GLP-1R responsiveness predicts individual gastric bypass efficacy on glucose tolerance in rats

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Abbreviations:

RYBG: Roux-en-Y Gastric Bypass
GLP-1: Glucagon-like protein 1
Ex4: Exendin 4
Abstract

Several bariatric operations are currently used to treat obesity and obesity-related comorbidities. These vary in efficacy, but most are more effective than current pharmaceutical treatments. Roux-en-Y Gastric Bypass (RYGB) produces substantial body weight (BW) loss, enhanced glucose tolerance, and is associated with increased secretion of the gut hormone GLP-1. Given the success of GLP-1-based agents in lowering blood glucose and BW, we hypothesized that an individual sensitivity to GLP-1 receptor agonism could predict metabolic benefits of surgeries associated with increased GLP-1 secretion. One hundred ninety-seven high-fat-diet-induced obese male Long-Evans rats were monitored for BW loss during Exendin-4 (Ex4) administration. Stable populations of responders and non-responders were identified.
based on Ex4-induced BW loss and GLP-1-induced improvements in glucose tolerance. Sub-populations of Ex4 extreme responders and non-responders received RYGB. Following RYGB, responders and non-responders showed similar BW loss compared to sham, but non-responders retained impaired glucose tolerance. These data indicate that the GLP-1 response tests may predict some but not all of the improvements observed after RYGB. These findings present an opportunity to optimize the use of bariatric surgery based on an improved understanding of GLP-1 biology and suggest an opportunity for a more personalized therapeutic approach to the metabolic syndrome.
Introduction.

Both developed and developing countries have seen increased rates of obesity over the last 30 years that has been paralleled by an unprecedented increase in the incidence of metabolic disturbances such as hypertension, dyslipidemia, and type-2 diabetes. The World Health Organization has described obesity as the greatest current threat to human health, based upon its association with numerous serious comorbidities (1; 2). Conventional therapies, such as dietary and lifestyle changes, have proven to be largely ineffective (3). Furthermore, current pharmacotherapies are only mildly efficacious (4). At this time, surgical intervention stands alone in the context of sustained treatment for severe obesity (5).

Among these surgeries, adjustable gastric banding (AGB), vertical sleeve gastrectomy (VSG), and Roux en-Y gastric bypass (RYGB) have become the most prevalent procedures (6). RYGB, the current gold standard, results in a loss of 60-70% excess BW on average(7) and is associated with a surprisingly rapid resolution of T2D in 62 - 80% of patients (8; 9). However, the precise mechanisms that underlie the robust effects of RYGB are poorly understood.

While a portion of the anti-diabetic effects of RYGB are secondary to reduced BW, altered gut hormone profile after RYGB is also likely to be a contributing factor.(10) Clinical studies have identified substantial changes in multiple circulating factors, including glucagon-like peptide-1 (GLP-1), following RYGB.(11) GLP-1 secretion is greatly elevated after RYGB, suggesting that it may function as a modulator of both BW and glucose homeostasis.(12) Given that GLP-1 receptor (GLP1-R) signaling is a target
for several approved diabetes therapies, modulation of the GLP-1 system may improve surgical therapies for weight loss and diabetes.

In this study, we investigated the hypothesis that GLP1-R agonists can be used to predict the efficacy of RYGB in a rat model of diet-induced obesity. We here show that sensitivity to GLP1-R agonists functions as a novel predictive biomarker for changes in glucose tolerance but not BW, food intake, or fat mass following RYGB. Our results suggest that a more personalized approach to bariatric surgery may be used to optimize the likelihood of beneficial effects such as improved glucose tolerance, while preventing unnecessary surgical intervention and side effect risk based on the use of a GLP1 challenge as a novel predictive biomarker.
Materials and Methods

Animals

Male, Long-Evans rats (n = 197; 250–300 g) obtained from Harlan Laboratories (Indianapolis, IN) were individually housed and maintained on a 12/12-hour light/dark cycle (lights off at 19:00) at 25°C and 50%-60% humidity. Rats were provided ad libitum access to water and a high-fat butter diet (HFD; 4.54 kcal/g, 41% fat; Research Diets, New Brunswick, NJ) previously shown to produce diet-induced obesity (DIO) and metabolic impairments(13). GLP1-R sensitivity studies were initiated after 8 weeks of high-fat feeding. All procedures for animal use were approved by the University of Cincinnati Institutional Animal Care and Use Committee.

Selection of responders and non-responders for RYGB

Long-Evans rats (n = 197) were fed HFD for 8 weeks. Upon reaching DIO (492.1 ± 2.6 g), all rats were administered Ex4 (50 µg/kg/d, i.p.) for 4 days. BW, food intake, and ad libitum blood glucose were measured as surrogates for GLP1-R agonist sensitivity. Response to GLP-1 challenge during an intraperitoneal glucose tolerance test (ipGTT) was also assessed. Rats were ranked first by body weight and then by GLP-1 response. The 25 most responsive rats and 25 least responsive rats were then subjected to RYGB surgery and characterized over the next 130 days. Ten rats of intermediate response were administered a sham procedure to serve as controls to the RYGB rats.

RYGB
RYGB was performed in anesthetized rats (isoflurane) as previously described\(^{14}\). Briefly, rats had a laparotomy and transection of the jejunum 30cm from the ligament of Treitz, establishing two distinct jejunal limbs. At the level of the distal limb, a small longitudinal incision was made 10 cm distal to its proximal end and the proximal limb was anastomosed to it, end-to-side, with a running 7x0 Vicryl absorbable suture (Ethicon Endo-surgery, Somerville, NJ) creating the “Y”. The gastric pouch was then made using an ETS-Flex 35mm staple gun (Ethicon Endo-Surgery) to resect most of the fundus and partition the gastric remnant across its waist, thereby creating a proximal gastric pouch ~10% of the original gastric size. Following incision of the gastric pouch that spared its vascular architecture, the efferent jejunal limb (alimentary limb) was connected to the gastric pouch creating a side-to-end gastro-jejunal anastomosis with a running 8-0 prolene, non-absorbable suture (Ethicon). The stomach and jejunum were then re-integrated into the peritoneal cavity and the abdominal wall was closed in layers.

For the sham surgery the jejunum was transected 30cm distal to the ligament of Treitz and re-anastomosed end-to-end with a running 7-0 Vicryl suture. Survival following this procedure yielded n = 7 sham, 12 responder, and 13 non-responder rats.

**Postoperative care**

High fat diet was replaced with Ensure Plus liquid diet (1.41 kcal/g, 29% fat; Abbott Nutrition, Columbus, OH) 24 hours prior to surgery. Liquid diet was continued for 120 hours and then replaced with HFD. Subcutaneous injections of Metacam (0.25 mg/100 g body weight once daily for 4 days), gentamicin (0.8 mg/100 g body weight on the day
of surgery), Buprenex (0.3 mL 2× per day for 5 days), and warm saline (10 mL and 5 mL 2× per day for days 0–3 and 4–5, respectively) were given to all postoperative rats.

**Body composition measurements**

Echo MRI Whole-body composition analysis (fat and lean mass) was performed on all rats on post-operative days 28, 59, and 90 (EchoMRI, Houston TX).

**Peptides**

Ex4 was obtained from American Peptide (Sunnyvale, CA). GLP-1 (7-36)-amide was obtained from polypeptide group (San Diego, CA).

**Insulin, Glucose, and Mixed-meal tolerance tests**

Insulin and glucose tolerance tests were performed by intraperitoneal (i.p.) injection of human insulin (1 unit/kg, 20% w/v d-glucose, Lilly Humalog, in 0.9% w/v saline) or glucose (2 g/kg, 20% w/v d-glucose, Sigma, in 0.9% w/v saline) after 6- (GTT) or 16-hour (ITT) fast. For mixed meal tolerance test rats were gavaged with 3 mL Ensure Plus Liquid diet. Blood was collected into tubes containing 20 µL antiproteolytic cocktail (4.65 g EDTA + 92 mg aprotinin + 40,000 U heparin in 50 mL saline). Active and total GLP-1 (7-36) measured by electrochemiluminescence assay (Meso Scale Discovery, Gaithersburg, MD). Blood samples were collected before and 15, 30, 60, 90 and 120 minutes after challenge. Blood glucose determined by TheraSense Freestyle Glucometer.
**Statistical analysis**

All data are represented as mean and standard error of the mean (SEM). Non-linear correlation with a Gaussian fit was used to determine normal distribution of Ex4-stimulated effects throughout the population. One-way and two-way ANOVA with Bonferroni’s Multiple Comparison post test, or student t test were performed to assess effects between study groups where appropriate. In all cases, statistical significance was assumed when $P < 0.05$ using GraphPad Prism software (San Diego, CA).
Results

Diversity in Response to GLP-1 Receptor Agonism

GLP1-R signaling enhances glucose metabolism and decreases BW in both humans (15) and rodent models (15). However, whether the response to GLP1-R agonists varies among different sub-populations has yet to be systematically examined. To test this hypothesis we assessed the sensitivity of an outbred population of DIO rats to Ex4 injections. Ex4 treatment stimulated BW loss (Figure 1a & b) that followed a Gaussian distribution (Figure 1c & d). Consistent with this effect on BW, food intake, final (d 4) blood glucose, and the change in ad libitum blood glucose (d 0-4) were marked by considerable variability within the population (Figure 2a, c, & e).

The diversity within this population of outbred rats suggested a natural variation in individual responsiveness to GLP1-R agonism. Indeed, comparison of the upper and lower 15th-percentiles for body-weight loss revealed a marked difference in the response to Ex4 treatment. Specifically, loss of BW in the upper 15th-percentile (responders) was found to be much greater (7.05% ± 0.15%) than that observed in the lower 15th-percentile (non-responders, 1.68% ± 0.010%) or the total population (4.32 ± 0.12%) (Figure 1a). To test the hypothesis that there is an innate variation in sensitivity to GLP1-R agonism; we analyzed food intake, glucose tolerance, and ad libitum blood glucose in sub-populations stratified according to change in BW. Food intake (Figure 2b) and blood glucose (Figure 2d & f) were decreased in responders compared to either the total population or non-responders. Conversely, non-responders had increased food intake and elevated ad libitum blood glucose compared to the other two populations (Figure 2). Following a 10-day washout period, Ex4 treatment was repeated in all rats to
ensure that the phenomena were reproducible. As in the initial screen, rats were stratified into populations of responders and non-responders based on body-weight loss (Supplemental Figure 1a). Rats identified in the second screen were similar to those identified in the first study, with no crossover of groups observed. Decreased BW in the responder groups was associated with decreased *ad libitum* blood glucose, whereas in the non-responder group there was a less pronounced weight loss and elevated blood glucose (Supplemental Figure 1b & c). However, unlike the initial screen, no significant difference in food intake was observed between the groups (Supplemental Figure 1d).

To ensure that the biological response to Ex4 was based on GLP1-R signaling and not an off-target effect of the drug, glucose tolerance was assessed in the presence and absence of exogenous GLP-1 (7-36). Following a 10-day washout, all rats were fasted for 6 hours and then administered 2 g/kg i.p. glucose. As with other surrogates of GLP1-R signaling, we found that in regards to innate glucose tolerance there was considerable variability within the population (Figure 3a). While fasting glycemia (Figure 3b) was similar in all groups, responders exhibited greater glucose tolerance at 15 and 30 minutes (*P* < 0.05, Figure 3c) and a reduced area under the curve (AUC) (*P* < 0.01, Figure 3d). In the presence of exogenous GLP-1 (7-36), responders displayed a greater enhancement of glucose clearance (Figure 3c) and a reduced AUC (*P* < 0.01, Figure 4d). Intriguingly, no effect of the GLP-1 was evident in the AUC of non-responders.

GLP-1 secretion by an oral carbohydrate challenge is attenuated in obese individuals (16), thus, one possible explanation for the difference in sensitivity to GLP1-R agonists may be attributed to the animal’s initial adiposity. To better understand the contribution of initial adiposity to Ex4 response, we analyzed the same variables according to the
animal’s BW prior to Ex4 treatment. We observed a significant correlation between body-weight loss and initial BW in the total population (P< 0.01, Supplemental Figure 2a). However, this effect was lost when the comparison was restricted to either the responder or non-responder groups (Supplemental Figure 2a). Furthermore, animals from both the responder and non-responder groups had initial BWs across the range of values observed in the total population (Supplemental Figure 2a), suggesting that initial BW did not determine the body-weight response to Ex4. Food intake was also correlated with initial BW (P< 0.01, Supplemental Figure 2b), such that animals with the greatest food intake also had the highest initial BW. This correlation was still observed when the analysis was restricted to the non-responder group (P< 0.05, Supplemental Figure 2b). However, when analysis was restricted to the responder group, there was a slight but significant negative correlation between food-intake response and initial BW (P< 0.05; Supplemental Figure 2b), suggesting that the food-intake response may drive the overall body-weight response. Glycemic response to Ex4 was not correlated with initial BW in responders, non-responders, or the population as a whole (Supplemental Figure 2c). Taken together, these data suggest that there is an inter-individual variation in innate GLP1-R sensitivity that is not explained by initial adiposity.

**Effects of RYGB on BW, food intake, & fat mass**

To test the hypothesis that sensitivity to GLP1-R agonists could be used to predict the outcome of RYGB, we compared the effects of RYGB in animals classed as responders and non-responders to Ex4. RYGB stimulated a characteristic decrease in BW in both responder and non-responder groups compared to sham-operated control animals
(Figure 4a). Maximum weight loss was observed at day 13 in responders (19.1%±1.6%) and day 22 in non-responders (20.8%±2.5%). In addition to this early weight loss, RYGB-treated animals displayed a sustained weight loss throughout the study, with neither group returning to its pre-operative BW (Figure 4a). A slight decrease in BW (6.5±1.1%) was observed in sham-treated animals over the first 6 days, followed by a rapid and sustained rebound (Figure 4a). Body composition assessed on days 28, 59, and 90 confirmed that the loss of BW was mirrored by similar effects on fat mass, with no difference between responder and non-responder groups (Figure 4b). Lean mass was initially reduced in the non-responders (p<0.05), with similar values observed at later time points in all groups (figure 4c). Analysis of food intake over the first 48 days of the study revealed reduced food intake in RYGB animals compared to sham controls. As with RYGB-induced loss of BW, no evidence of differential food intake was observed between the responder and non-responder groups (Figure 4d). Fasting-induced 24 h food intake was similar in responder-RYGB and sham groups, with a significant increase observed in the non-responder-RYGB rats (Figure 4e). Taken together, these data suggest that, regardless of prior GLP1-R sensitivity, RYGB stimulates loss of body and fat mass while reducing cumulative food intake in rats. Sensitivity to GLP1-R agonists therefore does not predict individual energy balance following RYGB surgery.

**Effects of RYGB on glucose metabolism**

In addition to its effects on food intake, BW, and fat mass; GLP-1 stimulates insulin secretion and increases insulin-independent glucose disposal (17), leading to subsequent enhancement of glucose homeostasis. We therefore addressed whether
glucose homeostasis differed in responders and non-responders after RYGB. When glucose homeostasis was challenged via i.p. glucose bolus (2 g/kg) 3 months after RYGB, we found that responders exhibited an enhanced glucose tolerance (Figure 4f) and reduced area under the curve (Table 1) when compared to non-responders. These rats displayed a similar trend for a reduced area under the curve (p = 0.09), when compared to shams. It should be noted that this enhanced tolerance was independent of BW or fat mass, as these parameters were identical in the two groups. Insulin-stimulated glucose clearance was assessed via i.p. ITT (1 unit/kg) following a 16 h fast (Figure 4g). This assessment elucidated a similar glucose clearance (Kd) over the initial 30 min in both RYGB groups that was enhanced as compared to sham controls (Table 1).

To evaluate humoral regulation in the postprandial state, we analyzed plasma insulin, glucagon, and GLP-1 levels before and 30 minutes after a mixed-meal challenge. At the time of the challenge, fasting glycemia was reduced in responders compared to non-responders with similar levels of insulin (Table 1). Insulin resistance determined by HOMA-IR tended to be lower (p=0.0775) in responders as compared to sham or non-responders (Table 1). Total plasma GLP-1 levels were elevated after the challenge, although no difference was observed between responder and non-responder groups (Table 1). However, we observed greater levels of active GLP-1 30 minutes after the challenge (Table 1). This increase in plasma GLP-1 was associated with elevation of plasma insulin levels (Table 1). In addition, plasma glucagon was increased in both responder and non-responder groups (Table 1). Taken together, these findings are consistent with the hypothesis that the level of GLP1-R sensitivity prior to surgical
intervention predicts glucose tolerance after RYGB and suggests potential use of a Ex4 challenge test as a biomarker to predict metabolic benefits resulting from bariatric surgery. Furthermore, these data suggest that the enhancements in glucose metabolism are associated with increased levels of active GLP-1 and subsequent plasma insulin levels in rats identified as responders.

Discussion

This study shows that responsiveness to a GLP1-R agonist predicts populations of individuals who will not display some of the beneficial metabolic effects of RYGB surgery. Our findings raise the hope of a novel tailored, and ultimately personalized, approach to the treatment of obesity. They also suggest that prediction of treatment outcome and combinations of pharmacological and surgical intervention may be utilized to achieve maximum efficacy with minimal invasiveness in individual patients.

Current medical and lifestyle interventions offer modest efficacy in the treatment of obesity (3; 4). Bariatric interventions, on the other hand, have proven to be highly efficacious and frequently carry the beneficial side effect of T2D resolution (8). However, not all of the currently available surgical options are equally effective. RYGB is the current gold-standard in terms of both weight loss and resolution of T2D, and is associated with a similar mortality risk as compared to adjustable gastric banding or vertical sleeve gastrectomy (ref needed). However, 20%–50% of patients fail to lose substantial weight and 20% - 40% fail to achieve diabetes resolution after RYGB (18). Thus, identifying predictors of treatment outcome would reduce unnecessarily invasive interventions in patients who will not benefit from the surgery.
We hypothesized that the superior efficacy of RYGB is due to the humoral reprogramming observed after RYGB (19). Circulating GLP-1, and therapies based upon it, modulates food intake, glucose homeostasis, BW (20), and fat mass (21). Furthermore, GLP-1-based therapies are unaffected by diabetic state (22). Thus, the variation in metabolic outcome after RYGB might involve variations in GLP-1 signaling that could be predicted by sensitivity to GLP1-R agonists before the procedure.

The hypothesis that GLP-1 response may hold a predictive value was based upon the observation that a sufficient and sustained degree of variability in response was clearly detectable within our model population. To our knowledge, this finding of such variable response to GLP1-R agonism, across different agonist ligands and measured endpoints, is the first such in vivo example. Specifically we found that sub-populations, which were either responsive or non-responsive to Ex4-stimulated weight loss, could be identified from a large population of outbred rats. It is important to note that the response in body weight change was associated with similar effects on food intake and glycaemia, suggesting that the variable sensitivity was pervasive to some, if not all, GLP-1 actions. While we were unable to observe a significant increase in glucose tolerance in our Responder cohort of RYGB rats, this may be attributed to our selection of sham animals. These shams were selected from rats of intermediate GLP1-R response and therefore were not matched to our specific Responder and Non-responder groups. Importantly, we were able to identify a significant deficit in the glucose homeostasis of Non-responders. While the paradigm used here does not allow to observation of clear differences between responder rats and sham rats, the data
suggests that an individual’s response to GLP1-R agonism offers a novel, and the currently the only, predictor of metabolic outcome following RYGB.

These observations are consistent with the finding that a single nucleotide polymorphism of the GLP1-R (human GLP-1R Met\textsuperscript{149}) is associated with reduced ligand-receptor affinity and intracellular signaling.(23) Likewise, the exchange of a single amino acid (E68) in GLP-1R markedly reduces receptor activity (24). Thus, it is tempting to speculate that GLP-1 responsive and nonresponsive sub-populations can also be identified in humans and that responsiveness to GLP1-R agonists would be similarly predictive.

Increased circulating levels of GLP-1 combined with enhanced insulin secretion are often suggested to play a role in the improved glucose metabolism observed following RYGB (19). Likewise, elevated plasma levels of GLP-1 have been described following vertical sleeve gastrectomy (VSG) (14), ileal transposition (25), and duodenal-jejunal bypass (26). Consistent with the observed incretin hypersecretion, all of these procedures have been found to improve glucose tolerance (14; 25; 26). A recent study by our group has suggested that GLP1-R signaling is not necessary for the beneficial effects of VSG on food intake, body weight, dietary fat preference, glucose tolerance (27). While this finding leads us to reconsider the role of GLP-1 as the primary mediator of the metabolic benefits of bariatric surgery, its relevance to this study are not as clear. Several caveats prevent us from drawing a straight line between these surgeries. The VSG and RYGB are not the same procedure with the primary difference being the nutrient exclusion that occurs in RYGB. The study conducted by Wilson-Perez et al. utilized mice, whereas we have chosen the rat model. Thus, it is possible that a species
dependent effect is undermining the interpretation. Furthermore, the GLP-1 deficient
mice used in this study develop in the absence of this important neuroendocrine factor.
Thus, compensatory effects due to this deficiency (i.e. increased GLP-1 and GIP) may
cloud the interpretation of these results. Moreover, an earlier report from our own group
using the GLP-1r antagonist, Exendin-9, demonstrated that surgery-induced
improvements in glucose homeostasis are attenuated when GLP-1 action is blocked.
Together we interpret these findings to suggest that GLP-1 in not necessary for the
effects of VSG on food intake, body weight, dietary fat preference, glucose tolerance.
However, its sufficiency to drive these outcomes cannot be concluded from this study
design. Thus, while the role of GLP-1 as the primary mediator of the pleiotropic
metabolic benefits of bariatric surgery requires reconsideration, its effect in the present
study suggest that it may be predictive of outcome after RYGB.

While the here presented work awaits confirmation in humans, it undoubtedly
offers translational potential for clinical diabetologists and obesity physicians observing
therapy responder- and non-responder- sub-populations. As differences in
responsiveness have been reported for bariatric surgery (7), as well as incretin therapy
(23; 28), our findings provide one possible path toward a more personalized metabolic
medicine. Incretin challenge tests may allow us to begin individually stratifying risk when
choosing the correct therapy for the metabolic syndrome.

In summary, our study in a rodent model of DIO indicates that distinct sub-
populations exhibit differential responses to GLP-1 receptor agonism and that these
responses predict glucose metabolism after RYGB. Taken together, our results suggest that the GLP-1 system may offer untapped potential as a novel biomarker for personalized approaches to the treatment of T2D and obesity. Clinical studies in obese and T2D patients will be required to test if this desirable novel biomarker shows the same, or even greater, promise in humans and can be used to predict benefits - as well as to prevent unnecessary risks - of bariatric surgeries.
Acknowledgments:

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RD, MHT, and DP-T have a collaborative association with Roche Research Laboratories pertaining to peptide-based therapeutics in metabolism and receive research support from Roche Pharmaceuticals. RJS is a Consultant for Ethicon Endo-Surgery, Novo Nordisk, Novartis, Angiochem, Zafgen, Takeda, & Eli Lilly; receives research support from Ethicon Endo-Surgery, Novo Nordisk, Ablaris, & Pfizer; is a paid speaker for Ethicon Endo-Surgery, Novo Nordisk, Merck, & Pfizer; and holds equity in Zafgen. KMHa, KMHe, SEA, JH, NO, CR, JB, MT, EB, TM, PTP, and SCB have no conflicts to disclose.

Author Involvement:

KMHa and MHT were responsible for study conception and design, data analyses and interpretation, and drafting the article; KMHe, SA, JH, NO, CR, JB, MT, and EB generated experimental data; TM, DP-T, PTP, SCB, RD, DD, and RJS advised study concept, conducted specific analyses and critical revision of the article. MHT is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Sponsor Involvement:

Ethicon endosurgery and its employees had no role in designing or conducting these studies.
References

Figure Legends.

**Figure 1:** Relative (a) and absolute (b) BW response to Ex4 treatment in DIO rats. n=197 for total (triangles) and 30 for both responder (circles) and non-responder (squares) groups. Data are represented as mean +/- SEM. *p< 0.05, **p< 0.01, ***p< 0.001.

Population distribution of Ex4 response during the 5 d treatment as a function of % body weight loss (c) or body weight change (d). Data are represented as number of rats per bin. Solid line and R^2 denote non-linear Gaussian fit of data. All data obtained in male Long-Evans rats maintained on HFD (40% butter-fat) for 8 weeks. n= 197 for total (triangles) and 30 for both responder (circles) and non-responder (squares) groups.

**Figure 2:** Population distribution of Ex4 response during the 5 d treatment as a function of food intake (a) d 4 *ad libitum* blood glucose (c), or d 0-4 change in *ad libitum* blood glucose (e). Data are represented as number of rats per bin. Solid line and R^2 denote non-linear Gaussian fit of data. Food intake (b), final *ad libitum* blood glucose (d), and *ad libitum* blood glucose change (f) of responders and non-responders identified by BW. n= 197 for total (triangles) and 30 for both responder (circles) and non-responder (squares) groups. Data are represented as mean +/- SEM. *p< 0.05, **p< 0.01, ***p< 0.001.

**Figure 3:** Population distribution (n = 197) as a function of glucose tolerance (a, area under the curve during ipGTT). Data are represented as number of rats per bin. Solid line and R^2 denote non-linear Gaussian fit of data. Fasting blood glucose (b) before; glucose excursion (c), and area under the curve (d) after glucose challenge in the
presence (open symbols) or absence (closed symbols) of GLP-1 in responders and non-responders identified by body weight during both phases of the Ex4 response study. All data obtained in male Long-Evans rats maintained on HFD (40% butter-fat) for 12 weeks. n= 197 for total (triangles) and 30 for both responder (circles) and non-responder (squares) groups. All data are represented as mean +/- SEM. *p< 0.05, **p< 0.01, *** p< 0.001.

Figure 4: Relative (a) BW loss, fat mass (b), lean mass (c) and food intake (d) following RYGB in previously identified responders and non-responders. 24-h fasting-induced food intake (e) 19 wk after RYGB in previously identified responders and non-responders. Blood glucose excursion during ipGTT (f) and ipITT (g) 12 w and 19 w after RYGB in responders and non-responders. n= 7 for Sham (triangles), 10 for responder (circles), and 13 for non-responder (squares) groups. All data are represented as mean +/- SEM. *p< 0.05, **p< 0.01, *** p< 0.001.

Table 1: Circulating factors in RYGB rats.

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<td>ipGTT AUC</td>
<td>13252 ± 1530</td>
<td>10464 ± 1187</td>
<td>17550 ± 1625**</td>
</tr>
<tr>
<td>ipITT Kd$_{30}$ (mg/ml/min)</td>
<td>0.70 ± 0.06</td>
<td>1.70 ± 0.12**</td>
<td>1.31 ± 0.16**</td>
</tr>
</tbody>
</table>

Table 1 legend: Fasting and post-prandial plasma collected 20 w post-op in response to a mixed-meal challenge. Data expressed as mean ± SEM (n=7-11). Rate of glucose disappearance (Kd$_{30}$) calculated over initial 30 min of ITT. Data obtained in male Long-Evans rats maintained on HFD (40% butter-fat). All data are represented as mean +/- SEM. * p < 0.05 compared to Responders, ** p < 0.01 compared to Responders, # p < 0.05 compared to Sham, ## p < 0.01 compared to Sham via ANOVA.
Figure 1:

(a) Body Weight (% of Initial Weight)

- Total
- Non-responders
- Responders

(b) Body Weight (g)

- Total
- Non-responders
- Responders

(c) Animals (n)

$R^2 = 0.7267$

(d) Animals (n)

$R^2 = 0.9205$
Figure 3:

(a) Aniomals (n) vs. Glucose Tolerance (AUC). The plot shows a distribution of animals' tolerance to glucose, with a coefficient of determination $R^2 = 0.7893$.

(b) Blood Glucose (mg/dl) vs. Total, Non-responders, and Responders. The comparison shows a scatterplot with groups distinguished by color and shape.

(c) Blood Glucose (mg/dl) vs. Minutes. The graph illustrates the blood glucose levels over time for different conditions, with markers indicating significant differences.

(d) GTT (AUC) vs. Responders-Veh, Non-responders-Veh, Responders+GLP, Non-responders+GLP. The chart displays AUC values for various conditions, with statistical significance indicated by asterisks.
Supplemental Figure 1: Replication of Ex4 Response: Relative (a) body weight response to 50 µg/kg/day Ex4 treatment in DIO Long-Evans rats. Final *ad libitum* blood glucose (b), *ad libitum* blood glucose change (c) and food intake (d) of responders and non-responders identified by body weight during the 5 d treatment. All data obtained in male Long-Evans rats maintained on HFD (40% butter-fat) for 10 weeks. n= 197 for total (triangles) and 30 for both responder (circles) and non-responder (squares) groups. All data are represented as mean +/- SEM. *p< 0.05, **p< 0.01, *** p< 0.001.

Supplemental Figure 2: Relative (a) BW loss, food intake (b), and *ad libitum* blood glucose change (c) in response to Ex4 as correlated to initial BW for each individual rat. All data obtained in male Long-Evans rats maintained on HFD (40% butter-fat) for 8 weeks. n= 197 for all rats (black triangles) and 30 for both responder (red circles) and non-responder (blue squares) groups. Lines denote linear regression analysis of corresponding groups.