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Research paper

## Simulation of gastric bypass effects on glucose metabolism and non-alcoholic fatty liver disease with the Sleeveballoon device

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## ABSTRACT

**Background:** Gastric bypass surgery is a very effective treatment of obesity and type 2 diabetes. However, very few eligible patients are offered surgery. Some patients also prefer less invasive approaches.

We aimed to study the effects of the Sleeveballoon – a new device combining an intragastric balloon with a connecting sleeve, which covers the duodenal and proximal jejunal mucosa – on insulin sensitivity, glycemic control, body weight and body fat distribution.

**Methods:** We compared the effects of Sleeveballoon, Roux-en-Y Gastric-Bypass (RYGB) and sham-operation in 30 high-fat diet (HFD) fed Wistar rats. Whole body and hepatic insulin sensitivity and insulin signaling were studied. Transthoracic echocardiography was performed using a Vevo 2100 system (FUJIFILM VisualSonics Inc., Canada). Gastric emptying was measured using gastrografin.

**Findings:** Hepatic ( $P = .023$ ) and whole-body ( $P = .011$ ) insulin sensitivity improved in the Sleeveballoon and RYGB groups compared with sham-operated rats. Body weight reduced in both Sleeveballoon and RYGB groups in comparison to the sham-operated group ( $503.1 \pm 8.9$  vs.  $614.4 \pm 20.6$  g,  $P = .006$  and  $490.0 \pm 17.7$  vs.  $614.4 \pm 20.6$  g,  $P = .006$ , respectively). Ectopic fat deposition was drastically reduced while glycogen content was increased in both liver and skeletal muscle. Gastric emptying ( $T_{1/2}$ ) was longer ( $157.7 \pm 29.2$  min,  $P = .007$ ) in the Sleeveballoon than in sham-operated rats ( $97.1 \pm 26.3$  min), but shorter in RYGB ( $3.5 \pm 1.1$  min,  $P < .0001$ ). Cardiac function was better in Sleeveballoon and RYGB versus sham-operated rats.

**Interpretation:** The Sleeveballoon reduces peripheral and hepatic insulin resistance, glycaemia, body weight and ectopic fat deposition to a similar level as RYGB, although the contribution of gastric emptying to blood glucose reduction is different.

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## 1. Introduction

Metabolic surgery (MS) is an effective and recommended therapeutic option for the management of type 2 diabetes (T2D) [1,2]. MS improves insulin resistance [3–6], which is a primary component of T2D [7]. In particular, Roux-en-Y Gastric Bypass (RYGB) results in T2D remission and reversion of insulin resistance [8–13]. After RYGB, a large portion of the stomach, the entire duodenum and the initial segment of the jejunum are bypassed, excluding them from food transit. The bypass of

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## Research in context

### Evidence before this study

Roux-en-Y Gastric Bypass (RYGB) results in type 2 diabetes remission and reversion of insulin resistance. However only ca. 1% of eligible patients undergo bariatric surgery. In RYGB bypass, the gastric volume is reduced to only 30 ml and the duodenum and the initial portion of the jejunum are bypassed from food transit. These anatomical changes induce many physiological and endocrine modifications that contribute to diabetes remission.

### Added value of this study

The Sleeveballoon is a new device combining an intragastric balloon with a connecting sleeve, which covers the duodenal and proximal jejunal mucosa. We compared the effects of the Sleeveballoon and RYGB with sham-operation in Wistar rats under a high-fat diet. The Sleeveballoon reduced peripheral and hepatic insulin resistance, glycaemia, body weight and ectopic fat deposition, to a similar level as RYGB.

### Implications of all the available evidence

The Sleeveballoon seems to be a safe and effective device mimicking the effects of RYGB that can be used to reduce body weight and improve glucose disposal and diabetes complications, such as non-alcoholic fatty liver. Theoretically, it should stay in place for 6 months to 1 year, however only a safety and efficacy clinical trial can establish the duration of the Sleeveballoon permanence in the gastro-intestinal tract.

the duodenum plays a central role in the improvement of glycaemic control. The duodenal liner, a fluoropolymer sleeve extending 60–80 cm into the small intestine, improves glycaemic control and insulin resistance in patients with T2D and obesity [14]. Similarly, the hydrothermal ablation of the duodenal mucosa results in improved glycaemic control in T2D [15].

The Sleeveballoon device consists of an intra-gastric balloon with a central channel to allow passage of food into the proximal small bowel which is covered by a sleeve, extending into the duodenum and proximal jejunum. This reduces gastric volume by 2/3, while the sleeve bypasses the proximal small bowel, delivering food directly to the mid-jejunum. We previously demonstrated that the infusion of a liquid meal into the mid jejunum significantly improves insulin sensitivity in subjects with obesity and in subjects with T2D [16].

The primary aim of the present study was to compare the effect of the Sleeveballoon and that of RYGB with sham operation in rats fed a high-fat diet (HFD) in terms of fasting insulin sensitivity. Secondary aims were the effects of Sleeveballoon or RYGB on glycaemia, body weight, body fat distribution, ectopic fat deposition in both liver and skeletal muscle, heart contractility, gastric emptying and insulin signaling.

## 2. Materials and methods

### 2.1. Animals

The primary aim of our study was to assess the effect of the Sleeveballoon on insulin sensitivity during HFD. The sample size was computed on the basis of a preliminary pilot study and under the hypothesis that the HOMA-IR would be 18 mg-dl<sup>-1</sup>/ng-ml<sup>-1</sup> in sham-operated rats (μ1, mean of population 1) and 9 mg-dl<sup>-1</sup>/ng-ml<sup>-1</sup> in rats with the Sleeveballoon (μ2, mean of population 2) with a σ (common standard deviation) of 6. Assuming a value of α (type I error rate) of 0.05 and a power of 0.90, the sample size for each sample separately was 10. We assumed a similar effect of RYGB on HOMA-IR and, thus, we studied further 10 rats operated of gastric bypass.

The study was not designed and powered to assess differences among Sleeveballoon and RYGB, thus comparisons between the two procedures are considered as merely indicative.

Secondary aims were the effects of Sleeveballoon or RYGB on body weight, body fat distribution, ectopic fat deposition in both liver and skeletal muscle, heart contractility, gastric emptying and insulin signaling.

All experimental procedures were approved by the Catholic University of Rome Institutional Animal Care Committee. Thirty Wistar rats aged 10 weeks were housed individually in a controlled room at 22 °C with a 12-h day/night cycle (lights on 0700–1900 h). The animals received a purified tripalmitin-based liquid HFD ad libitum (Rieper AG, Bolzano, Italy). The HFD was continued for 10 weeks before and 10 weeks after the operation. The animals were randomized 1:1:1 to RYGB, sleeveballoon placement or sham operation. Survival rates were 90% after sham operation, 90% after sleeveballoon placement and 75% after RYGB.

### 2.2. Interventions

Rats were randomly assigned to one of the three intervention groups. The rats were anesthetized using ketamine (75 mg/kg intramuscularly) and xylazine (10 mg/kg intramuscularly). Ten milliliters of sterile 0.9% NaCl were administered subcutaneously before surgery. Access to the peritoneal cavity was obtained by 3-cm laparotomy.

#### 2.2.1. Roux-en-Y gastric bypass (RYGB)

The length of the small intestine was measured and the ligament of Treitz identified. The jejunum was divided into approximately two halves. A pouch was created by transecting the stomach and an end-to-side jejuno-jejunostomy and a gastrojejunostomy created with a 7-0 polydioxanone suture. The laparotomy was closed with a 4-0 polypropylene suture.

#### 2.2.2. Sleeveballoon

Fig. 1 shows how the device was placed. A 1-cm gastrotomy was performed in order to introduce the device. The sleeve was introduced first followed by the balloon. Once the device was in the correct position, the gastric wall was sutured similar to the RYGB group.

#### 2.2.3. Sham-operation

A midline laparotomy was performed and the stomach was exposed. A 1-cm gastrotomy was performed and then closed similar to the sleeveballoon group. The abdominal cavity was kept open for the same amount of time as required to perform the other operations.

### 2.3. Postoperative care

At the end of the surgical procedures, all rats received sterile 0.9% NaCl 10 ml i.p. and 10 ml s.c. to maintain hydration during the postoperative period. The animals received ketoprofen 5 mg/kg as an analgesic. They were placed on a heated mat until they recovered and then were returned to their home cages. The rats were allowed to drink purified water 12 h after surgery, and a liquid diet containing 5% glucose and 0.2% KCl was provided for the next 48 h. Thereafter, they received the HFD in liquid formulation until 10 weeks after surgery.



**Fig. 1.** Sleeveballoon device. The device consists in a gastric balloon traversed by a channel that permits the passage of a limited amount of food. The channel continues with a duodenal sleeve, which prevents the contact of food with the duodenal mucosa.

#### 2.4. Echocardiography

Rats were anesthetized in a sedation chamber containing 96% O<sub>2</sub> and 4% isoflurane before mask ventilation with a mixture of 98.25% O<sub>2</sub> and 1.75% isoflurane. Transthoracic echocardiography was performed using a Vevo 2100 system (FUJIFILM VisualSonics Inc., Canada). End-systolic and end-diastolic dimensions, end-systolic and end-diastolic volumes and stroke volume were recorded in order to calculate the per cent fractional shortening (FS%) and ejection fraction (EF%). Each measure was repeated three times.

#### 2.5. Gastric emptying

Gastric emptying was measured by gastric gavage using *Gastrografin* (diatrizoate meglumine and diatrizoate sodium solution). The gastric emptying scan was performed by an X-ray camera. After taking consecutive X-ray radiographs, rats were returned to their cages, and free access to food and water allowed. The gastric emptying scan was performed at 9 weeks after sleeveballoon placement.

#### 2.6. Oral glucose tolerance test

The oral glucose tolerance test (OGTT) was performed at the end of the study. Animals were fasted overnight and then received a 50% D-glucose solution (1 g/kg body weight) by oral gavage. Blood was collected from the tail vein for measurement of glucose and insulin concentrations at 0, 15, 30, 60, 90, and 120 min at the end of the study. At the end of the OGTT, blood was obtained by cardiac puncture and placed in tubes containing EDTA, aprotinin, and a dipeptidyl peptidase 4 (DPP-4) inhibitor and analyzed for GLP-1. After centrifugation, plasma was divided into appropriate subsamples and stored at  $-20^{\circ}\text{C}$  until analyses.

#### 2.7. Analytical methods

Blood glucose levels were analyzed by glucometer (Accu-Chek, Roche Diagnostics Division, Grenzachstrasse, Switzerland). Plasma insulin was measured by a rat insulin ultrasensitive ELISA (EMD Millipore Corporation, Billerica, MA, USA), with a sensitivity of 0.1 ng/ml and an intra- and inter-assay precision of 1.9% and 7.6%, respectively. Plasma GLP1<sub>7–36</sub> was measured by Rat GLP1/Glucagon-Like Peptide 1 ELISA

Kit (LifeSpan Biosciences Inc., Seattle, WA), sensitivity was 1.17 pg/ml, Intra-Assay CV <10%.

#### 2.8. Histology

When the rats were killed, fresh portions of heart from each rat were cut, fixed in neutral buffered formalin (10%), and dehydrated using gradations of ethanol (70%, 80%, 90%, 95%, and 100%). Dehydration was followed by clearing the samples in two changes of xylene. The samples were then embedded in paraffin and cut with a microtome (3–4  $\mu\text{m}$ ). Hematoxylin and eosin staining was used.

#### 2.9. Lipid staining and glycogen storage

Liver and skeletal muscle were embedded in cryo-embedding media (OCT) and immediately frozen in liquid nitrogen. Biopsies were cut using a cryostat (3–4  $\mu\text{m}$ ) and stored at  $-20^{\circ}\text{C}$  until analyses.

Periodic acid Schiff staining was used to evaluate glycogen storage. Slides were fixed 20 min with 4% formalin, stained in Periodic Acid Solution for 5 min and in Schiff's Reagent for 15 min. Counterstain was performed with Hematoxylin solution. Oil Red O staining was performed to assess intracellular lipid accumulation. Slides were fixed overnight with 4% formalin, stained with Oil Red O solution for 1 h. Counterstain was performed with Hematoxylin solution. Photographs of stained sections were taken with an optical microscope (ZEISS Primo Star HAL/LED).

#### 2.10. Western blot analysis

Muscle and liver biopsy specimens were homogenized in RIPA buffer containing a mixture of protease inhibitors. Homogenates were cleared by centrifugation (13,000 rpm; 30 min,  $4^{\circ}\text{C}$ ). Protein content was determined using Bradford Protein Assay. Protein lysates (30  $\mu\text{g}$ ) were separated on 10% SDS-PAGE and transferred on PVDF membrane. Membranes were probed overnight with Plin2, phospho-AktSer473, phospho-GSK3 $\alpha\beta$  Ser21/9, and Tubulin. Membranes were stripped for 30 min at  $56^{\circ}\text{C}$  and re-probed overnight with total Akt or total GSK3 $\alpha\beta$ . Detection and analysis were performed respectively with Chemidoc XRS Image system and Image Lab 5.0 software (Bio-Rad Laboratories, Hercules, CA). Plin2 was normalized by Tubulin, while phospho-AktSer473 and phospho-GSK3 $\alpha\beta$  Ser21/9 resulted from the ratio of phosphorylated to total protein.

#### 2.11. Statistical analysis

Data are expressed as mean  $\pm$  SEM unless otherwise specified. HOMA-IR [17] was calculated as fasting blood glucose (mg/dl)  $\times$  fasting plasma insulin ( $\mu\text{U/mL}$ )/405, where the factor 405 accounts for measurement units. The areas under the curve (AUCs) were computed as incremental over basal by using the trapezoidal rule. Whole body insulin sensitivity was measured as the ratio  $\text{AUC}_{\text{gluc}}/\text{AUC}_{\text{ins}}$ , which indicates the glucose cleared per unit of insulin. Early glycaemic response to the OGTT (blood glucose peak at 30 min) was used as indicative of the gastric emptying. The  $\beta$ -cell function was assessed by HOMA Beta-cell (HOMA-B) [18]:

$$\text{HOMA-B} = 20 \cdot \text{fasting insulin (in } \mu\text{U/ml)} / (\text{fasting glucose [in mmol/l]} - 3.5)$$

The disposition index was computed by multiplying 1/HOMA-IR by HOMA-B. In fact, the inverse of HOMA-IR is an index of insulin sensitivity.

Intergroup differences were assessed by Mann-Whitney *U* test, corrected for multiple comparisons by the Bonferroni's inference method, due to lack of normal distribution assessed by Shapiro-Wilk test.

To predict the glycaemic response at 30 min after the OGTT (dependent variable) we used the GLP1 levels at the same time and the values of gastric emptying as independent variables in a multiple regression model.

Differences were considered significant at  $P < .05$ . Spearman correlation analysis was performed to detect correlations among variables.

### 3. Results

#### 3.1. Sleeveballoon improves hepatic insulin sensitivity similar to gastric bypass

The primary aim of our study was the effect of Sleeveballoon on hepatic insulin resistance. HOMA-IR, a measure of fasting insulin resistance and thus hepatic insulin resistance, was significantly lower in the animals with the Sleeveballoon as compared with sham-operated rats ( $9.1 \pm 2.3$  vs.  $24.2 \pm 5.7$ ,  $P = .023$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method), but similar to the values found in animals following RYGB ( $10.4 \pm 2.2$  vs.  $24.2 \pm 5.7$ ,  $P = .042$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method).

#### 3.2. Sleeveballoon induces sustained weight loss and reduction of visceral and subcutaneous fat

Thirty rats under HFD were randomized 1:1:1 to RYGB, Sleeveballoon procedure or sham operation.

Baseline weights were comparable among groups:  $281.0 \pm 3.05$  g in the sham-operated group,  $283.0 \pm 3.09$  g in the Sleeveballoon group and  $284.5 \pm 2.03$  g in the RYGB group ( $P = .649$  RYGB vs. sham;  $P = .867$  Sleeveballoon vs. Sham;  $P = .923$  Sleeveballoon vs. RYGB, Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method). At 10 weeks after the operation, the body weight reduced in both Sleeveballoon and RYGB groups in comparison to the sham-operated group ( $503.1 \pm 8.9$  vs.  $614.4 \pm 20.6$  g and  $490.0 \pm 17.7$  vs.  $614.4 \pm 20.6$  g,  $P = .006$ , respectively, Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method), while no difference was found between Sleeveballoon and RYGB rats ( $P = .60$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method), Fig. 2, Panel A.

Food intake was lower in both Sleeveballoon ( $877.5 \pm 184.9$  kcal per week,  $P = .04$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method) and RYGB ( $1057.0 \pm 158.4$  kcal per week  $P = .04$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method) groups in comparison with sham-operated rats ( $1526.0 \pm 14.2$  kcal per week), but no difference was detected between Sleeveballoon and RYGB.

As shown in Fig. 2, Panel B, mesenteric and retroperitoneal fat content was higher in the sham-operated group than in both Sleeveballoon and RYGB groups ( $38.1 \pm 3.0$  vs.  $16.3 \pm 1.9$  g,  $P = .002$  and  $38.1 \pm 3.0$  vs.  $13.3 \pm 2.1$  g,  $P = .0006$ , respectively, Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method).

Additionally, the difference in subcutaneous fat content was significantly higher in sham-operated rats than in both the Sleeveballoon and RYGB rats ( $18.1 \pm 1.6$  vs.  $10.2 \pm 0.4$  g  $P = .0004$  and  $18.1 \pm 1.6$  vs.  $9.6 \pm 0.6$ ,  $P = .002$ , respectively, Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method).

#### 3.3. Sleeveballoon improves whole-body insulin sensitivity similar to gastric bypass

Insulin resistance manifests long before the onset of type 2 diabetes and it represents the primary defect of this type of diabetes [7]. We measured whole body insulin sensitivity as the ratio of glucose AUC to insulin AUC after the OGTT. This ratio, in fact represents the glucose clearance mediated by insulin.

Then, we calculated hepatic insulin resistance by HOMA-IR and pancreatic  $\beta$ -cell glucose sensitivity using HOMA-B. The disposition index, which indicates how  $\beta$ -cells can adapt to the tissue insulin sensitivity by modulating the secretion of insulin, was also computed.

The glycemic curve in response to the oral glucose load in rats with Sleeveballoon shows a lower response level than in sham-operated rats and a flatter shape in comparison with RYGB rats (Fig. 2, Panel C). Using blood glucose at 30 min (peak for RYGB) as the dependent variable and GLP1 levels and gastric emptying as the independent variables at the same time, the multiple regression model had an  $R^2$  of 0.83 ( $P$  of the model  $< 0.0001$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method). Both GLP1 levels ( $\beta = -0.501$ ,  $P = .003$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method) and gastric emptying ( $\beta = -0.303$ ,  $P = .024$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method) negatively predicted blood glucose levels.

The profile of the insulin curve resembles that of the glycemic response in rats with the Sleeveballoon, while in the rats undergoing RYGB, there is a peak at 30 min followed by a rapid decline (Fig. 2, Panel D).

The blood glucose AUC was lower in the rats with Sleeveballoon than in sham-operated animals ( $5471.3 \pm 2237.6$  vs.  $15,690.1 \pm 2794.7$  mg/dl-min,  $P = .011$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method), as well as in RYGB rats as compared with sham operation ( $7877.2 \pm 1529.4$  vs.  $15,690.1 \pm 2794.7$  mg/dl-min,  $P = .032$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method).

The plasma insulin AUC was lower with the Sleeveballoon than with sham operation ( $373.3 \pm 53.1$  vs.  $562.0 \pm 61.9$  ng/ml-min,  $P = .033$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method), while no significant difference was found between RYGB and sham-operation ( $498.6 \pm 61.6$  vs.  $562.0 \pm 61.9$  ng/ml-min,  $P = .477$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method).

The ratio of blood glucose AUC to plasma insulin AUC, which is a measure of whole body insulin sensitivity, was lower in the rats with Sleeveballoon than in sham-operated rats ( $9.1 \pm 4.8$  vs.  $27.6 \pm 4.8$ ,  $P = .014$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method) as well as in RYGB compared to sham-operated rats ( $14.6 \pm 3.9$  vs.  $27.6 \pm 4.8$ ,  $P = .049$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method), consistent with a greater insulin-mediated glucose clearance.

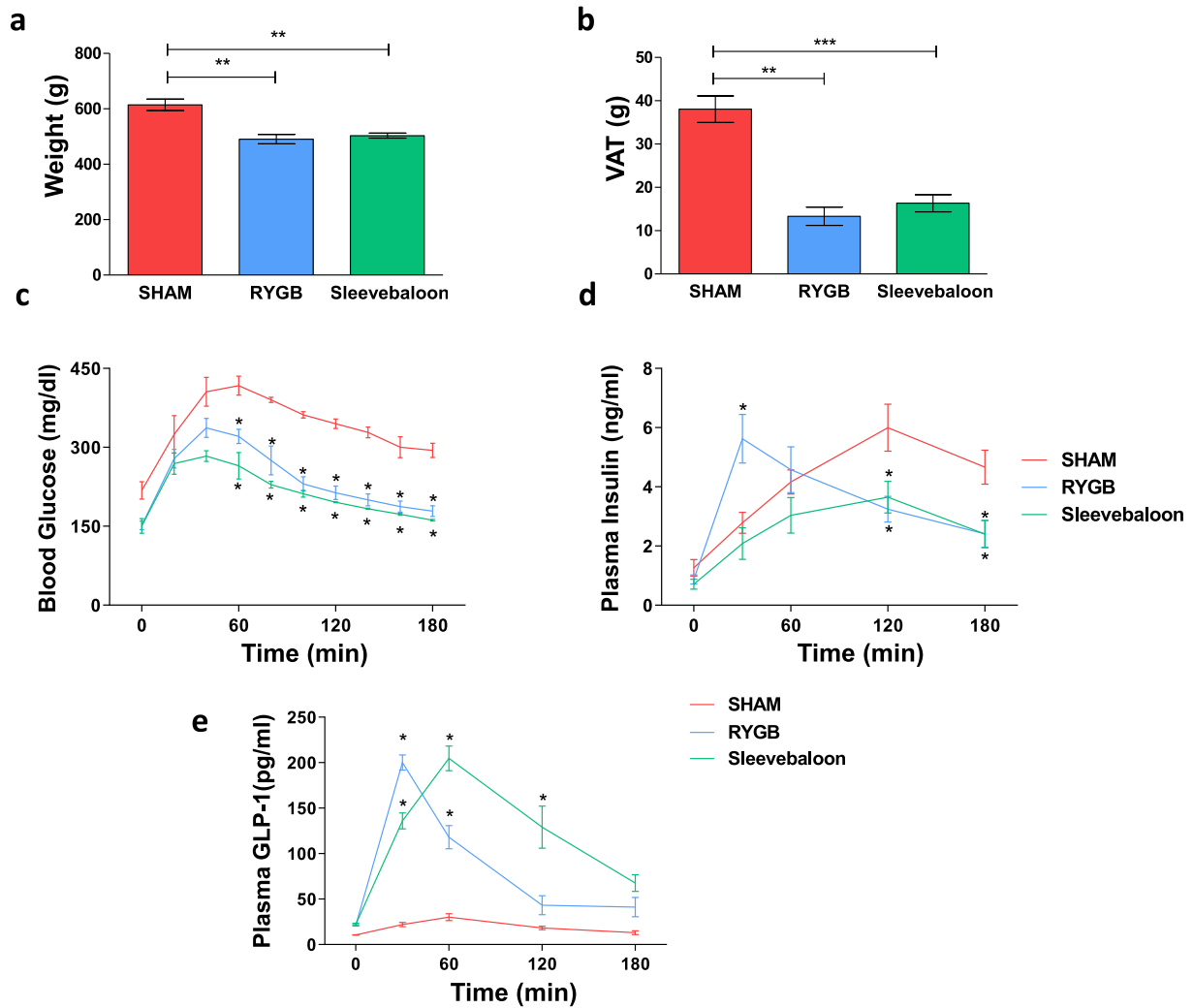
#### 3.4. Disposition index was significantly higher in the Sleeveballoon or RYGB than in sham-operated animals

HOMA-B was  $73.42 \pm 14.86$   $\mu\text{J/ml} \cdot (\text{mmol/l})^{-1}$  in the sham group,  $106.88 \pm 15.38$  in the RYGB group and  $81.33 \pm 18.10$  in the Sleeveballoon group. None of the comparisons was significant.

The disposition index was  $2.92 \pm 1.81$  in sham-operated rats vs.  $11.59 \pm 1.67$  in the RYGB group and  $11.44 \pm 2.12$  in the Sleeveballoon group ( $P = .002$  Sham vs. RYGB and  $P = .002$  Sham vs. Sleeveballoon,  $P = .998$  RYGB vs. Sleeveballoon, Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method). Therefore, the disposition index was significantly higher in the two groups of rats with Sleeveballoon or which underwent RYGB.

#### 3.5. GLP1 increases following RYGB and Sleeveballoon

GLP1 secretion is consistently increased after RYGB [19]. We measured circulating levels of GLP1 after the OGTT in the three intervention groups to verify whether also the Sleeveballoon stimulates secretion of GLP1.



**Fig. 2.** Sleeveballoon induces sustained weight loss and reduction of visceral fat. Panel a: Body weight was significantly lower in rats with the Sleeveballoon than in sham-operated rats ( $614.4 \pm 20.6$  vs.  $503.1 \pm 8.9$  g,  $P = .006$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method), while no difference was found in comparison with RYGB rats ( $490.0 \pm 17.7$  g,  $P = .60$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method). Panel b: Mesenteric and retroperitoneal fat (visceral fat) content was higher in sham-operated than in both Sleeveballoon and RYGB groups ( $38.1 \pm 3.0$  vs.  $16.3 \pm 1.9$  g,  $P = .002$  and  $38.1 \pm 3.0$  vs.  $13.3 \pm 2.1$  g,  $P = .0006$  respectively, Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method). Panels c,d: Time courses of blood glucose (c) and plasma insulin (d) after an oral glucose load ( $1$  g/kg<sub>bw</sub>) in sham, RYGB and Sleeveballoon rats. Rats undergone RYGB or Sleeveballoon placement significantly reduced both blood glucose and plasma insulin concentrations ( $P < .04$ ). Data are expressed as mean  $\pm$  SEM. Panel e: Time course of GLP1 after an oral glucose load ( $1$  g/kg<sub>bw</sub>) in sham, RYGB and Sleeveballoon rats. Rats undergone RYGB or Sleeveballoon placement significantly had a larger GLP1 secretion, although the peak in animals with the Sleeveballoon was anticipated. Data are expressed as mean  $\pm$  SEM.

**Fig. 2, Panel E** shows the time courses of GLP1 levels after the OGTT. In RYGB GLP1 levels peaked at 30 min, whereas the peak in rats with Sleeveballoon was observed at 60 min; in sham-operated rats the GLP1 curve was flat. The GLP1 AUCs in both RYGB and Sleeveballoon groups were similar and significantly higher than that in sham-operated rats ( $15,458 \pm 1604$  and  $23,396 \pm 2542$  pg/ml, respectively, vs.  $3660 \pm 376$  pg/ml;  $P < .001$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method).

### 3.6. Sleeveballoon delays gastric emptying

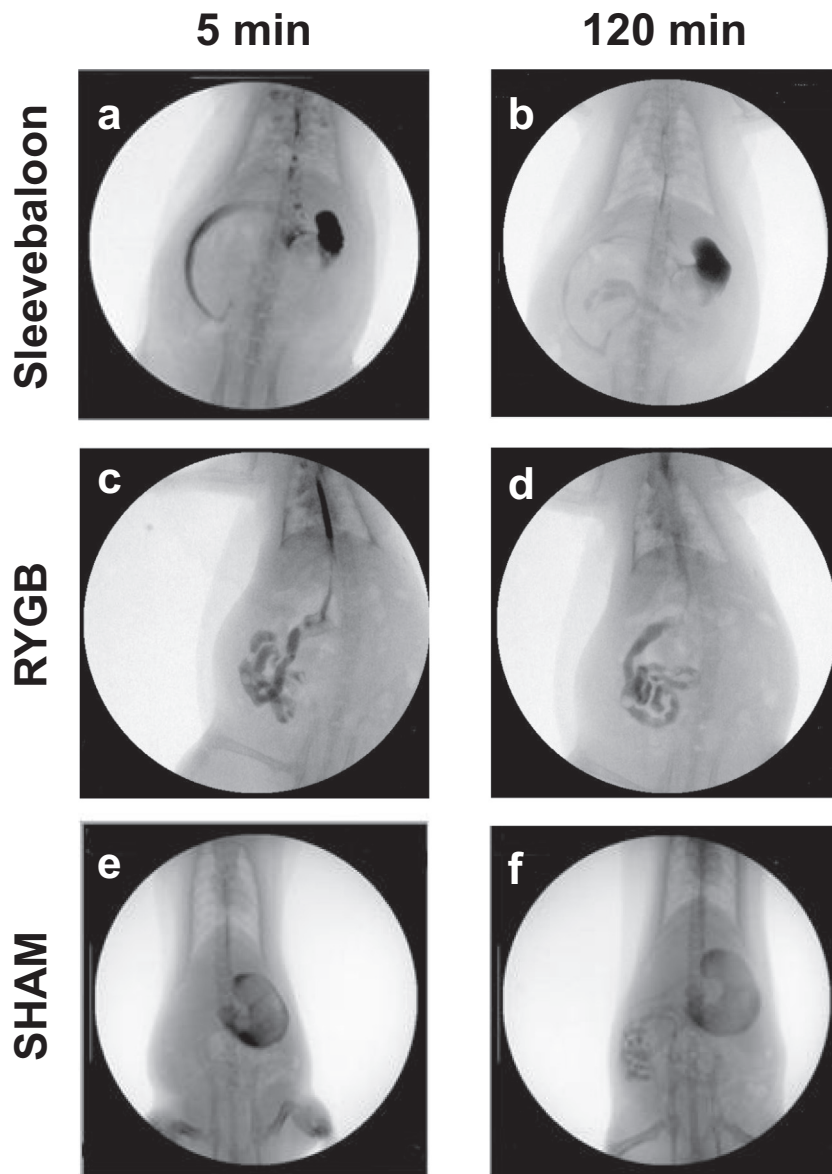
We studied the gastric emptying to verify if it was accelerated in rats with Sleeveballoon, as previously demonstrated with RYGB [20], or if, instead, it was delayed. Gastric emptying was significantly delayed in the group of rats with the sleeveballoon as compared with the group of sham-operated animals ( $T_{1/2}$   $157.7 \pm 29.2$  vs.  $97.1 \pm 26.3$  min (mean  $\pm$  SD),  $P = .007$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method). The gastric half-time emptying was very fast in the RYGB group ( $3.5 \pm 1.1$  vs.  $97.1 \pm$

$26.3$  min (mean  $\pm$  SD),  $P < .0001$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method). **Fig. 3, Panels A–C,** shows that the contrast medium enters the duodenal sleeves progressing distally over time in the animals with Sleeveballoon.

### 3.7. Insulin signaling was more efficient in the liver and in skeletal muscle tissue of rodents with the Sleeveballoon than in sham-operated rats

Insulin resistance is the primary defect of type 2 diabetes [7]. Akt signal regulates glucose metabolism in many tissues and organs, including liver, skeletal muscle and myocardium. The Akt downstream effector is GSK that, once phosphorylated, loses its inhibitory action on the enzyme glycogen synthase permitting the accumulation of glycogen in insulin-sensitive tissue and shifting the metabolism towards glucose utilization [21].

In both RYGB and Sleeveballoon groups Akt and GSK phosphorylation were significantly higher than in the sham-operated group. Akt Ser473 and GSK3 $\alpha$  $\beta$  Ser21/9 phosphorylation was higher in both liver



**Fig. 3.** Sleeveballoon delays gastric emptying. Panels a, b: Representative image of intestinal transit after 5 (a) and 120 (b) minutes from contrast medium administration in a rat with Sleeveballoon. Panels c, d: Representative image of intestinal transit after 5 (a) and 120 (b) minutes from contrast medium administration in RYGB rats. Panels e, f: Representative image of intestinal transit after 5 (a) and 120 (b) minutes from contrast medium administration in a sham-operated rat.

(Akt Ser473:  $0.9 \pm 0.2$  vs.  $0.3 \pm 0.1$ ,  $P = .02$ ; GSK3 $\alpha$  Ser21:  $0.2 \pm 0.04$  vs.  $0.1 \pm 0.01$ ,  $P = .01$ ; GSK3 $\beta$  Ser9:  $0.5 \pm 0.2$  vs.  $0.1 \pm 0.03$ ,  $P = .004$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method) (Fig. 4, Panels A and B) and skeletal muscle (Akt Ser473:  $1.2 \pm 0.1$  vs.  $0.7 \pm 0.1$ ,  $P = .02$ ; GSK3 $\alpha$  Ser21:  $1.9 \pm 0.3$  vs.  $1.0 \pm 0.1$ ,  $P = .014$ ; GSK3 $\beta$  Ser9:  $1.7 \pm 0.7$  vs.  $0.7 \pm 0.1$ ,  $P = .04$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method) of rats with Sleeveballoon than in sham-operated rats (Fig. 4, Panels C and D).

Moreover, Akt Ser473 and GSK3 $\alpha$  $\beta$  Ser21/9 phosphorylation was significantly higher in both liver (Akt Ser473:  $1.4 \pm 0.3$  vs.  $0.3 \pm 0.1$ ,  $P = .009$ ; GSK3 $\alpha$  Ser21:  $0.7 \pm 0.1$  vs.  $0.1 \pm 0.01$ ,  $P = .002$ ; GSK3 $\beta$  Ser9:  $0.5 \pm 0.1$  vs.  $0.1 \pm 0.03$ ,  $P = .008$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method) (Fig. 4, Panels A and B) and skeletal muscle (Akt Ser473:  $1.5 \pm 0.2$  vs.  $0.7 \pm 0.1$ ,  $P = .02$ ; GSK3 $\alpha$  Ser21:  $4.8 \pm 0.9$  vs.  $1.0 \pm 0.1$ ,  $P = .01$ ; GSK3 $\beta$  Ser9:  $2.9 \pm 0.6$  vs.  $0.7 \pm 0.1$ ,  $P = .0004$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method) of RYGB than in sham-operated rats (Fig. 4, Panels C and D). Fig. 5, Panels A-F,

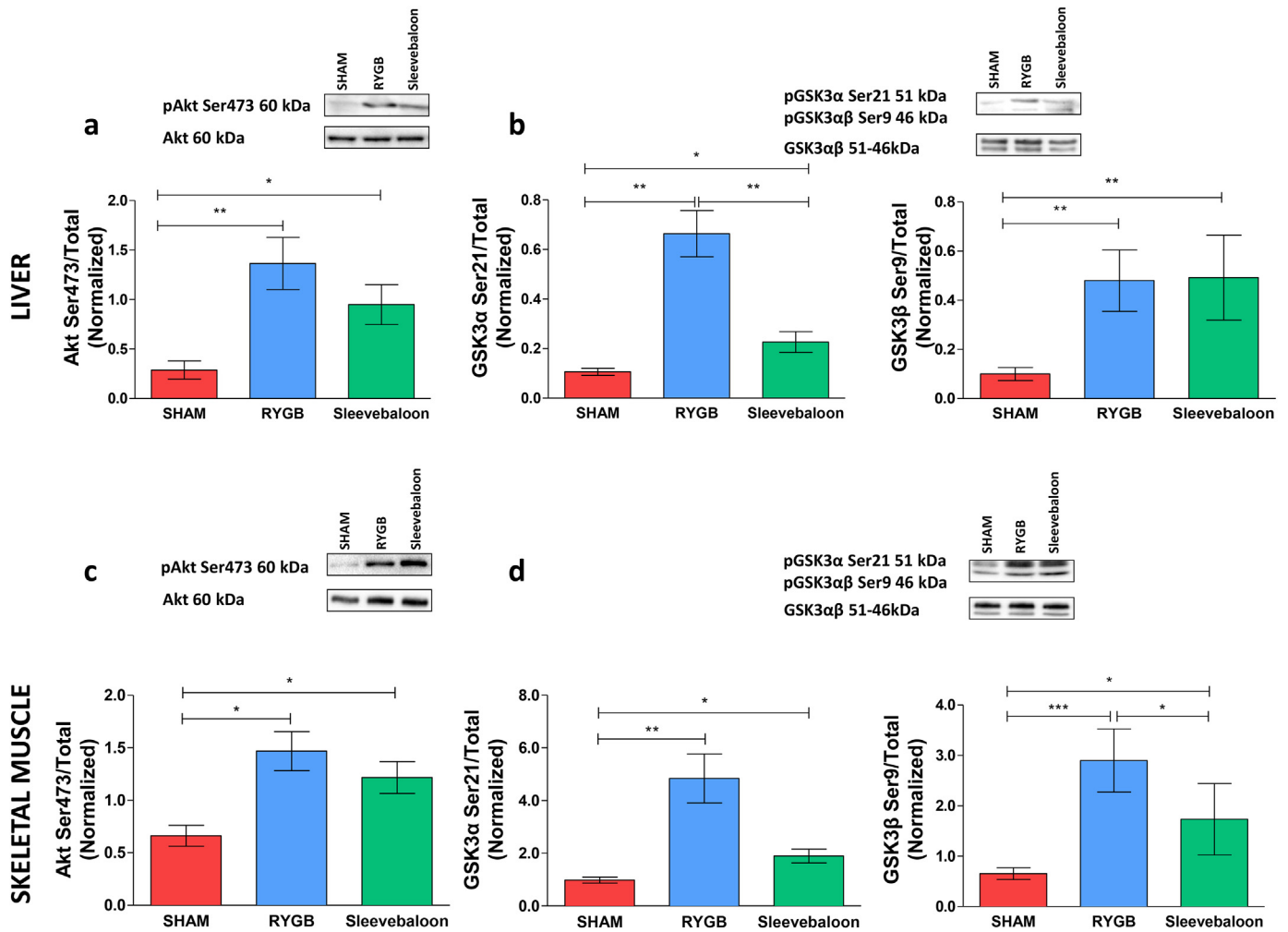
shows tissue PAS staining confirming that a more efficient insulin signaling leads to an increased glycogen accumulation in the liver and skeletal muscle of rats with either Sleeveballoon or RYGB as compared to sham-operated rats.

However, GSK3 $\alpha$  was significantly higher in the liver and GSK3 $\beta$  was significantly higher in the skeletal muscle of rats undergone RYGB than in those with the Sleeveballoon.

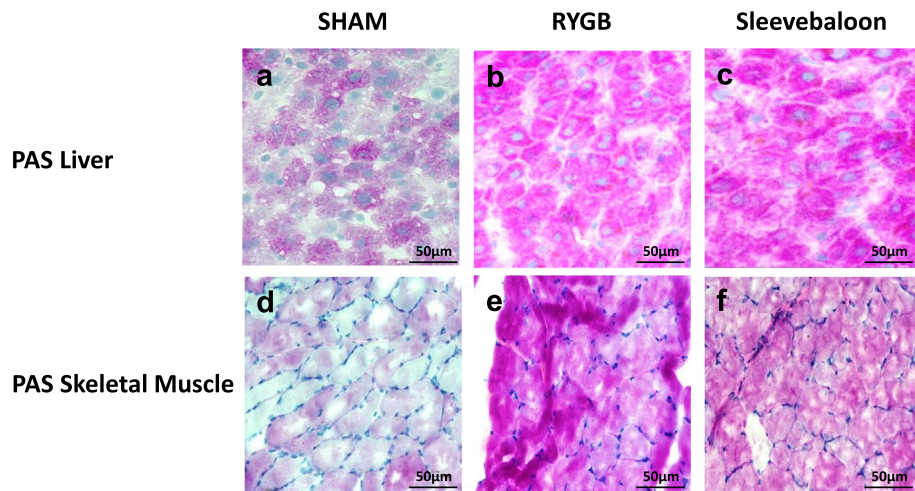
### 3.8. Sleeveballoon reduces fat accumulation and Plin2 protein expression in both liver and skeletal muscle

In many tissues, including the liver, lipid droplet coat proteins, perilipins, regulate lipid accumulation. Plin2 is an isoform expressed in the liver where it is the predominant protein surrounding cytoplasmic lipid droplets [22,23]. Plin2 deletion prevents diet-induced hepatic steatosis [24].

Therefore, we sought to measure Plin2 expression in rats with diet-induced obesity, which underwent RYGB, Sleeveballoon implantation or sham-operation.



**Fig. 4.** Insulin signaling was significantly more efficient in the liver and skeletal muscle tissue of rodents with the Sleeveballoon than in sham-operated rats. Panels a,b: Western blot analysis of Akt Ser473 and GSK3αβ Ser21/9 in the liver. Akt Ser473 and GSK3αβ Ser21/9 phosphorylation was significantly higher in the liver of both Sleeveballoon and RYGB rats. Panels c,d: Western blot analysis of Akt Ser473 and GSK3αβ Ser21/9 in the skeletal muscle. Akt Ser473 and GSK3αβ Ser21/9 phosphorylation was significantly higher in the skeletal muscle of both Sleeveballoon and RYGB rats. \* $P < .04$ , \*\* $P < .009$ , \*\*\* $P < .0004$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method. Data are expressed as mean  $\pm$  SEM.

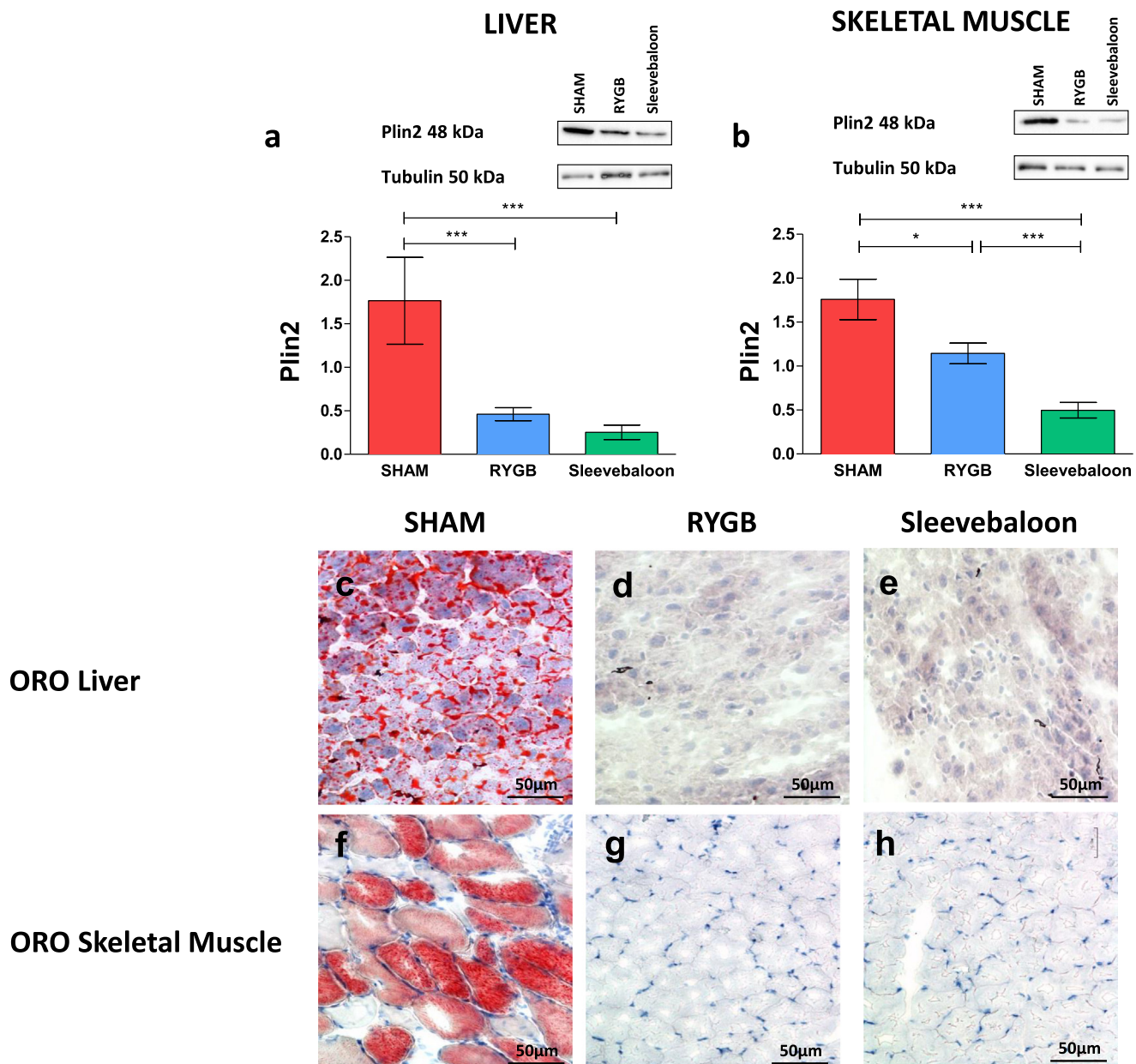


**Fig. 5.** RYGB and Sleeveballoon markedly increased hepatic glycogen depots. Panels a,b,c: Periodic acid-Schiff staining of liver biopsies from sham, RYGB and Sleeveballoon rats; both RYGB and Sleeveballoon have much larger hepatic glycogen depots. Panels d,e,f: Periodic acid-Schiff staining of skeletal muscle biopsies from sham, RYGB and Sleeveballoon rats; both RYGB and Sleeveballoon have much larger hepatic glycogen depots.

Plin2 protein expression in both liver and skeletal muscle was lower in rats with Sleeveballoon than in sham-operated rats (Liver:  $0.2 \pm 0.08$  vs.  $1.7 \pm 0.5$   $P = .0004$ ; Skeletal muscle  $0.5 \pm 0.1$  vs.  $1.8 \pm 0.2$  relative expression to  $\beta$ -actin,  $P = .0004$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method) (Fig. 6, Panels A and B). Also RYGB rats had a lower Plin2 content in both liver and skeletal muscle tissue than sham-operated rats (Liver:  $0.5 \pm 0.07$  vs.  $1.7 \pm 0.5$   $P = .0004$ ; Skeletal muscle  $1.1 \pm 0.1$  vs.  $1.8 \pm 0.2$  relative expression to  $\beta$ -actin,  $P = .02$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method) (Fig. 6, Panels A and B). Fig. 6, Panels C–H, shows the ORO staining of liver and skeletal muscle tissue. Decreased expression of Plin2 is associated with reduced lipid accumulation in both liver and skeletal muscle of rats with Sleeveballoon or RYGB as compared to sham-operated ones.

### 3.9. Sleeveballoon improves cardiac function

Obesity impairs left ventricular ejection function [25] and is an independent predictor of left ventricular hypertrophy and diastolic dysfunction, but also of cardiac failure [26]. For these reasons we performed echocardiography to study *cardiac morphology and function*. The cardiac ejection fraction was  $47.8 \pm 4.3\%$  in sham-operated rats as compared to  $82.2 \pm 5.5\%$  in the group with Sleeveballoon ( $P = .006$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method) and  $76.8 \pm 3.2\%$  in the RYGB group ( $P = .026$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method). The fractional shortening was  $26.9 \pm 7.8\%$  in the sham group versus  $44.8 \pm 8.1\%$  in the Sleeveballoon group ( $P = .013$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference



**Fig. 6.** Sleeveballoon drastically reduces fat accumulation and Plin2 protein expression in both liver and skeletal muscle. Panels a,b: Western blot analysis of Plin2 protein level, in liver and skeletal muscle biopsies of sham, RYGB and Sleeveballoon rats. Plin2 protein expression in both liver and skeletal muscle is significantly lower in rats with Sleeveballoon and RYGB than in sham-operated rats. Panels c,d,e: Oil Red O staining of liver biopsies from sham, RYGB and Sleeveballoon rats. RYGB and Sleeveballoon drastically reduced hepatic fat accumulation. Panels f, g,h: Oil Red O staining of skeletal muscle biopsies from sham, RYGB and Sleeveballoon rats. RYGB and Sleeveballoon drastically reduced skeletal muscle fat accumulation. \*\*\* $P < .0005$ , \* $P < .03$ . Data are expressed as mean  $\pm$  SEM, Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



method) and  $42.2 \pm 9.7\%$  in the RYGB group ( $P = .026$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method).

Fatty infiltration of the myocardium is regarded as an arrhythmogenic factor [27] and often preludes to cardiac failure. Consequently, we have studied the intra-myocytic fat content and Plin2 expression.

Plin2 protein expression in the heart was lower in rats with Sleeveballoon than in sham-operated rats ( $0.7 \pm 0.1$  vs  $3.5 \pm 0.6$  relative expression to  $\beta$ -actin,  $P = .014$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method). RYGB rats also had a lower myocardial Plin2 content ( $0.5 \pm 0.05$  vs  $3.5 \pm 0.6$  relative expression to  $\beta$ -actin,  $P = .014$  Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method) (Fig. 7, Panel A).

The ORO staining shows a lower intra-myocytic fat content in rats with both Sleeveballoon and RYGB as compared to sham-operated animals (Fig. 7, Panels B–D).

#### 4. Discussion

The Sleeveballoon device mimics the effects of RYGB on insulin sensitivity, glycaemic control, body weight, body fat distribution and ectopic fat accumulation. The combination of weight reduction together with the bypass of the duodenum concur to the net amelioration of both hepatic and peripheral insulin resistance observed in our study.

The disposition index, a tool to assess the efficiency of glucose homeostasis, i.e. the  $\beta$ -cells ability to regulate insulin secretion in relation to the degree of insulin sensitivity, was significantly higher in both RYGB and Sleeveballoon groups than in sham-operated rats. These

data suggest that  $\beta$ -cells glucose sensitivity was ameliorated with both RYGB and Sleeveballoon.

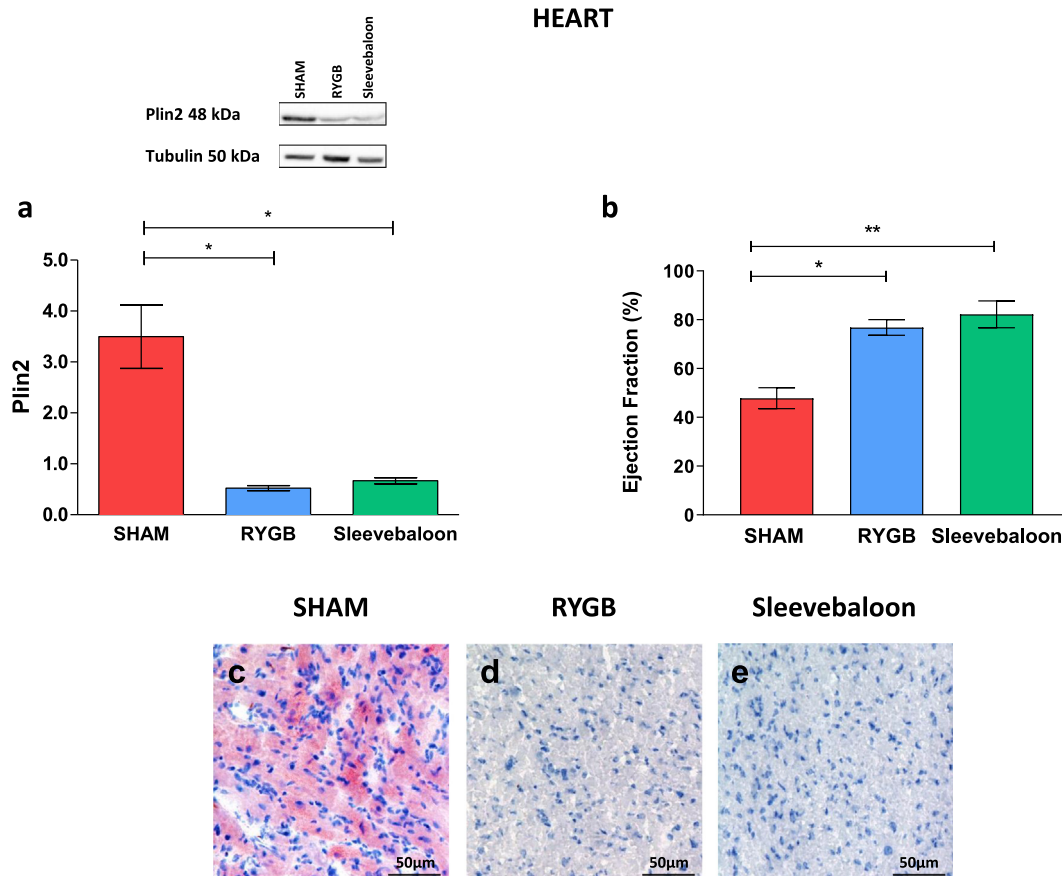
It is acknowledged that a high-fat diet induces insulin resistance by impairing the insulin-Akt signaling pathway [28]. Both Sleeveballoon and RYGB enhance Akt phosphorylation, which is mainly expressed in insulin-responsive tissues, such as the skeletal muscle where it promotes the translocation of glucose transporter 4 (GLUT4) to the cell membrane and glucose uptake [29]. The downstream effector of phosphorylated Akt is glycogen synthase kinase 3 (GSK3). Once phosphorylated, the action of GSK is inhibited and glycogen production enhanced [30]. In fact, we found a higher phosphorylation of GSK in both surgical groups and a concomitant increase of glycogen deposition in skeletal muscle and liver in comparison with sham-operated rodents.

Interestingly, rats undergone RYGB had a significantly higher GSK3 isoform in both the liver and the skeletal muscle tissue and, in fact, although only a qualitative analysis, the glycogen content seemed to be higher after RYGB than after Sleeveballoon implantation.

Improved hepatic insulin resistance with Sleeveballoon or RYGB improves also NAFLD through the reduction of liver steatosis. It was previously shown that drugs that improve insulin resistance, such as metformin [31] and the GLP1 agonist liraglutide [32] reduce liver steatosis and even improve inflammation in non-alcoholic steatohepatitis (NASH).

Weight loss and the reduction of myocyte fat deposition contributed also to the improvement of myocardial contractility. Myocardial lipid accumulation is, in fact, associated with impairment of cardiac function [33–35].

However, the shape of the glucose and insulin curves following the oral glucose load was different in rats with a Sleeveballoon compared



**Fig. 7.** Sleeveballoon reduces myocytic fat accumulation and Plin2 levels and improves left ventricular contractility function. Panel a: Plin2 levels are much reduced in the heart of rats undergone sleeveballoon placement or RYGB. Panel b: Left ventricular ejection fraction (%) is significantly higher in the RYGB and Sleeveballoon groups than in sham-operated controls. Panels c,d,e: Fat accumulation is much lower in the heart sections of rats undergone Sleeveballoon placement or RYGB. \* $P < .03$ , \*\* $P < .009$ . Data are expressed as mean  $\pm$  SEM, Mann-Whitney  $U$  test, corrected for multiple comparisons by the Bonferroni's inference method.

with RYGB. While RYGB was associated with a rapid increase in both glucose and insulin, the glucose and insulin responses were attenuated in rodents with the Sleeveballoon.

As shown by Chambers et al. [20], RYGB, where the anatomy of the stomach and duodenum is unaltered although functionally excluded, is characterized by a rapid emptying of nutrients from the gastric pouch into the jejunum. In contrast, we found that gastric emptying was delayed in the rats with Sleeveballoon in comparison with sham-operated animals. The net improvement of glucose disposal in the rats with Sleeveballoon can be attributed to the combined delayed gastric emptying and increased GLP1 secretion that explain most of the variability of the glycaemic peak following the oral glucose load.

Gastric emptying is a major determinant of postprandial glycaemia in both patients with or without T2D, explaining approximately 35% of the variance in both peak and AUC of circulating glucose [36,37]. A series of medical interventions have been employed in an attempt to reduce the rate of gastric emptying in T2D. Increased dietary fibre intake [38] or the addition of guar gum to food [39] have been demonstrated to moderately slow gastric emptying and to reduce postprandial glucose in patients with T2D. As well as improving  $\beta$ -cell glucose sensitivity, both GLP1 and GLP1 receptor agonists (GLP1-RA) slow gastric emptying [40]. Amylin and its synthetic analogue, pramlintide, also reduce gastric emptying while improving glycaemia [41]. Therefore, the mechanical slowing of gastric emptying is an important feature of the Sleeveballoon, contributing to its positive effect on glucose metabolism.

The metabolic effects of the Sleeveballoon share many similarities with those of RYGB, but the former procedure shows also some relevant advantages on RYGB. In fact, RYGB determines a rapid rise of postprandial blood glucose levels and consequently stimulates early insulin secretion with possible hypoglycaemia, a side-effect frequently described after this type of surgery [42]. In isolate cases, severe hypoglycaemia with neuroglycopenia can occur requiring drastic measures, such as partial pancreatectomy [43].

Although several studies show that laparoscopic bariatric surgery is associated with low mortality and morbidity rates [44,45], surgery still is a serious intervention, which can be fatal; for this reason minimally invasive procedures can represent a viable alternative. Indeed, devices to treat obesity and/or type 2 diabetes can remain in place only for a limited time, in general up to 1 year. However, alternating the use of devices with anti-obesity drugs can represent a valid alternative to bariatric surgery at least in high-risk patients for whom gastrointestinal surgery can be contraindicated or for patients preferring a more conservative approach.

Similarly to the EndoBarrier, the Sleeveballoon has a duodenal liner however it overcomes some safety issues arose from the former device. The most serious adverse event of the EndoBarrier is an incidence of liver abscesses ranging from 1.2% to 3.5% [46].

The EndoBarrier has anchors with barbs nitinol at its proximal end to take in place the device at the duodenal bulb distal to the pylorus. Lesions of the duodenal mucosa may have permitted the translocation of bacteria through the portal vein system to the liver causing abscesses. A concomitant cause could be the use of proton pump inhibitors reducing the efficiency of the gastric acidic barrier.

Sleeveballoon overcomes this problem since it stays in place thanks to the gastric balloon. In addition, the gastric balloon induces gastric distention and early satiety.

In spite of the robustness of the results, our study has several limitations. The Sleeveballoon is not designed to stay in place for >6–12 months with possible weight regain and vanishing of metabolic benefits. We did not investigate how long could the metabolic benefits persist after the removal of the device since this would have required a second operation. Body composition was measured post mortem and thus we did not followed its changes over time. Dual-Energy X-ray Absorptiometry (DEXA) or magnetic resonance imaging (MRI), which are non-invasive and non-destructive techniques, would have permitted to monitor body composition in the same animals.

Insulin sensitivity was assessed using a surrogate marker, HOMA-IR, instead of the golden standard euglycaemic hyperinsulinemic clamp with stable isotope infusion to measure endogenous glucose production and glucose rate of disappearance. However, changes in HOMA-IR, as a marker of hepatic insulin resistance, are extensively used to calculate the sample size in clinical trials and in animal studies [47,48]. Finally, our study was not designed and powered to assess differences among Sleeveballoon and RYGB, thus comparisons between the two interventions are considered as merely indicative.

In conclusion, the Sleeveballoon increases peripheral and hepatic insulin sensitivity, reduces body weight, slows gastric emptying and excludes food from the proximal small bowel thus reducing glycaemia, decreases ectopic fat deposition and ameliorates cardiac function.

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## Author contributions

Giovanni Casella, James Casella-Mariolo and Lidia Castagneto-Gissey, and Giulia Angelini designed the study.

James Casella-Mariolo and Lidia Castagneto-Gissey, Giovanni Casella, Pierluigi Marini, conceived and planned the experiments.

Giovanni Casella, James Casella-Mariolo, Lidia Castagneto-Gissey and Geltrude Mingrone wrote the manuscript.

Giulia Angelini performed the gene expression analyses and histology.

Andrea Zoli contributed to the analyses.

Stefan R. Bornstein, Dimitrios Pournaras, Carel W Le Roux, Francesco Rubino provided critical feedback and helped shape the research, analysis and manuscript.

## Declaration of Competing Interest

James Casella-Mariolo, Lidia Castagneto-Gissey, Giulia Angelini, Andrea Zoli, Pierluigi Marini, Stefan R. Bornstein and Giovanni Casella declares no conflict of interest.

Dimitrios Pournaras reports personal fees from Johnson & Johnson, personal fees from Novo Nordisk, other from Keyron Ltd., outside the submitted work.

Francesco Rubino reports grants and other from Keyron, during the conduct of the study; grants from NIHR- UK, grants from Ethicon Endosurgery, grants from Medtronic, personal fees from GI Dynamics, personal fees from Fractyl, personal fees from Ethicon Endosurgery, outside the submitted work.

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Guarantors' names: Giulia Angelini, Giovanni Casella and Geltrude Mingrone.

## References

- [1] Davies MJ, D'Alessio DA, Fradkin J, et al. Management of hyperglycemia in type 2 diabetes, 2018. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care* 2018;41(12):2669–701.
- [2] Davies MJ, D'Alessio DA, Fradkin J, et al. Management of hyperglycaemia in type 2 diabetes, 2018. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetologia* 2018;61(12):2461–98.
- [3] Harris LLS, Smith GI, Mittendorfer B, et al. Roux-en-Y gastric bypass surgery has unique effects on postprandial FGF21 but not FGF19 secretion. *J Clin Endocrinol Metab* 2017;102(10):3858–64.
- [4] Pourmaras DJ, Nygren J, Hagström-Toft E, et al. Improved glucose metabolism after gastric bypass: evolution of the paradigm. *Surg Obes Relat Dis* 2016;12(8):1457–65.
- [5] Casella G, Soricelli E, Castagneto-Gissey L, et al. Changes in insulin sensitivity and secretion after sleeve gastrectomy. *Br J Surg* 2016;103(3):242–8.
- [6] Guidone C, Manco M, Valera-Mora E, et al. Mechanisms of recovery from type 2 diabetes after malabsorptive bariatric surgery. *Diabetes* 2006;55(7):2025–31.
- [7] DeFronzo RA, Tripathy D. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes Care* 2009;32(2):S157–63.
- [8] Mingrone G, Panunzi S, De Gaetano A, et al. Bariatric surgery versus conventional medical therapy for type 2 diabetes. *N Engl J Med* 2012;366(17):1577–85.
- [9] Mingrone G, Panunzi S, De Gaetano A, et al. Bariatric-metabolic surgery versus conventional medical treatment in obese patients with type 2 diabetes: 5 year follow-up of an open-label, single-centre, randomised controlled trial. *Lancet* 2015;386(9997):964–73.
- [10] Schauer PR, Kashyap SR, Wolski K, et al. Bariatric surgery versus intensive medical therapy in obese patients with diabetes. *N Engl J Med* 2012;366(17):1567–76.
- [11] Schauer PR, Bhatt DL, Kirwan JP, et al. STAMPEDE Investigators. Bariatric surgery versus intensive medical therapy for diabetes – 5-year outcomes. *N Engl J Med* 2017;376(7):641–51.
- [12] Ikramuddin S, Korner J, Lee WJ, et al. Lifestyle intervention and medical management with vs without Roux-en-Y gastric bypass and control of hemoglobin A1c, LDL cholesterol, and systolic blood pressure at 5 years in the diabetes surgery study. *JAMA* 2018;319(3):266–78.
- [13] Castagneto Gissey L, Casella Mariolo JR, et al. 10-year follow-up after laparoscopic sleeve gastrectomy: outcomes in a monocentric series. *Surg Obes Relat Dis* 2018;14(10):1480–7.
- [14] Jirapinyo P, Haas AV, Thompson CC. Effect of the duodenal-jejunal bypass liner on glycemic control in patients with type 2 diabetes with obesity: a meta-analysis with secondary analysis on weight loss and hormonal changes. *Diabetes Care* 2018;41:1106–15.
- [15] Rajagopalan H, Cherrington AD, Thompson CC, et al. Endoscopic duodenal mucosal resurfacing for the treatment of type 2 diabetes: 6-month interim analysis from the first-in-human proof-of-concept study. *Diabetes Care* 2016;39(12):2254–61.
- [16] Salinari S, Carr RD, Guidone C, et al. Nutrient infusion bypassing duodenum-jejunum improves insulin sensitivity in glucose-tolerant and diabetic obese subjects. *Am J Physiol Endocrinol Metab* 2013;305(1):E59–66.
- [17] Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28(7):412–9.
- [18] Levy JC, Matthews DR, Hermans MP. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. *Diabetes Care* 1998;21(12):2191–2.
- [19] Steierner RE, Feinle-Bisset C, Asarian L, Horowitz M, Beglinger C, Geary N. Ghrelin, CCK, GLP-1, and PYY(3-36): secretory controls and physiological roles in eating and glycemia in health, obesity, and after RYGB. *Physiol Rev* 2017;97(1):411–63.
- [20] Chambers AP, Smith EP, Begg DP, et al. Regulation of gastric emptying rate and its role in nutrient-induced GLP-1 secretion in rats after vertical sleeve gastrectomy. *Am J Physiol Endocrinol Metab* 2014;306(4):E424–32.
- [21] Woodgett JR, Cohen P. Multisite phosphorylation of glycogen synthase. Molecular basis for the substrate specificity of glycogen synthase kinase-3 and casein kinase-II (glycogen synthase kinase-5). *Biochim Biophys Acta* 1984;788(3):339–47.
- [22] Liu X, Xue R, Ji L, et al. Activation of farnesoid X receptor (FXR) protects against fructose-induced liver steatosis via inflammatory inhibition and ADRP reduction. *Biochem Biophys Res Commun* 2014;450(1):117–23.
- [23] Imai Y, Varela GM, Jackson MB, Graham MJ, Crooke RM, Ahima RS. Reduction of hepatosteatosis and lipid levels by an adipose differentiation-related protein anti-sense oligonucleotide. *Gastroenterology* 2007;132(5):1947–54.
- [24] Libby AE, Bales E, Orlicky DJ, McManaman JL. Perilipin-2 deletion impairs hepatic lipid accumulation by interfering with sterol regulatory element-binding protein (SREBP) activation and altering the hepatic Lipidome. *J Biol Chem* 2016;291(46):24231–46.
- [25] Blomstrand P, Sjöblom P, Nilsson M, Wijkman M, Engvall M, Länne T, et al. Overweight and obesity impair left ventricular systolic function as measured by left ventricular ejection fraction and global longitudinal strain. *Cardiovasc Diabetol* 2018;17(1):113.
- [26] Ndumele CE, Matsushita K, Lazo M, Bello N, Blumenthal RS, Gerstenblith G, et al. Obesity and subtypes of incident cardiovascular disease. *J Am Heart Assoc* 2016;5(8):e003921.
- [27] Pantanowitz L. Fat infiltration in the heart. *Heart* 2001;85(3):253.
- [28] Whiteman EL, Cho H, Birnbaum MJ. Role of Akt/protein kinase B in metabolism. *Trends Endocrinol Metab* 2002;13(10):444–51.
- [29] Wang Q, Somwar R, Bilan PJ, et al. Protein kinase B/Akt participates in GLUT4 translocation by insulin in L6 myoblasts. *Mol Cell Biol* 1999;19(6):4008–18.
- [30] Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. *Cell* 2007;129(7):1261–74.
- [31] Feng W, Gao C, Bi Y, et al. Randomized trial comparing the effects of gliclazide, liraglutide, and metformin on diabetes with non-alcoholic fatty liver disease. *J Diabetes* 2017;9(8):800–9.
- [32] Armstrong MJ, Gaunt P, Aithal GP, et al. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. *Lancet* 2016;387(10019):679–90.
- [33] Finck BN, Lehman JJ, Leone TC, et al. A high fat diet increases mitochondrial fatty acid oxidation and uncoupling to decrease efficiency in rat heart. *Basic Res Cardiol* 2011;106(3):447–57.
- [34] Jones KL, Horowitz M, Carney BI, et al. Gastric emptying in early noninsulin-dependent diabetes mellitus. *J Nucl Med* 1996;37(10):1643–8.
- [35] Ishii M, Nakamura T, Kasai F, et al. Altered postprandial insulin requirement in IDDM patients with gastroparesis. *Diabetes Care* 1994;17(8):901–3.
- [36] Chandalia M, Garg A, Lutjohann D, et al. Beneficial effects of high dietary fiber intake in patients with type 2 diabetes mellitus. *N Engl J Med* 2000;342(19):1392–8.
- [37] Russo A, Stevens JE, Wilson T, et al. Guar gum attenuates fall in postprandial blood pressure and slows gastric emptying of oral glucose in type 2 diabetes. *Dig Dis Sci* 2003;48(7):1221–9.
- [38] Mihai BM, Mihai C, Cijevski-Prelipcean C, et al. Bidirectional relationship between gastric emptying and plasma glucose control in normoglycemic individuals and diabetic patients. *J Diabetes Res* 2018;2018:1736959.
- [39] Samsom M, Szarka LA, Camilleri M, et al. Pramlintide, an amylin analog, selectively delays gastric emptying: potential role of vagal inhibition. *Am J Physiol Gastrointest Liver Physiol* 2000;278(6):G946–51.
- [40] Capristo E, Panunzi S, De Gaetano A, et al. Incidence of hypoglycemia after gastric bypass vs sleeve gastrectomy: a randomized trial. *J Clin Endocrinol Metab* 2018;103(6):2136–46.
- [41] Patti ME, McMahon G, Mun EC, et al. Severe hypoglycaemia post-gastric bypass requiring partial pancreatectomy: evidence for inappropriate insulin secretion and pancreatic islet hyperplasia. *Diabetologia* 2005;48(11):2236–40.
- [42] Lent MR, Benotti PN, Mirshahi T, et al. All-cause and specific-cause mortality risk after Roux-en-Y gastric bypass in patients with and without diabetes. *Diabetes Care* 2017;40(10):1379–85.
- [43] le Roux CW, Heneghan HM. Bariatric surgery for obesity. *Med Clin North Am* 2018;102(1):165–82.
- [44] Ruban A, Ashrafian H, Teare JP. The EndoBarrier: duodenal-jejunal bypass liner for diabetes and weight loss. *Gastroenterol Res Pract* 2018;2018:7823182.
- [45] Bowden Davies KA, Sprung VS, Norman JA, et al. Short-term decreased physical activity with increased sedentary behaviour causes metabolic derangements and altered body composition: effects in individuals with and without a first-degree relative with type 2 diabetes. *Diabetologia* 2018;61(6):1282–94.
- [46] Gagnon C, Daly RM, Carpentier A, et al. Effects of combined calcium and vitamin D supplementation on insulin secretion, insulin sensitivity and  $\beta$ -cell function in multi-ethnic vitamin D-deficient adults at risk for type 2 diabetes: a pilot randomized, placebo-controlled trial. *PLoS One* 2014;9(10):e109607.