matched shams; GMS, glycemic-matched shams; RYGB, Roux-en-Y gastric bypass; SG, vertical sleeve gastrectomy. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

RYGB and SG rats. Nevertheless, in *week 26*, three more RYGB rats died or had to be euthanized due to severe hypoglycemia (<1 mmol/l); consequently, glucose monitoring was further intensified to twice daily, i.e., before every insulin injection. This revealed surprising differences of up to 20 mmol/l in blood glucose between morning (0900–1100) and evening (1700–1900) measurements within the same rat (Fig. 2D), suggesting that the previously applied insulin dose regimen with seemingly stable conditions was no longer appropriate to steadily keep blood glucose within the target range. However, in spite of twice-daily blood glucose measurement and frequent reevaluation of the insulin doses in GMS, RYGB, and SG rats, it was progressively more difficult to regain the same level of glycemic control achieved earlier in the study (Fig. 2, *A*, *B*, and *D*).

Two more RYGB rats developed hypoglycemia (<2.5 mmol/l) aged 28 wk (despite having ad libitum access to food) but were successfully treated with glucose injections (2.5 ml of 20% glucose ip and sc).

Irrespective of these problems, average measured values of blood glucose was still significantly higher in ALS and BMS rats compared with GMS, RYGB, and SG rats during *phase 2* ($P < 0.001$), but there was no significant difference between GMS, RYGB, and SG rats. As shown in Fig. 2B, it appears that BMS rats required slightly less insulin and had lower blood glucose levels compared with ALS rats; this is indicative of an improved glycemic control, illustrating the well-known beneficial effect of weight loss on insulin sensitivity. The average area under the curve of blood glucose during the entire postsurgical period was also significantly higher in ALS and BMS rats ($P < 0.001$), while there was no difference between GMS, RYGB, and SG rats (Fig. 2C, Table 1C). The average daily insulin dose remained much higher in GMS compared with RYGB and SG (38.1 ± 1.2 vs. 3.3 ± 1.1 and 7.7 ± 2.4 U/day, respectively; $P < 0.001$), while there was no significant difference between RYGB and SG ($P = 0.10$). However, in the last week before termination (aged 30 wk), SG rats required significantly more insulin than RYGB rats (7.3 ± 2.9 vs. 0.6 ± 0.2; $P < 0.05$; Fig. 2B).

Urine collected at the time of death was analyzed for its glucose concentration. Consistent with the improved control of glycemia after RYGB, RYGB rats [23.0 ± 3.6 mmol/l ($n = 7$); $P < 0.001$] had a much lower level of glucosuria than ALS [151.0 ± 1.0 ($n = 5$)] or BMS rats [150.2 ± 1.9 ($n = 8$)] and glucosuria in RYGB did not differ from GMS [2.3 ± 0.3 ($n = 8$)] or lean ZDF (*fa*/+) control rats [2.8 ± 0.2 ($n = 5$)]. Surprisingly, glucosuria in SG [112.7 ± 22.0 ($n = 5$); $P < 0.001$ vs. RYGB; $P < 0.05$ vs. ALS or BMS, resp.] was higher than in RYGB despite similar levels of measured glycemia.

DISCUSSION

This study illustrates the very significant technical challenges involved in investigating the effects of bariatric surgery on glycemic control in rodent models of T2DM. We used ZDF rats with advanced T2DM to compare the effects of RYGB and SG surgery, body weight matching, and medical combination therapy-based approaches to glycemic control. We show that both RYGB and SG surgery led to a marked decrease in the insulin dose required to maintain mid-light-phase blood glucose levels below 15 mmol/l compared with rats that were body weight-matched or that received medical combination therapy. We did not find a difference in the initial efficacy of SG vs. RYGB in terms of improving glycemic control. However, SG rats had a higher food intake and higher weight regain than RYGB rats, which was associated with higher insulin requirements in SG at the tail end of the study. RYGB, but not SG, completely resolved glucosuria. Severe hypoglycemia occurred in several RYGB rats, but notably was not observed in any SG rats.

ZDF rats are hyperphagic due to a spontaneous mutation of the leptin receptor (*fa*) gene; because they also suffer from hyperglycemia and urinary glucose losses, a direct interpretation of their food intake and weight development compared with the other groups is challenging. For example, hyperphagia in ALS rats was not accompanied by a significant weight gain during the first weeks of our study; this effect was likely due to the excessive urinary calorie loss subsequent to glycosuria. Similarly, the lack of a more marked weight gain in BMS rats despite the strong increase in food intake of these rats around *weeks 24–27* of the study probably reflects the consequence of severe glucosuria with subsequent calorie loss. Nonetheless,
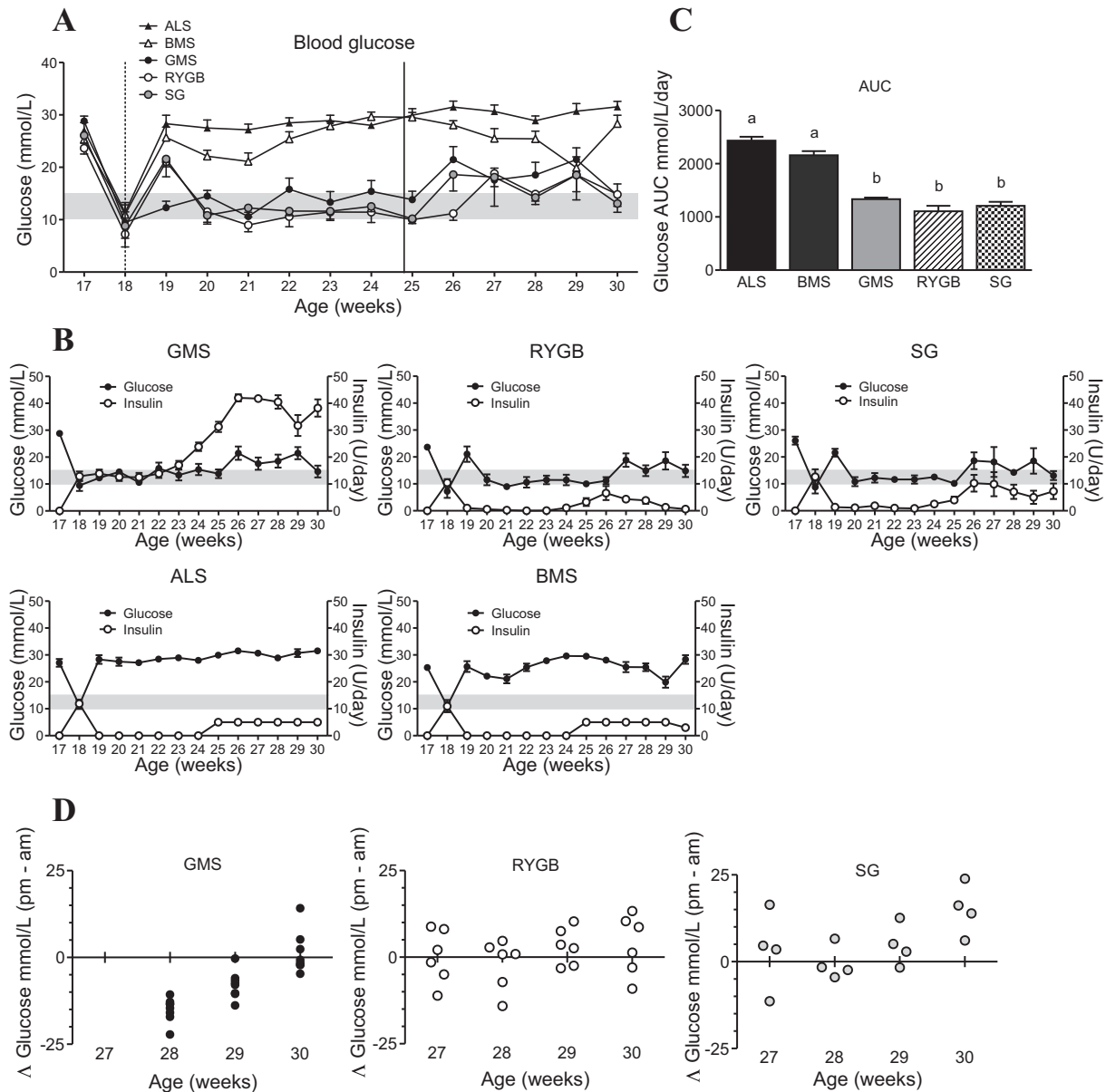
Fig. 2. Blood glucose level (*A*), blood glucose level and daily insulin dose (*B*), and area under the curve for postsurgical blood glucose levels (*C*). *D*: difference between evening and morning blood glucose levels within individual rats. Values below zero indicate higher morning blood glucose levels. Different letters (a, b) indicate statistical significance ($P < 0.05$).

more food had to be given to the animals temporarily to maintain their body weight near that of the RYGB rats.

Despite the combined treatment with insulin, liraglutide, and metformin, GMS rats needed much higher insulin doses than RYGB and SG-operated rats; the latter two groups did not receive liraglutide and metformin. Histological examination of the pancreas in ZDF rats treated with liraglutide and pioglitazone has shown that improvement in glycemic control is not necessarily accompanied by changes in β-cell mass (20). Whether improved glycemic control in RYGB- and SG-operated rats in our study was accompanied by increased β-cell mass or prevention of pancreatic islet degeneration will need to be assessed in future studies because a limitation of our study was that no pancreas histology was done in these rats. However, pancreas islet morpohology in Goto-Kakizaki rats (a

non-obese model of T2DM) after duodeno-jejunal exclusion showed increased β-cell mass and decreased islet fibrosis. Together, these data suggest that the improved glycemic control observed in RYGB- and SG-operated rats could also be associated with improved pancreatic islet morphology. Importantly, the high-dose insulin therapy led to pronounced weight gain in GMS rats, which per se can possibly be detrimental (26). The GMS rats ate fewer calories compared with the ALS group, but as they did not present with the hyperglycemia-induced osmotic polyuria and glucosuria and concomitant calorie loss, as observed in the ALS group, the increased calorie efficiency also contributed to their weight gain. The weight gain in GMS rats occurred despite the treatment with liraglutide. Liraglutide may have attenuated the body weight gain in these rats; however, because GMS rats that did not

receive liraglutide were not included in this study, we do not have an objective and unconfounded measure for liraglutide's action.

Our data support increasing evidence that both RYGB and SG are effective treatments for obesity-associated and poorly controlled T2DM (9, 39). There was no difference in the average daily insulin requirements or in the insulin dose ranges between RYGB and SG rats during the first 11 wk of the study; however, SG rats required progressively higher doses during the last week of the study, which was not the case in RYGB rats. Our data are, therefore, consistent with the human randomized controlled trial, suggesting that RYGB may have marginally better long-term effects on glycemic control, although in this model, this benefit is clearly offset by the incidence of hypoglycemia. The increasing insulin requirement in SG rats paralleled weight regain, consistent with several studies, suggesting that weight regain is a major risk factor for diabetes relapse after surgery (3, 6, 16). Weight regain was not a feature of the RYGB rats in our study, which is consistent with findings of a 20-yr weight loss maintenance in the Swedish Obese Subjects study (41). The finding of marked glucosuria at study end in SG vs. RYGB rats was surprising because measured blood glucose concentrations were similar. It seems unlikely that SG had a direct effect on the renal threshold for maximal glucose reabsorption. We presume that SG rats had much larger circadian fluctuations of glycemia than RYGB rats, including extended periods of hyperglycemia. However, we did not perform continuous glucose measurements in this experiment that would allow detection of such fluctuations, so that the underlying mechanisms of glucosuria in SG remain unclear at present.

In the second phase of the study, i.e., about 6 wk after surgery when the rats had reached an age of 24 wk, four RYGB rats died presumably of hypoglycemia and two additional RYGB rats developed severe hypoglycemia, which required parenteral glucose. All of these rats died 3 to 4 h into the dark phase, just prior to the scheduled evening insulin injection. Since rats consume large meals shortly after dark onset, we suspect that these meals were followed by episodes of severe hypoglycemia and would be consistent with reports of postprandial hypoglycemia in patients after RYGB surgery possibly reflective of an increased incretin response and/or relative of increases in β-cell mass of a magnitude greater than that observed in animals that underwent SG (27, 35, 37). Notably, clinical studies indicate that postprandial GLP-1 release does not differ significantly between RYGB and SG (28, 34). Further, a recent pilot experiment in our laboratory using real-time telemetric recordings of blood glucose levels in nondiabetic Wistar rats indicated that RYGB rats had much larger fluxes in glycemia compared with sham-operated controls, which extended into the hypoglycemic range (unpublished data), which is also consistent with continuous glucose monitoring in humans after RYGB (17). The late onset of hypoglycemia in our rats is also consistent with the severe hypoglycemia in humans that typically only occurs years after RYGB surgery (25).

Finally, as evident by randomized controlled trials in humans (1, 2, 13, 18, 28), we also found that weight loss due to caloric restriction alone is inferior to RYGB and SG, but that calorie restriction may need to be combined with insulin treatment to improve glycemic control similarly to RYGB and

SG. This adds to the current discussion about whether the early effects of RYGB and SG surgery on obesity-associated comorbidities, such as T2DM and cardiovascular disease, can be solely attributed to the significant postoperative calorie restriction, as suggested by a number of recent studies (15, 24, 42, 47). BMS rats consumed more food than RYGB rats in this study, because the BMS rats had higher glucose levels and, thus, lost many of consumed calories in their urine in contrast with the RYGB rats that had higher energy efficiency, as their glucose levels did not exceed the renal threshold and, thus, fewer calories were lost in the urine. The improved glycemic control observed after RYGB compared with BMS (with similar weight loss) suggests possible weight loss-independent mechanisms of RYGB surgery on glucose control consistent with some findings in humans (31, 38). Two intestinal effects of RYGB, the nutrient exclusion from the duodenum (30, 31) and the early delivery of partially digested nutrients to the mid-jejunum (7, 25, 40), have been proposed to mediate the enhanced effects of RYGB on glucose homeostasis through augmented secretion of different gut peptides, such as GLP-1. Because of its well-known incretin properties, GLP-1 has been postulated as a key antidiabetic mediator after RYGB in rats and humans (8, 18, 19, 21). RYGB has also been associated with increased circulating levels of bile acids, which, in addition to their effect on lipid digestion, modulate energy and glucose homeostasis (1, 30, 33). Finally, it was shown recently that glycemic control after RYGB in rats occurred through fundamental physiological changes of glucose metabolism within the alimentary limb (36).

The limitations of this study include the high mortality rate of the rats in the RYGB and SG groups. The microsurgery can be successfully performed through a laparotomy, but the risks of a leak and subsequent peritonitis remain significant. In our study, we introduced food as soft mash too early to the rats after SG, and although the RYGB rats had fewer problems, a period of liquid food as is the custom in humans after RYGB and SG should reduce the risk of staple line breakdown. Management of increasing glycemia in RYGB rats in attempts to restore normoglycemia in *phase 2* of the experiment was probably pursued too aggressively and likely contributed to the severe and sometimes fatal postprandial hypoglycemia. Finally, the introduction of insulin to both the ALS and BMS groups was not originally planned but had to be considered to reduce the mortality presumed to be related to HONC or DKA in these groups. The requirement to do so compromised slightly the sharp distinctions that we wished to draw between body weight-matching and glycemia-matching groups and which we intended would allow for clean interrogation of the independent influence of weight loss on glycemic control following RYGB and SG.

In summary, we compared the long-term glycemic control effects of medical combination therapy, body weight matching, and bariatric surgery in a rodent model of advanced T2DM. We found that rats after RYGB and SG required much less exogenous insulin than sham-operated animals to maintain stable blood glucose levels. Furthermore, we were able to reproduce several findings observed in human studies, e.g., differences between short- and long-term glycemic control, the impact of weight regain on glycemia, postprandial hypoglycemia as a late complication of RYGB, and improvement of hyperglycemia by caloric restriction when combined with in-

sulin therapy. Therefore, this study provides a guide to the intricacies of mimicking observations in clinical practice within a rodent model to investigate the impact and mechanisms involved in long-term glycemic control following RYGB and SG. Subsequent studies using the model will focus on comparing the effects of individual bariatric procedures and intensive medical therapy on microvascular complications of diabetes. The reasons for glucosuria in SG but not in RYGB rats, despite similar levels of glycemia, will require further studies.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: K.A., N.G.D., T.A.L., R.M., and C.W.l.R. conception and design of research; K.A., C.C., and R.M. performed experiments; K.A., C.C., N.G.D., C.B., T.A.L., R.M., and C.W.l.R. analyzed data; K.A., T.A.L., R.M., and C.W.l.R. interpreted results of experiments; K.A. and C.C. prepared figures; K.A., C.C., C.B., T.A.L., and R.M. drafted manuscript; K.A., T.A.L., R.M., and C.W.l.R. edited and revised manuscript; K.A., C.C., N.G.D., C.B., T.A.L., R.M., and C.W.l.R. approved final version of manuscript.

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