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Increased Glucagon Immunoreactivity in a Rat Model of Diet-Induced Obesity following Sleeve Gastrectomy

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Highlights

- Sleeve gastrectomy (SG) resulted in significant reductions in the body mass and insulin resistance of obese rats.
- SG increased the glucagon-like peptide-1, GIP, and glucagon responses to a standard test meal.
- The intracellular liver lipid content of the SG rats was lower than that of obese rats.

Keywords
Sleeve gastrectomy · Glucagon-like peptide-1 · Glucose-dependent insulinotropic polypeptide · Glucagon

Abstract
Objective: Bariatric surgery is currently the most effective treatment for obesity, and procedures such as Roux-en-Y gastric bypass and sleeve gastrectomy (SG) also result in rapid improvements in insulin sensitivity and glucose tolerance. In addition, these procedures cause changes in the secretion of various gut-derived hormones. The role these hormones play in the mechanism of the beneficial effects of bariatric surgery is still debated, but nonetheless, their importance provides inspiration for novel obesity-targeted pharmacotherapies. ***Methods:*** Male Sprague-Dawley rats were fed either regular chow or a cafeteria diet to induce obesity. A sub-group of the obese animals then underwent either sham surgery or SG. ***Results:*** Following a 4-week recovery period, SG rats weighed significantly less than obese or sham-operated rats. Improvements in glucose tolerance and insulin sensitivity also occurred in the SG group, but these

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were not always statistically significant. We measured the intracellular lipid content of liver samples and found that obese rats showed signs of non-alcoholic fatty liver disease, which were significantly ameliorated by SG. There were significantly higher glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide responses to a standard mixed meal in the SG group, as well as paradoxically higher glucagon secretion. ***Conclusion:*** These data highlight the need for more specific anti-glucagon antibodies to characterize the changes in proglucagon-derived peptide concentrations that occur following SG. Further studies are required to determine whether these peptides contribute to the therapeutic effects of SG.

Introduction

Obesity is a debilitating chronic condition that causes or exacerbates other comorbidities, such as type 2 diabetes mellitus (T2DM), non-alcoholic fatty liver disease (NAFLD), cardiovascular disease, musculoskeletal disorders, and cancer [1]. Owing to the rapid global increase in the prevalence of this condition, there is an urgent need for more effective pharmacotherapy. Currently, the most efficacious treatment for obesity is bariatric surgery [2]. Procedures such as Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG) not only cause long-lasting weight loss but also often result in rapid improvements in glucose tolerance and insulin sensitivity [3]. These rapid improvements in glycemic and appetite control are thought to, at least in part, be the result of changes in gut hormone release [4].

Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), the so-called incretin hormones, are released from the gastrointestinal tract in response to nutrient ingestion and potentiate insulin secretion in a glucose-dependent manner [5]. Both hormones have pleiotropic effects. For example, GLP-1 suppresses both appetite and glucagon secretion, and GIP is an important regulator of bone and lipid metabolism [6]. A loss of the effects of both GLP-1 and GIP is an early feature of T2DM [7]. However, although pharmacological concentrations of GLP-1 potentiate insulin secretion in patients with T2DM, the same is not true for GIP [8], and several GLP-1 receptor agonists are currently used to treat both T2DM and obesity [9]. Both RYGB and SG cause a large increase in the GLP-1 response to feeding, but the extent to which this contributes to improving glucose control and weight loss is still being investigated. However, data generated in studies using the GLP-1 receptor antagonist exendin (9–39) should be interpreted carefully because GLP-1 also suppresses glucagon secretion [10].

High concentrations of glucagon contribute to the pathology of T2DM by promoting hepatic gluconeogenesis and glycogenolysis [11]. Surprisingly, several recent studies of the changes in glucagon secretion following bariatric surgery have shown a paradoxical increase in glucagon secretion [12, 13]. Although glucagon causes an increase in blood glucose concentration, it also causes increases in metabolic rate and energy expenditure [14]. The administration of GLP-1 is thought to be balanced by the effect of glucagon on glycemia [15]. Furthermore, the naturally occurring peptide oxyntomodulin acts as an agonist at both the GLP-1 and glucagon receptors [16].

In the present study, we have quantified the changes in GLP-1, GIP, and glucagon responses to a standard mixed meal in a rat model of diet-induced obesity (DIO). We hypothesized that SG would ameliorate the adverse metabolic effects of cafeteria diet feeding and cause increases in the GLP-1, GIP, and glucagon responses to a standard mixed meal.

Materials and Methods

Study Design

Male Sprague-Dawley rats were supplied by and maintained at the Health Sciences Center Animal Resource Center. At the age of 4–5 weeks, the rats were divided into two groups: a control group and a DIO group. The rats were housed in cages in pairs under 23 ± 1°C and a 12:12 h light/dark regime. The number of animals per group was determined based on the results of a previous study [17]. The primary endpoint was a 10% decrease in the body weight in the SG group compared to the sham-operated group. In this case, the mean body weight of the previous sham group was 576.3 +/− 41 g. The result of power calculations gave a sample size of $n_1 = 8$, where $\alpha = 0.05$, $\beta = 0.2$, and power = 80%. Unfortunately, one of the SG animals died during oral glucose tolerance testing (OGTT), resulting in the number of animals in each being control = 8, obese = 8, SG = 7, and sham = 8. The control rats ($n = 8$) were provided with regular rat chow and tap water ad libitum, and the DIO group (obese, SG, and sham groups combined, $n = 23$) was provided with a cafeteria diet (a high-fat, high-carbohydrate diet) ad libitum, as previously described [17]. Briefly, this diet included a variety of highly palatable foods, flavored milk, and 20% sugar water (online suppl. Table. 1, 2; for all online suppl. material, see https://doi.org/10.1159/000533746). The body mass and food intake of the rats were determined weekly for 25 weeks during the mid-portion of the light cycle. Pre-weighed food was placed in the food hoppers, and food intake was calculated on a per-cage basis as the number of grams consumed per week (online suppl. Tables 3 and 4). The rats were fed their respective diets for 17 weeks. The study was performed as per the guidelines of the Ethics Committee of the Research, Health Sciences Center, Kuwait University, for the care and use of animals.

Bariatric Surgery and Euthanasia

After 21 weeks, the rats in the DIO group were randomly subdivided into DIO-obese ($n = 8$), DIO-sham ($n = 8$), and DIO-SG ($n = 7$) groups. The DIO rats weighed at least 20% more than the control rats. They were housed individually in raised wire-floored cages suspended over a collection tray (Tecniplast®, Buguggiate, Italy); solid food and water were withdrawn for 12–15 h and 2–3 h, respectively, prior to surgery. All surgical procedures were performed using a strict aseptic technique with the aid of a surgical microscope (Leica Wild M690, Leica Microsystems, Wetzlar, Germany). An electric blanket was used to maintain the rectal temperatures of the rats at 37°C ± 0.5°C throughout the procedure. Artificial tear ointment was applied to the eyes of the rats to prevent corneal abrasion and infection. Anesthesia was induced and maintained using 5% and 2.5% isoflurane, respectively. Baytril® (Bayer Healthcare, Berlin, Germany) (enrofloxacin 10%) 5 mg/kg, Tramal® (Grunenthal GmbH, Aachen, Germany) 0.1 mg/kg, and 20 mL 0.9% saline were injected subcutaneously.

SG was performed as previously described [18], with minor modifications. Briefly, the cranial abdominal skin of the rats was shaved and cleaned with a povidone-iodine scrub solution and 70% alcohol, and the animals were wrapped with sterile Sterogauze Tubular Gauze Bandage #56 (Medisave, Weymouth, UK). A 3-cm midline skin incision was made, which was followed by laparotomy. The stomach was exposed and exteriorized, and a retaining suture was placed at the edge of the glandular part of the greater curvature. The omental bursa was bluntly dissected, and the caudal surface of the stomach was reflected. The stomach, greater curvature, and fundus were mobilized using 8–0 metric size 4 suture ligatures, and another two stay sutures were inserted into the antrum and fundus of the freed greater curvature to stretch and flatten the stomach. The stomach from the antrum to the rumen was captured between two staggered rows of DST Series™ (Covidien GIA stapler, Mansfield, MA, USA) titanium staples (60 mm in length, 2.5 mm initial, and 1 mm closed height [Covidien LLC]) inserted using a linear cutting stapler to resect 90% of the rumen and 70% of the glandular stomach along the greater curvature. The omentum was placed over the staple line of the resected stomach for the adhesions formed to help seal the stomach and prevent leakage. The abdominal wall was then sutured in two layers using 0/3 silk. The mean duration of the SG surgery was 39.4 ± 4.2 min. Sham surgery consisted only of laparotomy.

Following surgery, the animals were returned to wire-floored cages and were administered saline subcutaneously for 72 h; sips of water were given after 72 h and water was fully reintroduced ad libitum after 96 h. After 120 h, a liquid diet and semi-solid cafeteria food were introduced, and after 7 days, the rats were reintroduced to the solid cafeteria diet until 30 days post-surgery, as described previously. According to Shah and Shin [18], both bariatric and sham surgery cause decreases in food intake for approximately 3–4 weeks, during which period the animals recover from the surgery and resume their normal eating pattern. We used the same protocol in previous studies to differentiate the effects of SG from those of initial post-surgery caloric restriction [17, 19].

After 25 weeks, the rats were euthanized in accordance with the ethical guidelines. Briefly, they were anesthetized and then decapitated; their livers were then dissected, placed into tubes containing 1× sterile, filtered phosphate-buffered saline (pH 7.4 ± 0.2), and fixed in 10% neutral buffered formalin for 24–48 h prior to paraffin embedding.

Blood Sampling and Collection

OGTT was performed after 24 weeks of diet feeding. Briefly, after a designated fasting period of 8–12 h, a bolus of glucose (2 g/kg) was delivered to the stomach via polyurethane feeding tubes (16-gauge, 38-mm long, Instech Laboratories, Inc., Plymouth Meeting, PA, USA), and then blood samples were collected by tail snip 0, 15, 30, 60, and 120 min later. Blood glucose concentration was measured using an On Call Advanced Blood Glucose Meter (ACON Laboratories, Inc., San Diego, CA, USA). After 24 weeks, the rats were anesthetized using 5% and maintained using 2% isoflurane inhalation (Parkland Scientific® V3000PK), and a silastic catheter filled with heparin-glycerol lock solution was inserted into the left jugular vein until the meal challenge was performed after 25 weeks.

The meal challenge was performed after 25 weeks of diet feeding, as described previously. Briefly, after a designated fasting period of 8–12 h, a bolus of chocolate milk (5 mL) was delivered into the stomach using a polyurethane feeding tube (16-gauge, 38-mm long, Instech Laboratories, Inc.) and 400 µL of blood was collected after 0, 15, 30, 60, and 120 min. These samples were immediately centrifuged at 2,500 *g* for 10 min and plasma samples were obtained and stored at −80°C until assayed.

Plasma GLP-1 concentration was measured using a commercially available enzyme-linked immunosorbent assay (Cat no: EZGLP1T-36K, Millipore, Billerica, MA, USA), according to the manufacturer's instructions. Glucagon, insulin, and GIP concentrations were measured using a Milliplex Map Rat Metabolic Hormone Magnetic Bead Panel (Cat no: RMHMAG-84K, Millipore), according to the manufacturer's protocol.

Histologic Analysis

Formalin-fixed paraffin-embedded liver samples were sectioned (4–5 µm), hematoxylin and eosin-stained, and their histology analyzed. Briefly, sections were deparaffinized in xylene (Sigma-Aldrich, St. Louis, MO, USA) and rehydrated by passing through a concentration gradient of ethanol (100%, 95%, and 75%) (Fisher Scientific, Waltham, MA, USA). After 15 min of immersion in distilled water, the sections were stained in hematoxylin solution (Sigma-Aldrich) for 10 min, washed in running tap water for 5 min, immersed in eosin solution for 1 min, and washed several times in distilled water. Dehydration was then performed by immersing the sections in an ethanol concentration gradient (75%, 95%, and 100%), and then they were immersed in xylene. Finally, the sections were mounted using DPX solution and examined using a light microscope.

Data Analysis

The areas under the curves (AUCs) were calculated using the trapezoid rule with the baseline set at time zero with GraphPad Prism 8.0 (San Diego, CA, USA) software. Differences between groups were analyzed using one-way analysis of variance (ANOVA) and Tukey's post-hoc test using GraphPad Prism 8.0. Results were considered statistically significant when $p < 0.05$.

Results

The rats were weighed weekly while consuming either a cafeteria diet or regular chow ad libitum. After 21 weeks, the rats fed the cafeteria diet weighed

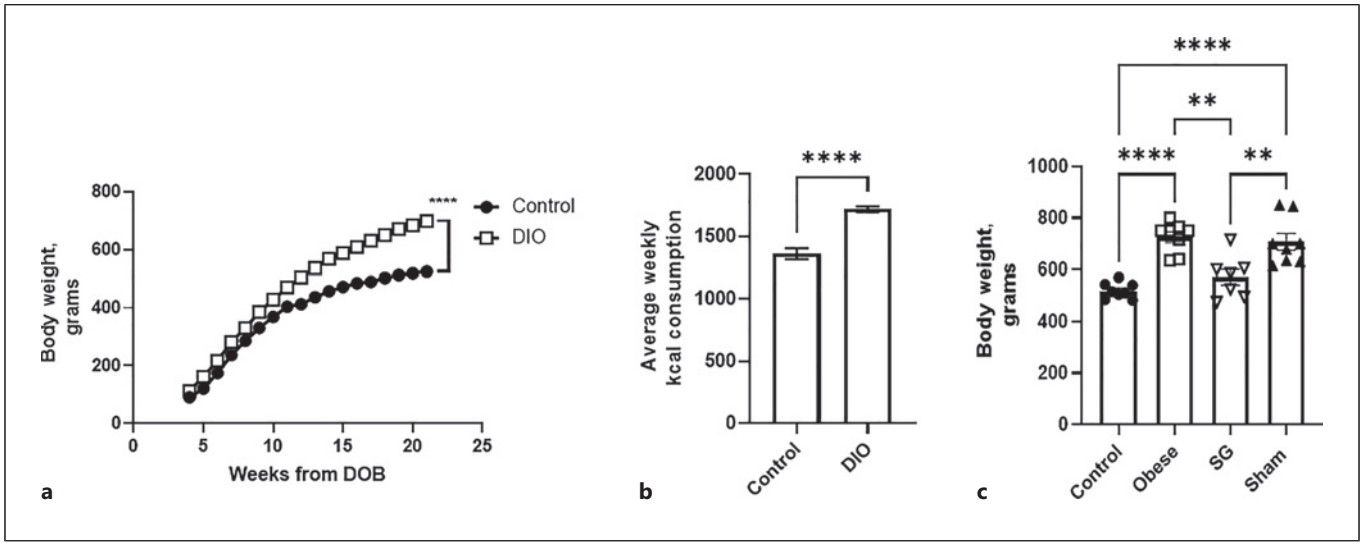

Fig. 1. Body mass before and after surgery. **a** Body mass over time of rats fed regular chow (control group) and those fed a cafeteria diet (diet-induced obesity group). **b** Mean weekly food consumption expressed in kcal. Data shown are the mean ± standard error of the mean for at least seven animals. **c** Rats in the DIO group were allocated to obese, sham, and SG groups. Body mass at week 25, 30 days post-surgery. Data shown are the individual values, with the mean ± standard error of the mean shown as error bars. $^{**}p < 0.01$, $^{****}p < 0.001$.

significantly more than the control rats ($p < 0.0001$) and were consuming significantly more food in terms of kcal ($p < 0.0001$) (Fig. 1a, b). Four weeks post-surgery, there was no significant difference in the body masses of the rats in the groups *vs*. their preoperative masses, except with respect to the SG group, which weighed significantly less ($p < 0.05$) than prior to surgery. Following surgery, the SG rats weighed significantly less than either obese controls or sham-operated rats ($p < 0.01$). There was no significant difference in the body masses of the lean control or SG rats (Fig. 1c).

Oral Glucose Tolerance Testing

OGTT was performed on rats in all the groups at week 24. The AUCs of the blood glucose concentration against time curves were calculated (Fig. 2a, b). The AUC for the obese rats was significantly ($p < 0.005$) larger than that for the control rats, as were the AUCs for the sham and SG rats, although to a lesser extent ($p < 0.05$). There were no significant differences between the fasting blood glucose concentrations between groups, but those of the SG rats at the 15-min time point were significantly ($p < 0.01$) larger than those of the other rats, and at the 120-min time point, the obese group had significantly ($p < 0.01$) higher blood glucose concentrations than the control group.

Responses to a Standard Test Meal

The blood glucose concentrations of the rats following a standard test meal, plotted against time, are shown in Figure 3a. The AUCs were calculated for these data (Fig. 3b), and the AUC for the obese group was significantly ($p < 0.01$) larger than those for both the control and sham groups but not for the SG group. There was no significant difference between the AUC for the control group and that for the SG group. The insulin response to a standard test meal, plotted against time, is shown in Figure 3c. The AUC for the sham group was significantly ($p < 0.05$) larger than those for the control and SG groups (Fig. 3d), but the time course for this response differed. The SG group showed a peak at 15 min, unlike the control group, and the insulin concentrations between 30 and 120 min had returned to normal. The insulin concentrations of the obese and sham groups remained high and were significantly ($p < 0.05$) higher than those of the control group after 120 min.

Although there were no significant differences in the fasting glucose concentrations between the groups (calculated using the data shown in Fig. 3a), the fasting insulin concentrations of the sham group were significantly higher than those of the control and SG groups ($p < 0.05$) (Fig. 3e). Insulin resistance assessed using the homeostasis model of assessment-insulin resistance (HOMA-IR) showed a similar trend, with the sham group being significantly ($p < 0.05$) more insulin resistant than both the control and SG groups (Fig. 3f).

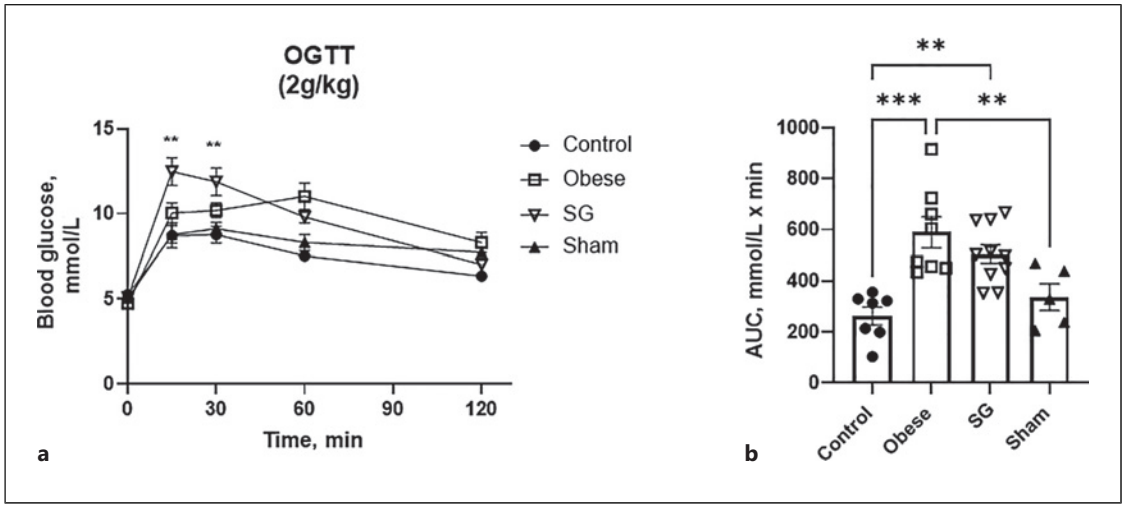

Fig. 2. a Results of oral glucose tolerance testing (OGTT) performed on the control, obese, SG, and sham groups of rats. The blood glucose concentrations were significantly higher (**$p < 0.01$) at the 15- and 30-min time points for the SG group than for all the other groups. **b** Area under the curve (AUC), derived from Figure 2A. Values are the mean ± standard error of the mean. *$p < 0.05$, ***$p < 0.005$.

The AUC for the GIP response was significantly ($p < 0.05$) larger in the SG group than in the control and sham groups but not in the obese group. However, the GIP concentrations were significantly ($p < 0.05$) larger for the SG group at the 15- and 30-min time points than for all other groups (Fig. 4a, b). Similarly, there was a significantly larger AUC ($p < 0.05$) for the GLP-1 response to a standard test meal (Fig. 4c, d) for the SG group than for all the other groups. The plasma concentrations of glucagon in response to a standard test meal are shown in Figure 4e. The AUC for the glucagon response was significantly ($p < 0.05$ or $p < 0.01$) larger for the SG group than for all the other groups (Fig. 4e).

Hematoxylin and eosin staining of liver samples showed that rats fed a cafeteria diet had significantly ($p < 0.001$) higher intracellular lipid content than lean control animals, which is indicative of NAFLD. SG resulted in significantly lower intracellular liver lipid content *vs*. the obese and sham groups ($p < 0.01$ or $p < 0.001$), but this was still significantly higher ($p < 0.005$) than that of the control group (Fig. 5a–i).

Discussion

In the present study, we used a rat model of DIO to characterize the effects of SG on the insulin, incretin hormone, and glucagon responses to a standard test meal and on the extent of lipid accumulation in the liver. Rats that had ad libitum access to a cafeteria diet weighed approximately 35% more than those fed regular chow. Following surgery, the rats that had undergone SG weighed significantly less than the obese controls or sham-operated rats. These data are consistent with those of previous studies that used this model or studies that used other animal models to study the effects of bariatric surgery [17, 20]. However, it is unclear if the weight loss that occurred in the SG group was the result of lower calorie intake or if SG had a direct effect on increasing energy expenditure. Two limitations of the present study were that food consumption was not recorded following the surgery and that we did not have access to equipment for the measurement of changes in energy expenditure. The areas under the OGTT curves for the obese and sham-operated group animals were significantly larger than those for lean control rats, suggesting that the cafeteria diet induced impairment in glucose tolerance. The AUC for the SG group did not significantly differ from those for the obese or sham groups and was significantly larger than that for the lean control group. In addition, the glucose excursion profiles were quite different. The SG group had significantly higher blood glucose concentrations at the 15-min time point than the other groups, probably because the surgery caused an increase in glucose absorption. This is consistent with the findings of previous studies [17].

A standard test meal (5 mL chocolate milk) was used to characterize the hormonal responses to feeding because, as well as glucose, amino acids and lipids also stimulate

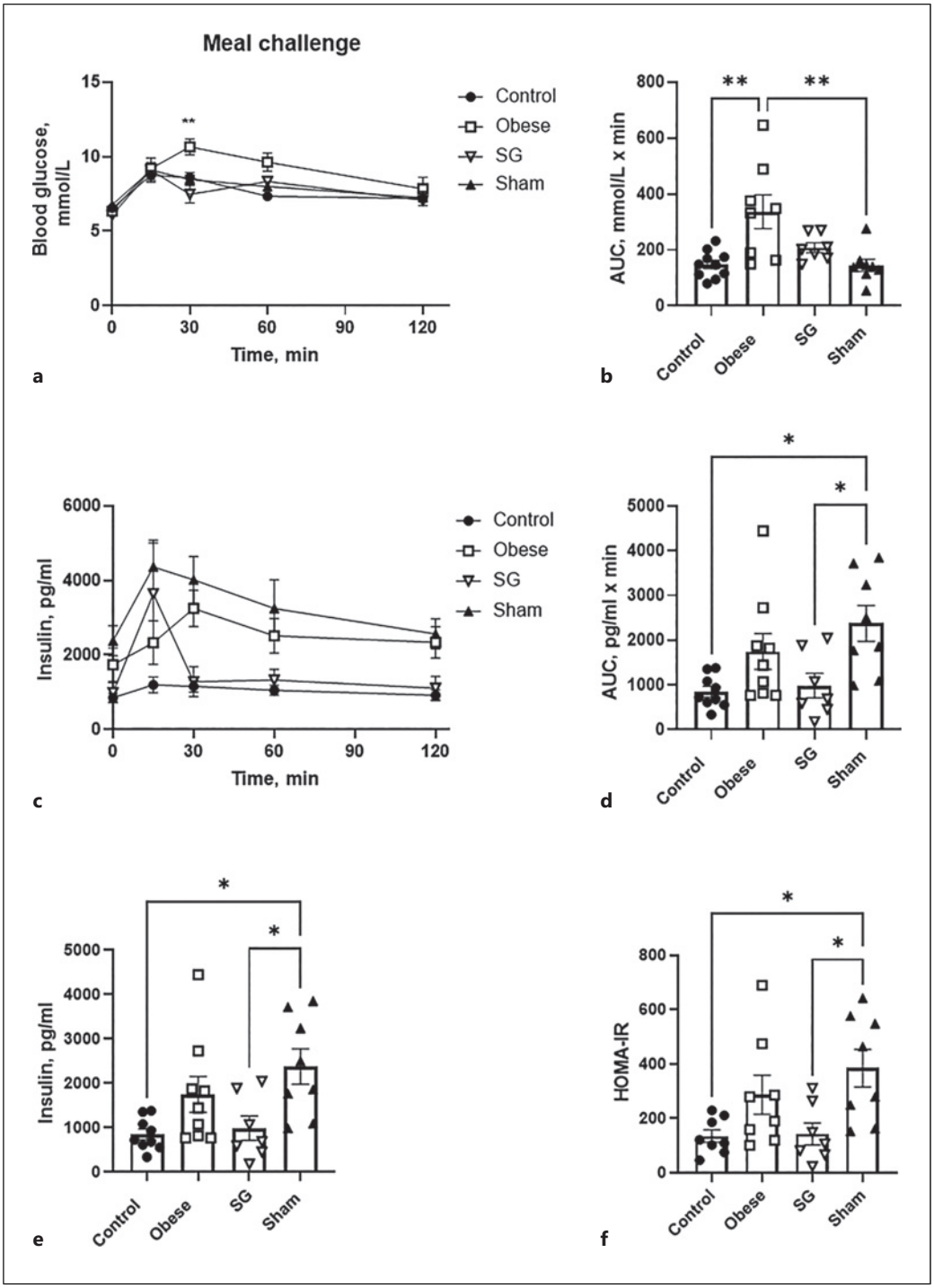

Fig. 3. a Blood glucose concentration against time for the control, obese, sham, and SG groups, following a standard test meal. **b** AUC derived from the data shown in Figure 3A. **c** Insulin concentration against time for the control, obese, SG, and sham groups following a standard test meal. **d** AUC derived from Figure 3C. **e** Fasting insulin concentrations of the control, obese, sham, and SG groups. **f** Homeostatic model assessment-insulin resistance (HOMA-IR) calculated using the fasting insulin concentrations shown in Figure 3E and the fasting glucose concentrations shown in Figure 3A. Values are the mean ± standard error of the mean. *$p < 0.05$, **$p < 0.01$.

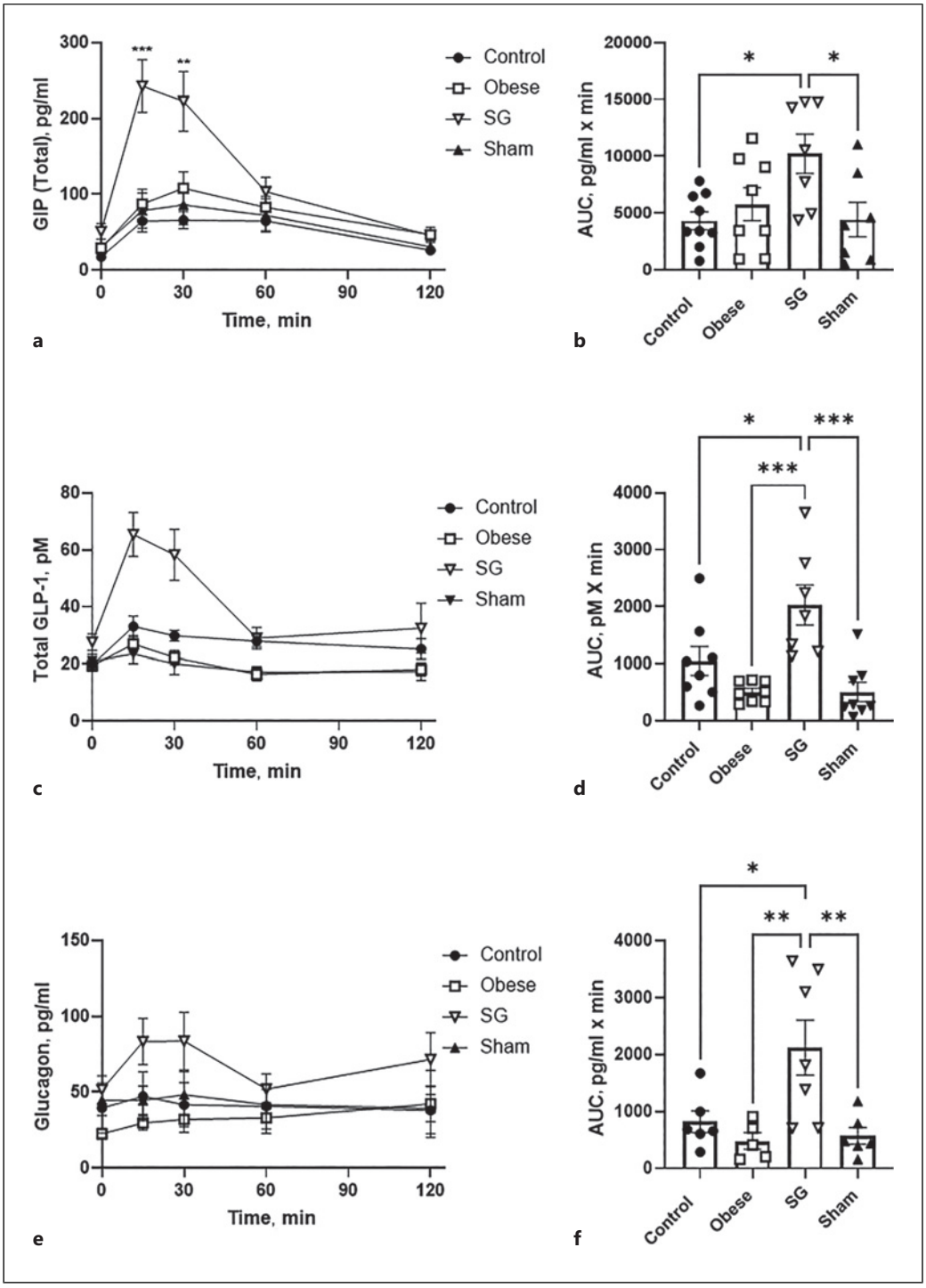

Fig. 4. a GIP concentration against time for the control, obese, SG, and sham groups following a standard test meal. The GIP concentrations were significantly higher at the 15- (***$p < 0.005$) and 30- (**$p < 0.01$) min time points for the SG group than for all the other groups. **b** AUC derived from Figure 4A. **c** Total GLP-1 concentration against time for the control, obese, SG, and sham groups following a standard test meal. **d** AUC derived from Figure 4C. **e** Glucagon concentration against time for the control, obese, sham, and SG groups following a standard test meal. **f** AUC derived from Figure 4E. Values are the mean ± standard error of the mean. *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$.

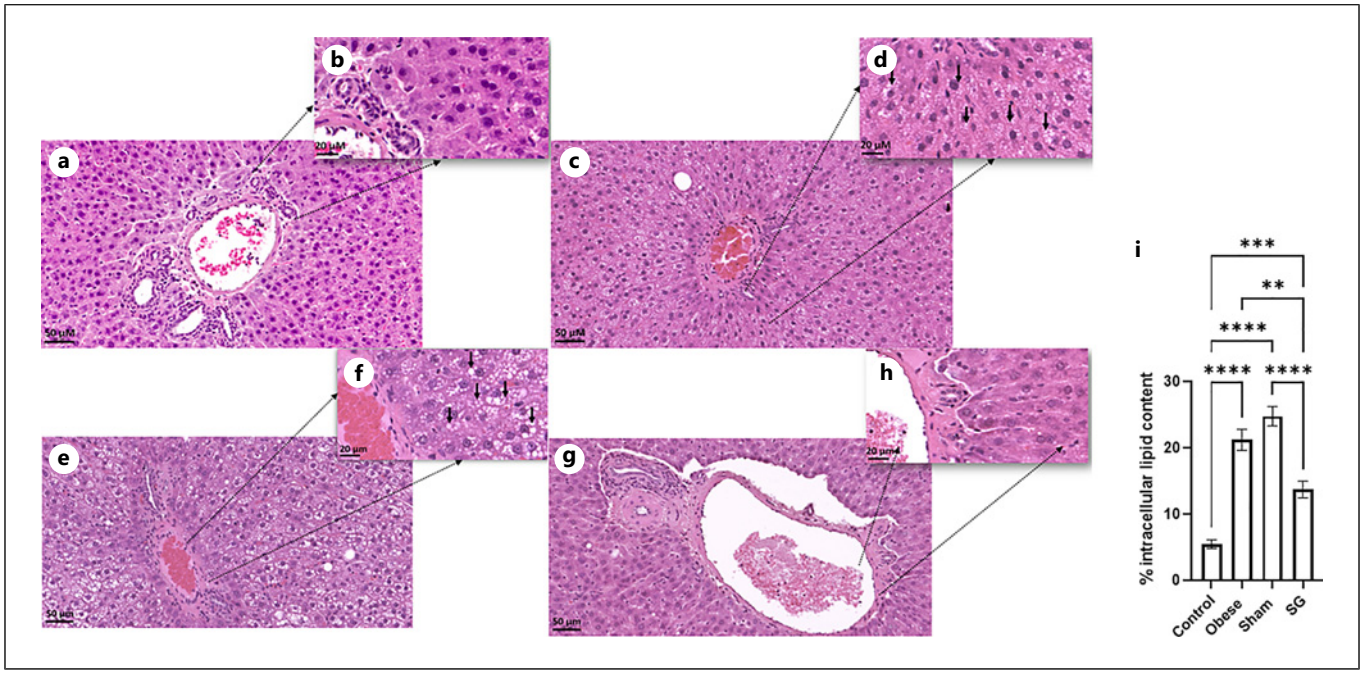

Fig. 5. a Hematoxylin and eosin (H&E)-stained liver section from a control rat (×20). **b** ×63 magnification of the region shown with arrows. This illustrates the low intracellular lipid content in the form of very few cytoplasmic perinuclear white lipid vacuoles. **c** H&E-stained liver section from an obese rat (×20). **d** ×63 magnification of the region shown with arrows. This illustrates the presence of substantial intracellular lipid content in the form of many cytoplasmic perinuclear white lipid vacuoles. **e** H&E-stained liver section from a sham-operated rat (×20). **f** ×63 magnification of the region shown with arrows. This illustrates the presence of substantial intracellular lipid content in the form of many cytoplasmic perinuclear white lipid vacuoles. **g** H&E-stained liver section from the SG group (×20). **h** ×63 magnification of the region shown with arrows. This shows significantly less intracellular lipid content, in the form of fewer cytoplasmic perinuclear white lipid vacuoles, than in the obese and sham-operated groups. **i** Percentage intracellular lipid droplet content of liver tissue. Values are the mean ± standard error of the mean. $^{**}p < 0.01$, $^{****}p < 0.001$. Scale bars: 50 μm for ×20 images and 20 μm for ×63 images.

the release of incretin hormones [21]. In contrast to the results of OGTT, only the obese group had a significantly larger blood glucose AUC than lean controls. It is unclear why the sham group did not have a large blood glucose AUC. However, one possible explanation is that the stress of the sham surgery caused an exaggerated insulin response to the standard test meal (see below). Although we did not expect the SG group to show a large blood glucose AUC, previous work has shown a peak in blood glucose concentration at the 15-min point, which is followed by a sharp decrease [17]. Such extreme changes in blood glucose concentrations over time were not identified in the SG group in the present study.

Although the AUC for insulin tended to be larger for the obese group than for lean controls, it was only significantly larger for the sham group. A similar pattern was obtained for fasting insulin concentration and insulin resistance, assessed using HOMA-IR. SG appeared to ameliorate insulin resistance because the fasting insulin concentration, HOMA-IR, and AUC for this group were significantly lower than those for the sham group. There were no significant differences in these parameters between the obese and sham groups. It is possible that the deterioration in insulin sensitivity was only more significant for the sham group than for the SG and control groups, owing to a combination of cafeteria diet consumption and the stress of the sham surgery. In addition, there was a rapid spike in insulin concentration at the 15-min time point in the SG group, which was most likely a result of increased incretin hormone release induced by SG.

An increase in GLP-1 response is a well-documented result of SG [4]. Consistent with these findings, the GLP-1 AUC was significantly larger in the SG group than in all the other groups. The contribution that GLP-1 makes to the early, weight loss-independent improvements in β-cell function and insulin sensitivity following bariatric surgery is still debated [10]. Studies using the GLP-1 receptor antagonist exendin (9–39) in patients that had undergone RYGB surgery suggested that this may be the

case, but the fact that GLP-1 also inhibits glucagon release should be considered in interpreting these data. In contrast, the effect of bariatric surgery on post-prandial GIP secretion is unclear. Some studies have shown an increase, others a decrease, and others no change [17, 22]. We have previously reported no change in GIP response following SG in the same rat model of DIO [17]; however, in the present study, we found a significant increase in GIP response, especially at the 15- and 30-min time points. The reasons for this discrepancy are unclear, but the differences may result from differences in the surgical technique or the overall health of the animals.

We identified a paradoxical increase in glucagon secretion following SG. Although other studies have also shown an increase in glucagon secretion following SG, its significance is only beginning to be appreciated [12]. However, these observations must be interpreted carefully because GLP-1 and glucagon are both post-translational products of the proglucagon gene. In pancreatic α-cells, tissue-specific post-translational processing by prohormone convertase (PC) gives rise to glucagon (PC 2). In contrast, in the L-cells of the ileum, both GLP-1 and oxyntomodulin are produced (PC 1/3) [23, 24]. There are also other proglucagon peptides whose functions are at present unclear [25]. It is possible that the antibodies against glucagon used in the multiplex assay may have detected peptides other than glucagon, such as glucagon 1–61, glicentin, and oxyntomodulin.

Another critical question is whether the measured glucagon immunoreactivity is related to peptides released from the pancreas or the gut. Previous studies of mice with specific deletions of PC 1/3 or 2 have shown that pancreatic α-cells produce small amounts of GLP-1 and intestinal endocrine cells produce glucagon [26, 27]. However, further studies are needed to identify the exact nature of the glucagon immunoreactivity that SG increases and whether this plays a role in the weight loss by increasing the metabolic rate.

We also identified a significant improvement in the diet-induced accumulation of intracellular lipids in the livers of the rats following surgery. Both bariatric surgery and GLP-1 receptor agonists have been shown to ameliorate NAFLD [28, 29]. This is unlikely to be the result of a direct effect of GLP-1 on the liver because GLP-1 receptors are not expressed in this organ [30]. The precise mechanism by which GLP-1 improves hepatic insulin signaling remains to be determined. Because glucagon receptors are expressed in the liver, further work is required to investigate whether the increase in glucagon secretion following SG plays a role in the amelioration of NAFLD.

Conclusions

Rodents fed a cafeteria diet gained a significant amount of weight and displayed signs of glucose intolerance, insulin resistance, and NAFLD. SG ameliorated these signs and resulted in a significant increase in the incretin hormone response to mixed meal challenge. We also identified a paradoxical increase in glucagon release in response to a mixed meal. While a high post-prandial glucagon concentration may contribute to the therapeutic effects of bariatric surgery, it is also possible that this may be explained by another proglucagon-derived peptide. Further studies are needed to determine the identity of this glucagon-like immunoreactive peptide and whether it plays a role in the therapeutic effects of SG.

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Statement of Ethics

The study was performed as per the guidelines of the Ethics Committee of the Research, Health Sciences Center for the care and use of animals.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Data Availability Statement

The data supporting the findings of this study are available from the corresponding authors upon request.

References

1 Srivastava G, Apovian CM. Current pharmacotherapy for obesity. Nat Rev Endocrinol. 2018 Jan;14(1):12–24.
2 Madsen LR, Baggesen LM, Richelsen B, Thomsen RW. Effect of Roux-en-Y gastric bypass surgery on diabetes remission and complications in individuals with type 2 diabetes: a Danish population-based matched cohort study. Diabetologia. 2019 Apr;62(4):611–20.
3 Borgeraas H, Hofsø D, Hertel JK, Hjelmesæth J. Comparison of the effect of Roux-en-Y gastric bypass and sleeve gastrectomy on remission of type 2 diabetes: a systematic review and meta-analysis of randomized controlled trials. Obes Rev. 2020 Jun;21(6):e13011.
4 Holst JJ, Madsbad S, Bojsen-Møller KN, Svane MS, Jørgensen NB, Dirksen C, et al. Mechanisms in bariatric surgery: gut hormones, diabetes resolution, and weight loss. Surg Obes Relat Dis. 2018 May;14(5):708–14.
5 Kim W, Egan JM. The role of incretins in glucose homeostasis and diabetes treatment. Pharmacol Rev. 2008 Dec;60(4):470–512.
6 Del Prato S, Gallwitz B, Holst JJ, Meier JJ. The incretin/glucagon system as a target for pharmacotherapy of obesity. Obes Rev. 2022 Feb;23(2):e13372.
7 Holst JJ, Knop FK, Vilsbøll T, Krarup T, Madsbad S. Loss of incretin effect is a specific, important, and early characteristic of type 2 diabetes. Diabetes Care. 2011 May 1;34(Suppl 2):S251–7.
8 Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R, Creutzfeldt W. Preserved incretin activity of glucagon-like peptide 1 [7–36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. J Clin Invest. 1993 Jan 1;91(1):301–7.
9 Nauck MA, Quast DR, Wefers J, Meier JJ. GLP-1 receptor agonists in the treatment of type 2 diabetes: state-of-the-art. Mol Metab. 2021 Apr;46:101102.
10 Hindsø M, Svane MS, Hedbäck N, Holst JJ, Madsbad S, Bojsen-Møller KN. The role of GLP-1 in postprandial glucose metabolism after bariatric surgery: a narrative review of human GLP-1 receptor antagonist studies. Surg Obes Relat Dis. 2021 Jul;17(7):1383–91.
11 Hædersdal S, Lund A, Knop FK, Vilsbøll T. The role of glucagon in the pathophysiology and treatment of Type 2 diabetes. Mayo Clin Proc. 2018 Feb;93(2):217–39.
12 Eickhoff H, Louro T, Matafome P, Seiça R, Castro e Sousa F. Glucagon secretion after metabolic surgery in diabetic rodents. J Endocrinol. 2014 Dec;223(3):255–65.
13 Jorsal T, Wewer Albrechtsen NJ, Christensen MM, Mortensen B, Wandall E, Langholz E, et al. Investigating intestinal glucagon after Roux-en-Y gastric bypass surgery. J Clin Endocrinol Metab. 2019 Dec 1;104(12):6403–16.
14 Scott RV, Bloom SR. Problem or solution: the strange story of glucagon. Peptides. 2018 Feb;100:36–41.
15 Tan TM, Field BCT, McCullough KA, Troke RC, Chambers ES, Salem V, et al. Coadministration of glucagon-like peptide-1 during glucagon infusion in humans results in increased energy expenditure and amelioration of hyperglycemia. Diabetes. 2013 Apr 1;62(4):1131–8.
16 Wynne K, Park AJ, Small CJ, Patterson M, Ellis SM, Murphy KG, et al. Subcutaneous oxyntomodulin reduces body weight in overweight and obese subjects: a double-blind, randomized, controlled trial. Diabetes. 2005 Aug 1;54(8):2390–5.
17 Al-Sabah S, Alasfar F, Al-Khaledi G, Dinesh R, Al-Saleh M, Abul H. Incretin response to a standard test meal in a rat model of sleeve gastrectomy with diet-induced obesity. Obes Surg. 2014 Jan;24(1):95–101.
18 Shah H, Shin AC. Meal patterns after bariatric surgery in mice and rats. Appetite. 2020 Mar;146:104340.
19 Jamal MH, Abu-Farha M, Al-Khaledi G, Al-Sabah S, Ali H, Cherian P, et al. Effect of sleeve gastrectomy on the expression of meteorin-like (METRNL) and Irisin (FNDC5) in muscle and brown adipose tissue and its impact on uncoupling proteins in diet-induced obesity rats. Surg Obes Relat Dis. 2020 Dec;16(12):1910–8.
20 Patrikakos P, Toutouzas KG, Perrea D, Menenakos E, Pantopoulou A, Thomopoulos T, et al. A surgical rat model of sleeve gastrectomy with staple technique: long-term weight loss results. Obes Surg. 2009 Nov;19(11):1586–90.
21 Carr RD, Larsen MO, Winzell MS, Jelic K, Lindgren O, Deacon CF, et al. Incretin and islet hormonal responses to fat and protein ingestion in healthy men. Am J Physiol Endocrinol Metab. 2008 Oct;295(4):E779–84.
22 Korner J, Bessler M, Inabnet W, Taveras C, Holst JJ. Exaggerated glucagon-like peptide-1 and blunted glucose-dependent insulinotropic peptide secretion are associated with Roux-en-Y gastric bypass but not adjustable gastric banding. Surg Obes Relat Dis. 2007 Nov;3(6):597–601.
23 Holst JJ, Wewer Albrechtsen NJ. Methods and guidelines for measurement of glucagon in plasma. Int J Mol Sci. 2019 Oct 30;20(21):5416.
24 Wewer Albrechtsen NJ, Kjeldsen SAS, Jensen NJ, Rungby J, Veedfald S, Bojsen-Møller KN, et al. On measurements of glucagon secretion in healthy, obese, and Roux-en-Y gastric bypass operated individuals using sandwich ELISA. Scand J Clin Lab Invest. 2022 Feb 1;82(1):75–83.
25 Wewer Albrechtsen NJ, Kuhre RE, Hornburg D, Jensen CZ, Hornum M, Dirksen C, et al. Circulating glucagon 1-61 regulates blood glucose by increasing insulin secretion and hepatic glucose production. Cell Rep. 2017 Nov;21(6):1452–60.
26 Ugleholdt R, Zhu X, Deacon CF, Ørskov C, Steiner DF, Holst JJ. Impaired intestinal proglucagon processing in mice lacking prohormone convertase 1. Endocrinol. 2004;145(3):1349–55.
27 Webb GC, Dey A, Wang J, Stein J, Milewski M, Steiner DF. Altered proglucagon processing in an alpha-cell line derived from prohormone convertase 2 null mouse islets. J Biol Chem. 2004 Jul;279(30):31068–75.
28 Mantovani A, Petracca G, Beatrice G, Csermely A, Lonardo A, Targher G. Glucagon-like peptide-1 receptor agonists for treatment of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: an updated meta-analysis of randomized controlled trials. Metabolites. 2021 Jan 27;11(2):73.
29 Esquivel CM, Garcia M, Armando L, Ortiz G, Lascano FM, Foscarini JM. Laparoscopic sleeve gastrectomy resolves NAFLD: another formal indication for bariatric surgery? Obes Surg. 2018 Dec;28(12):4022–33.
30 Panjwani N, Mulvihill EE, Longuet C, Yusta B, Campbell JE, Brown TJ, et al. GLP-1 receptor activation indirectly reduces hepatic lipid accumulation but does not attenuate development of atherosclerosis in diabetic male ApoE–/– mice. Endocrinol. 2013 Jan 1;154(1):127–39.