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RESEARCH ARTICLE | *Nutrient Sensing, Nutrition, and Metabolism*

Customization of biliopancreatic limb length to modulate and sustain antidiabetic effect of gastric bypass surgery

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Pal A, Rhoads DB, Tavakkoli A. Customization of biliopancreatic limb length to modulate and sustain antidiabetic effect of gastric bypass surgery. *Am J Physiol Gastrointest Liver Physiol* 314: G287–G299, 2018. First published November 2, 2017; doi:10.1152/ajpgi.00276.2017.—Although Roux-en-Y Gastric Bypass (RYGB) remains the most effective treatment for obesity and type 2 diabetes (T2D), many patients fail to achieve remission, or relapse. Increasing intestinal limb lengths of RYGB may improve outcomes, but the mechanistic basis for this remains unclear. We hypothesize biliopancreatic (BP) limb length modulates the antidiabetic effect of RYGB. Rats underwent RYGB with a 20-cm (RYGB-20cm) or 40-cm (RYGB-40cm) BP limb and were compared with control animals. After 2 and 4 wk, portal and systemic blood was sampled during intestinal glucose infusion. Portosystemic gradient was used to calculate intestinal glucose utilization (G_{util}), absorption (G_{absorp}), and hormone secretion. Intestinal morphology and gene expression were assessed. At 2 wk, G_{absorp} progressively decreased with increasing BP limb length; this pattern persisted at 4 wk. G_{util} increased ≈70% in both RYGB-20cm and -40cm groups at 2 wk. At 4 wk, G_{util} progressively increased with limb length. Furthermore, Roux limb weight, and expression of hexokinase and preproglucagon, exhibited a similar progressive increase. At 4 wk, glucagon-like peptide-1 and -2 levels were higher after RYGB-40cm, with associated increased secretion. We conclude that BP limb length modulates multiple antidiabetic mechanisms, analogous to the dose-response relationship of a drug. Early postoperatively, a longer BP limb reduces G_{absorp}. Later, G_{util}, Roux limb hypertrophy, hormone secretion, and hormone levels are increased with longer BP limb. Sustained high incretin levels may prevent weight regain and T2D relapse. These data provide the basis for customizing BP limb length according to patient characteristics and desired metabolic effect.

NEW & NOTEWORTHY Biliopancreatic limb length in gastric bypass modulates multiple antidiabetic mechanisms, analogous to the dose-response relationship of a drug. With a longer biliopancreatic limb, Roux limb hypertrophy, increased glucose utilization, reduced glucose absorption, and sustained high incretin levels may prevent weight regain and diabetes relapse.

diabetes; incretin

INTRODUCTION

Roux-en-Y Gastric Bypass (RYGB) remains the most effective treatment for obesity and type 2 diabetes (T2D) (18). However, despite impressive rates of diabetes remission, over 30% of patients continue to require medical treatment after surgery. Furthermore, of patients that achieve initial remission, 35% relapse within five years (1). Weight regain is also an issue, with overall regain of 23% (7). Reduced incretin levels have been proposed to play a role (17).

Diabetes relapse and weight regain have led to various strategies of surgical revision following RYGB, including shortening the common limb (4), lengthening the biliopancreatic (BP) limb (5, 12), and lengthening the Roux limb (19).

Even for primary RYGB, representing a much larger patient group, intestinal limb lengths have not been standardized. This group is heterogeneous, including diabetic and nondiabetic patients, with a wide body mass index range and prevalence of comorbidities. This heterogeneity suggests that customizing the surgery based on patient characteristics could improve overall outcomes. This hypothesis has been studied by varying the length of Roux limb according to body mass index (6, 10, 11). A subsequent review of available data has concluded that longer Roux limb in super obese patients leads to a modest weight loss advantage, but this is short lived (22). The role of BP limb and impact on weight loss and diabetes remission is, however, less well studied. In a study of diabetic superobese patients, both the BP and Roux limbs lengths were increased, leading to improved T2D remission. Although such data suggest that BP length can have a role in modulating metabolic outcomes of RYGB, mechanistic studies are lacking in diabetic patients (15). Long limb surgeries carry a greater risk of malabsorptive complications (4), so the metabolic benefits need to be carefully balanced against the risk of malabsorption and consequent malnutrition and intractable diarrhea. A stronger evidence base would more adequately inform the specifics of surgical strategies for individual patients.

Previously, we showed that glucose sensing in the proximal intestine modulates the glucose absorptive and endocrine functions of the more distal small intestine (14, 21), and that RYGB has several interacting antidiabetic effects (A. Pal, D. Rhoads, and A. Tavakkoli unpublished observations), including changes in intestinal glucose fluxes (i.e., absorption and utilization), systemic glucagon-like peptide (GLP) levels, and hepatic degradation of GLPs. We hypothesize that the length of the BP limb will affect these antidiabetic effects of RYGB.

Using a rat model, we simultaneously sampled portal and systemic blood to measure the effects of BP limb length on intestinal glucose fluxes, intestinal hormone secretion, and intestinal and hepatic gene expression. We specifically limited

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this study to normal rats to examine the impact of limb length in the absence of underlying pathology. Our study reveals a mechanistic basis for customizing RYGB to optimize metabolic outcomes.

METHODS

Surgical Procedures

Animal studies were performed in accordance with protocols prospectively approved by the Harvard Medical Area Standing Committee on Animals. Male Sprague-Dawley (220–240 g; Harlan) rats were acclimatized for 7 days under a 12:12-h light-dark cycle (lights on 7:00 AM) with ad libitum access to standard rat chow (Purina 5053). After an overnight fast, they were anesthetized using isoflurane (1–3% in oxygen) and underwent either control surgery or RYGB (Fig. 1).

RYGB

RYGB was performed as previously described by our group (3, 20). In summary, the stomach was divided using a linear stapler to create the gastric pouch and gastric remnant. The gastric pouch was joined to a 10-cm Roux limb. The gastrojejunal (GJ) and jejunojejunal (JJ) anastomoses were hand-sewn using interrupted sutures (PDS 6/0). Two BP limb lengths were fashioned: 16 or 36 cm from the ligament of Treitz to JJ anastomosis so, with a duodenal length of 4 cm, the overall BP length was 20 cm (RYGB-20cm, $n = 7$ and 7 at 2 and 4 wk postoperative, respectively) and 40 cm (RYGB-40cm, $n = 5$ and 6 at 2 and 4 wk postoperative, respectively). Total small intestinal length was 90 cm. Animals were provided a liquid diet for 5 days postoperatively and then switched to a solid diet with ad libitum access to standard rat chow (Purina 5053). Daily measurements were made of postoperative weights and food intake while rats were on the solid diet.

Control Surgery

In the Control group ($n = 5$ and 8 at 2 and 4 wk postoperative, respectively), to control for the effects of anesthesia and intestinal anastomosis, the small intestine was divided (16 cm from the ligament of Treitz) and then anastomosed (PDS 6/0 interrupted sutures).

Portal and Systemic Sampling Experiments

After a 2- or 4-wk recovery period, animals underwent portal and systemic sampling experiments. Overnight-fasted rats were anesthetized using isoflurane (1–3% in oxygen). Experiments were consistently started at 9:00 AM to minimize diurnal variation in intestinal absorptive function as a confounding factor.

Portal and systemic venous blood was sampled before and during an intestinal glucose bolus to measure glucose and hormone levels as previously described (14). In brief, for systemic sampling, a silastic catheter (ID 0.02 in.; Dow Corning) was advanced through the right jugular vein into the right atrium, allowing sampling of mixed systemic venous blood. For portal sampling, a Silastic catheter (ID 0.012 in.; Dow Corning) was inserted through the superior mesenteric vein (SMV) into the portal vein. For this, a short length of SMV was controlled using curved vascular clamps (Fine Science Tools, Foster

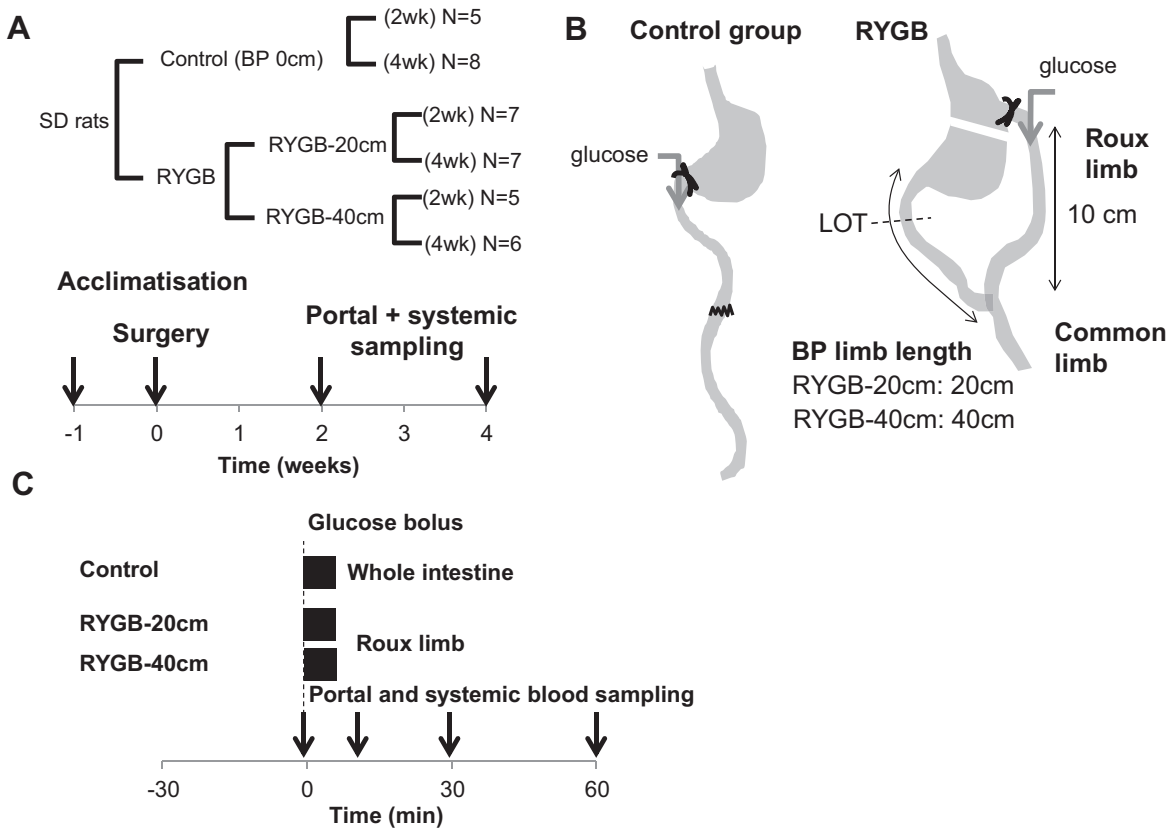

Fig. 1. Experimental timeline and surgical procedures. *A*: experimental timeline. Animals were acclimatized for 1 wk and then underwent Roux-en-Y Gastric Bypass (RYGB) or control surgery, followed 2 or 4 wk postoperatively by portal and systemic sampling experiments. No. of animals in each group is given. *B*: control surgery involved intestinal transection and anastomosis. RYGB involved formation of a standard (20-cm) or long (40-cm) biliopancreatic (BP) limb and a 10-cm Roux limb. The arrow indicates the point of glucose bolus. *C*: timeline of infusions and blood sampling. Control rats received a whole intestinal glucose bolus. The pylorus was ligated to prevent backflow. RYGB rats received a Roux limb glucose bolus. The gastrojejunal (GJ) junction was ligated to prevent backflow.

Table 1. *Primers used for real-time PCR*

Gene	Protein	Primers
SGLT1	Sodium-glucose cotransporter-1	F: 5′-CCAAGCCCATCCCAGACGTACACC R: 5′-CTTCCTTAGTCATCTTCGGTCCTT
SGLT3b	Sodium-glucose cotransporter-3b	F: 5′-GAACATGTCCCACGTGAAGGC R: 5′-TGCAGAAGATGGCAAGCAAGAAC
PPGlu	Preproglucagon	F: 5′-CTCTGGTGGCAAGGTTATCG R: 5′-CATTCACAGGGCACATTCAC
DPP4	Dipeptidyl peptidase-4	F: 5′-TCCCAACTCCAGAGGACAAC R: 5′-CAGGGCTTTGGAGATCTGAG
PEPCK	Phospho*enol*pyruvate carboxykinase	F: 5′-CTCACCTCTGGCCAAGATTGGTA R: 5′-GTTGCAGGCCCAGTTGTTGA
G6Pase	Glucose-6-phosphatase	F: 5′-AACGTCTGTCTGTCCCGGATCTAC R: 5′-ACCTCTGGAGGCTGGCATTG
ChREBP	Carbohydrate responsive element-binding protein	F: 5′-GGGACATGTTTGATGACTATGTC R: 5′-AATAAAGGTCGGATGAGGATGCT
GS	Glycogen synthase	F: 5′-CTGCATGGGAAGCTGAAAGACTCCCCG R: 5′-CATGGCTCTACTTGAGTCTTCACATTA
GP	Glycogen phosphorylase	F: 5′-CCAAAGATCCAGACTGCTTCAAGGATG R: 5′-CCAGAGCAGGCTATATTTCTGATCACCTTT

F, forward; R, reverse.

City, CA), the SMV was punctured (27-gauge needle), and the catheter was inserted and advanced to the portal vein. Portal and systemic blood was sampled at 0 min and then 10, 30, and 60 min after the glucose bolus (200 μl from each catheter at each time point) and analyzed for glucose and hormone levels.

In control animals, the intestinal catheter was placed directly in the duodenum and secured with a silk suture. A glucose bolus of 2 g/kg of glucose (dissolved in distilled water), an amount equivalent to that given to rats in an oral glucose tolerance test, was then administered directly to the intestinal lumen over 5 min.

In RYGB animals, the intestinal catheter was placed in the Roux limb (just distal to the GJ anastomosis) and secured with a silk suture, and a 2 g/kg glucose bolus was administered in the Roux limb.

Tissue Harvest and Storage

At the end of each experiment, the intestine and liver were harvested. The entire small intestine was weighed (total intestinal weight), and then four intestinal segments were weighed and mucosal samples taken: BP limb (5–15 cm distal to the ligament of Treitz), Roux limb (entire segment of the intestine), common limb (0–10 cm distal to JJ anastomosis), and terminal ileum (0–10 cm proximal to the cecum). Corresponding segments of intestine were harvested from control animals. Samples were rapidly frozen in liquid N_2 and stored at −80°C.

Blood Analyses

Portal and systemic blood glucose levels were measured using a glucometer (LifeScan OneTouch). For hormonal analysis, blood was centrifuged at 4°C at 4,000 *g* for 15 min, and the plasma was aspirated and stored at −80°C. Plasma GLP-1 (active) and glucose-dependent insulinotropic peptide (GIP) (total) levels were measured in duplicate using the Milliplex rat metabolic hormone panel (catalog no. RMHMAG-84K; Millipore, EMD Millipore). Plasma GLP-2 levels were measured using the Millipore ELISA kit (catalog no. EZGLP2–37K; EMD Millipore).

Real-Time PCR for mRNA Expression

Expression of the mRNAs for intestinal proteins and several liver proteins (Table 1) involved in glucose metabolism were determined relative to β-actin (internal reference). See Supplemental Methods for details (Supplemental data for this article may be found on the journal website.). RNA was extracted from tissue samples using the mirVana mRNA Isolation Kit (Ambion, Grand Island, NY) and quantified

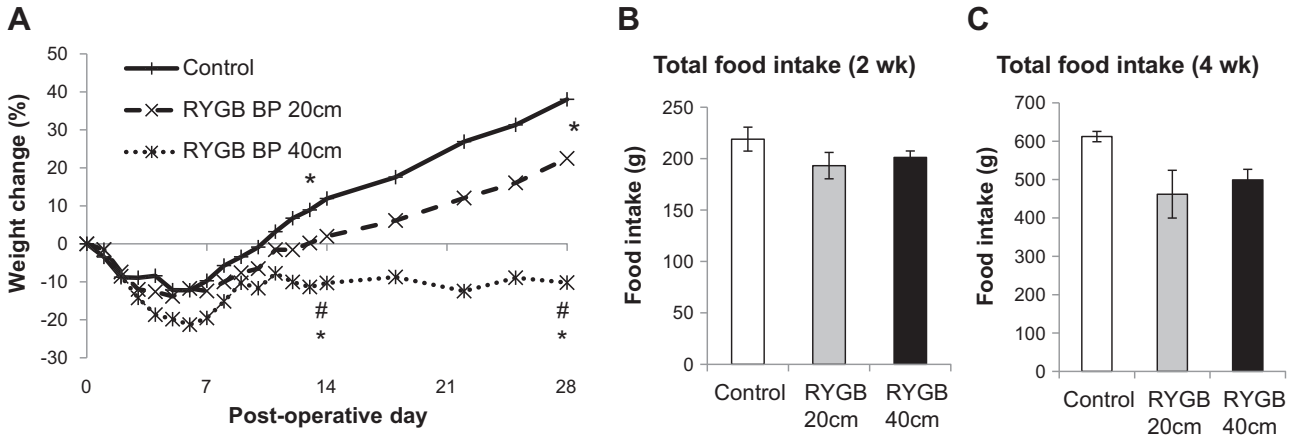

Fig. 2. Postoperative weight changes and food intake. *A*: there were significant differences in weight between the three groups. *B* and *C*: there was no difference in food intake. *$P < 0.05$ vs. Control; #$P < 0.05$ vs. RYGB with a 20-cm BP limb (RYGB-20cm).

(Spectramax M5; Molecular Devices). RNA (2 μg) was reverse transcribed (Superscript III and oligo(dT); Invitrogen-Life Technologies) to generate cDNA. Real-time PCR was then performed (ABI 7900HT; Applied Biosystems) on a 384-well plate using SYBR Green (Life Technologies).

Portal Flow Measurement

Portal blood flow was measured using a transabdominal ultrasound Doppler (Vevo 2100; VisualSonics, Toronto, Canada). The portal vein, which runs from the splenic vein-SMV confluence to its bifurcation in left and right branches, was identified. The mean diameter was calculated from two measurements (at the confluence and the bifurcation). The mean flow velocity was measured in the sagittal section view. Portal blood flow was calculated as the product of vein area and mean velocity. Portal vein diameter was 2.2 ± 0.2 mm. Portal flow was 9.6 ± 0.7 ml/min. No difference was observed between control and RYGB rats.

Calculation of Intestinal Glucose Fluxes

Intestinal glucose fluxes were calculated as previously described (2, 14) using the portosystemic glucose gradient (G_{PS}):

$$G_{PS} = G_P - G_S$$

where G_P and G_S are portal and systemic glucose level, respectively, at a given time point.

At baseline (0 min), before the intestinal glucose infusion was started, G_{PS} was negative (i.e., $G_P < G_S$), reflecting the net use of glucose by the intestine in the fasting state. Glucose utilization (G_{util}; mg/h) was calculated:

$$G_{util} = G_{PS0} \times 60 \times PBF \times 10^{-2}$$

where G_{PS0} is portosystemic glucose gradient at 0 min and PBF is portal blood flow (ml/min).

After the intestinal glucose infusion was started, G_{PS} became positive, indicating glucose absorption by the intestine in the portal bloodstream. The area under the G_{PS} curve (2), corrected for baseline

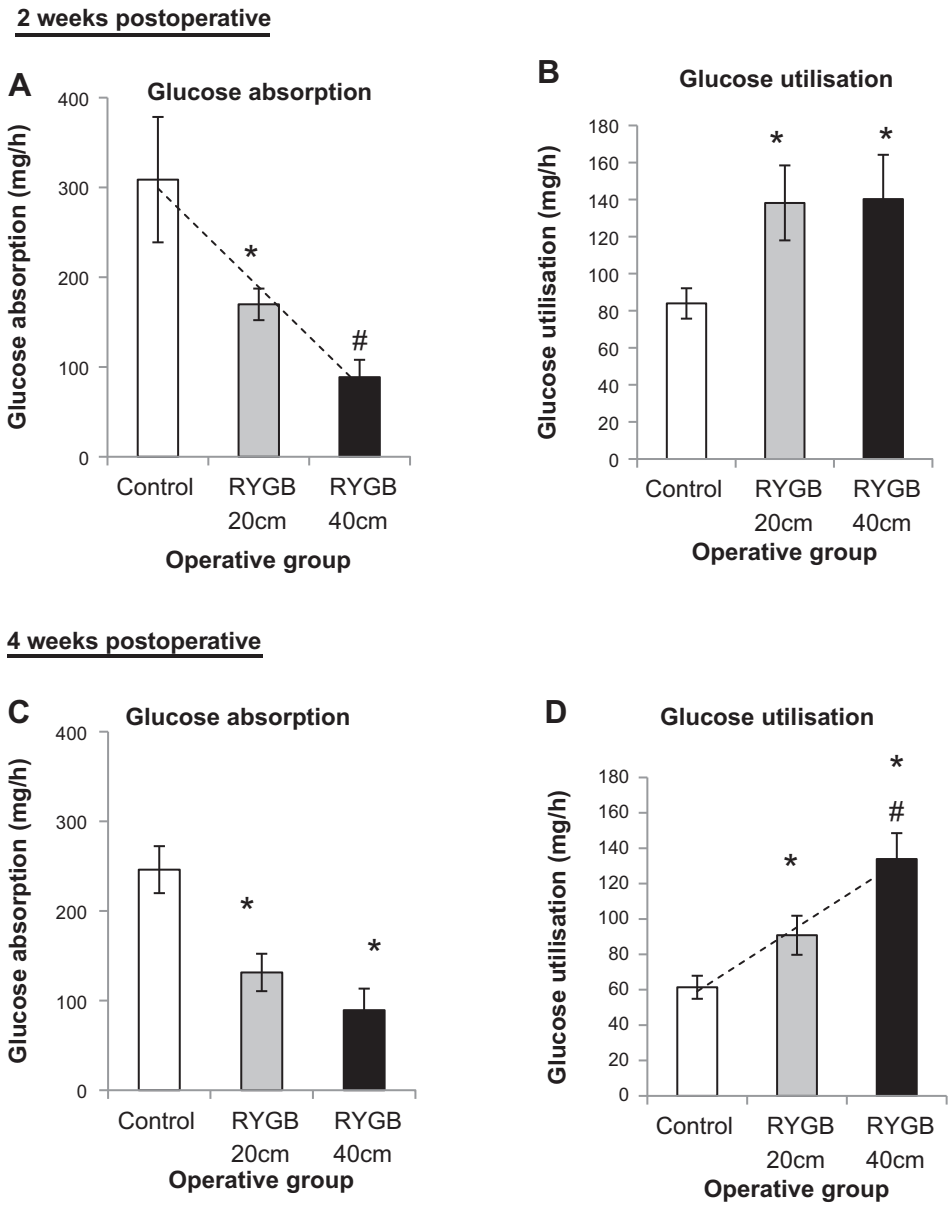

Fig. 3. Intestinal glucose fluxes. Intestinal glucose absorption and utilization at 2 (*A* and *B*) and 4 (*C* and *D*) wk. *$P < 0.05$ vs. Control; #$P < 0.05$ vs. RYGB-20cm.

gradient, was used to estimate intestinal glucose absorption (G_{absorp}; mg/h) as follows:

$$G_{absorp} = G_{PS}(AUC) \times PBF \times 10^{-2}$$

where $G_{PS}(AUC)$ is area under the curve of G_{PS} from 0 to 60 min ($mg \cdot dl^{-1} \cdot min^{-1}$).

Calculation of Hormone Secretion

Hormone levels in portal and systemic blood were determined at 0, 10, 30, and 60 min.

The portosystemic hormone gradient was calculated:

$$GLP\text{-}1_{PS} = GLP\text{-}1_{P} - GLP\text{-}1_{S}$$

where $GLP\text{-}1_{P}$ and $GLP\text{-}1_{S}$ are portal and systemic GLP-1 levels (pg/ml), respectively.

$GLP\text{-}1_{PS}$ increased following glucose infusion, indicating GLP-1 secretion by the intestine in the portal blood. The area under the $GLP\text{-}1_{PS}$ curve was used to calculate intestinal GLP-1 secretion ($GLP\text{-}1_{secrete}$; ng) as follows:

$$GLP\text{-}1_{secrete} = GLP\text{-}1_{PS}(AUC) \times PBF \times 10^{-3}$$

where $GLP\text{-}1_{PS}(AUC)$ is area under the curve of $GLP\text{-}1_{PS}$ from 0 to 60 min ($pg \cdot ml^{-1} \cdot min^{-1}$).

Similar calculations were used for GLP-2 and GIP.

Statistical Analysis

Data analysis was performed using Excel. The two-tailed unpaired *t*-test was used for planned comparison of two groups. For comparing several groups, analysis of variance with post hoc analysis was used. Where a statistically significant relation was present across the three groups, linear regression was used to model the "length-response" relationship. Multivariate regression was used to analyze the effects of weight, type of surgery, and postoperative time on hormone levels. Data are presented as means ± SE.

RESULTS

Weight Loss and Food Intake

Weight in the RYGB groups was lower than in the Control group, and weight loss was greater after RYGB-40cm than RYGB-20cm (*day 14*: 301 ± 9 vs. 251 ± 11 vs. 233 ± 9 g, $P < 0.05$; *day 28*: 368 ± 4 vs. 295 ± 14 vs. 226 ± 14 g, $P < 0.05$, Fig. 2). Of interest, there was no difference in food intake in either group at 2 wk but a trend toward reduced food intake at 4 wk postoperatively ($P = 0.07$) (Fig. 2, *B* and *C*). None of

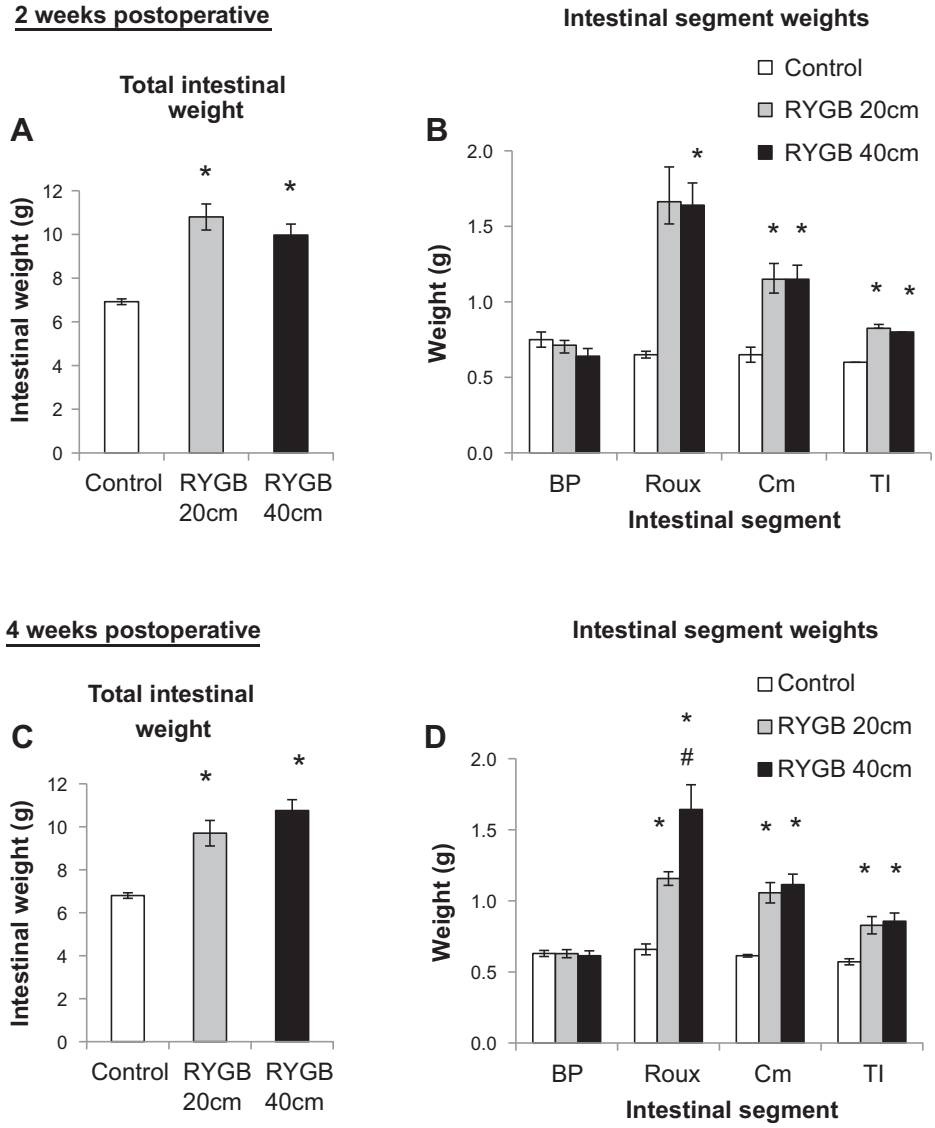

Fig. 4. Intestinal weight. Intestinal weight of entire small intestine (from pylorus to ileocecal valve), Roux limb (10-cm segment), and intestinal segments at 2 (*A* and *B*) and 4 (*C* and *D*) wk. Cm, common limb; TI, terminal ileum. *$P < 0.05$ vs. Control; #$P < 0.05$ vs. RYGB-20cm.

the animals developed diarrhea, or other suggestion of intestinal malabsorption.

Intestinal Glucose Fluxes

At 2 wk, G_{absorp} was reduced by about one-half in RYGB-20cm (170 ± 18 mg/h vs. 309 ± 70 in Controls; $P < 0.05$) and by ~70% in RYGB-40cm (89 ± 19 mg/h, $P < 0.05$) (Fig. 3*A*), giving an apparent G_{absorp} length-response relationship of -5.5 mg·h^{-1}·cm^{-1}. G_{util} increased by ~60% after either RYGB-20cm or RYGB-40cm (Fig. 3*B*).

At 4 wk (Fig. 3, *C* and *D*), G_{absorp} decreased in RYGB-20cm (246 ± 26 vs. 131 ± 21 mg/h, Control vs. RYGB-20cm; $P < 0.05$); there was a further decrease in RYGB-40cm that was not significant compared with RYGB-20cm (89 ± 24 mg/h, $P = 0.18$). G_{util} increased in RYGB-20cm (61 ± 7 vs. 91 ± 11 mg/h, Control vs. RYGB-20cm, $P < 0.05$), and there was a further increase in RYGB-40cm (134 ± 15 mg/h, $P < 0.05$), giving an apparent G_{util} length-response relationship of $+1.8$ mg·h^{-1}·cm^{-1}.

Intestinal Morphology and Gene Expression

At 2 wk, total intestinal weight increased in both RYGB groups (RYGB-20cm, 10.8 ± 0.6 g; RYGB-40cm, 10.0 ± 0.6 g; and Control, 6.9 ± 0.3; $P < 0.001$, RYGB vs. Control; Fig. 4*A*). A similar pattern was seen for Roux limb weight, common limb, and terminal ileum (Fig. 4*B*).

At 4 wk, total intestinal weight increased in both RYGB groups (6.8 ± 0.2 vs. 9.7 ± 0.3 vs. 10.8 ± 0.2 g, Control vs. RYGB-20cm vs. RYGB-40cm). Roux limb weight was highest in RYGB-40cm (1.20 ± 0.05 vs. 1.64 ± 0.67 g, RYGB-20cm vs. RYGB-40cm, $P < 0.05$) (Fig. 4, *C* and *D*).

Intestinal mRNA expression was measured at 4 wk in the Roux limb (Fig. 5, *A* and *B*). There was a length-response relationship for hexokinase expression, with highest expression in RYGB-40cm. Preproglucagon expression was increased in RYGB-40cm ($P < 0.05$ vs. Control). Expression of PEPCK and SGLT3 was reduced. These expression changes were not seen in the BP limb (Fig. 5, *C* and *D*).

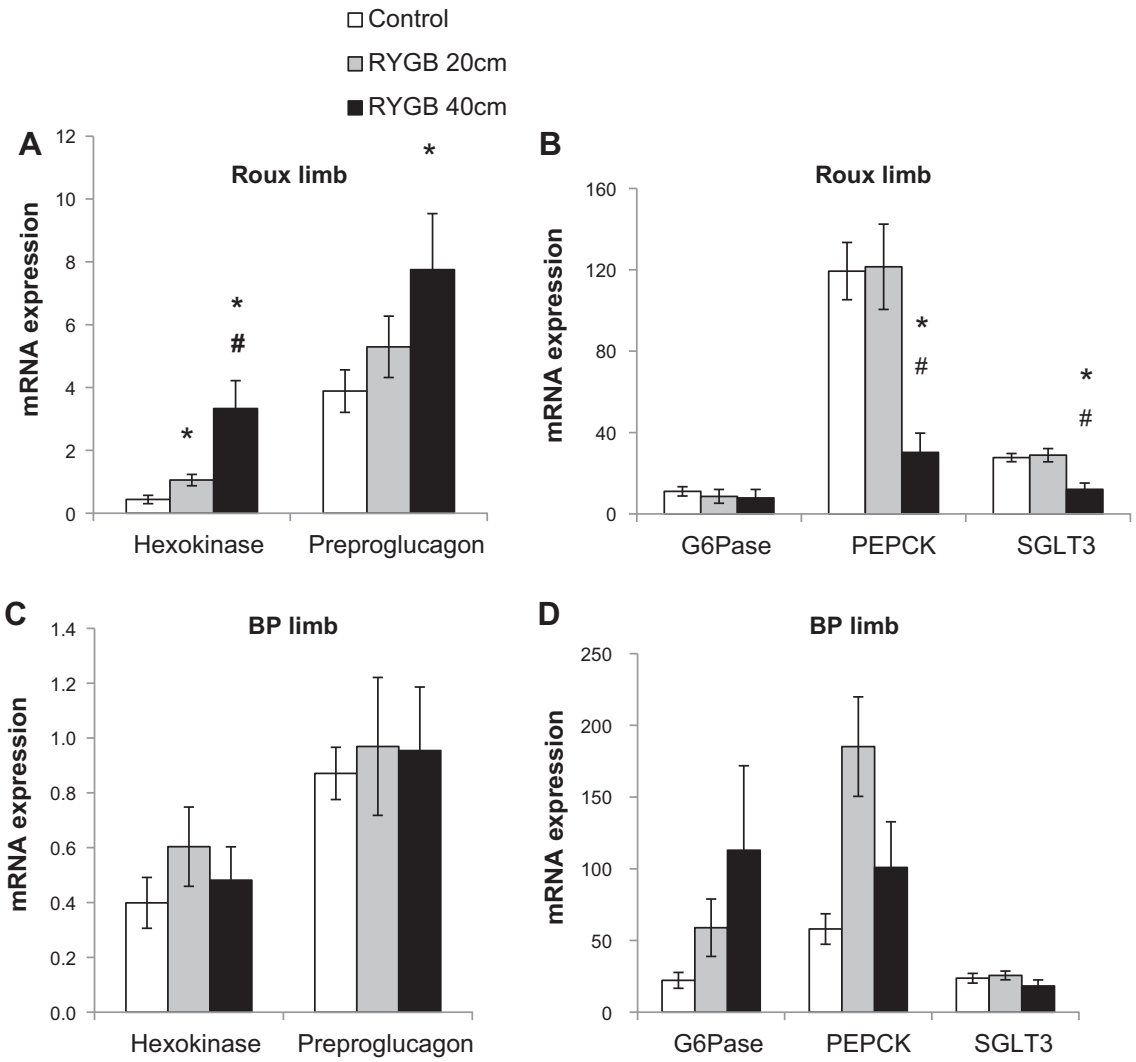

Fig. 5. Intestinal gene expression at 4 wk postoperative. mRNA expression in Roux limb (*A* and *B*) and BP limb (*C* and *D*). *$P < 0.05$ vs. Control; #$P < 0.05$ vs. RYGB-20cm.

Hormone Levels and Secretion

To examine the impact on hormone secretion of glucose infusion in the intestine, a bolus was infused in the Roux limb (duodenum for Control rats). Glucose infusion led to increased levels of GLP-1, GLP-2, and GIP in both portal and systemic blood in all three groups (Figs. 6–11), In addition, the portosystemic gradient also increased in all groups.

GLP-1. At 2 wk (Fig. 6), there were no differences in baseline GLP-1 levels. At 60 min, portal and systemic GLP-1 levels were significantly higher after RYGB-20cm and RYGB-40cm compared with Control. GLP-1 secretion was higher after RYGB-40cm (246 ± 118 vs. 538 ± 48 ng/h, Control vs. RYGB-40cm, $P < 0.05$).

At 4 wk (Fig. 7), a different pattern emerged. Portal and systemic GLP-1 levels at 0 min were higher in both RYGB groups compared with Control. At 60 min, portal and systemic GLP-1 levels were significantly higher only after RYGB-40cm, with no difference between Control and RYGB-20cm. Secretion was higher after RYGB-40cm compared with the other groups (266 ± 45 vs. 283 ± 99 vs. 618 ± 163 ng/h, Control vs. RYGB-20cm vs. RYGB-40cm).

GLP-2. At 2 wk (Fig. 8), baseline GLP-2 levels were lower in RYGB-40cm compared with Control. However, at 60 min, portal and systemic GLP-2 levels were significantly higher after RYGB-40cm compared with Control. There was no difference in secretion between the groups.

At 4 wk (Fig. 9), a different pattern emerged. Portal and systemic GLP-2 levels at 0 min were higher in both RYGB groups, and significantly so after RYGB-40cm compared with Control, a similar pattern to that seen above for GLP-1. At 60 min, portal and systemic GLP-2 levels were significantly higher after RYGB-40cm, as was secretion.

GIP. At 2 wk (Fig. 10), baseline and 60-min portal and systemic GIP levels showed a length-response relationship, with levels significantly higher after RYGB-40cm. There was no difference in secretion, suggesting reduced degradation.

At 4 wk (Fig. 11), a similar pattern remained. Baseline and 60-min portal and systemic GIP levels showed a length-

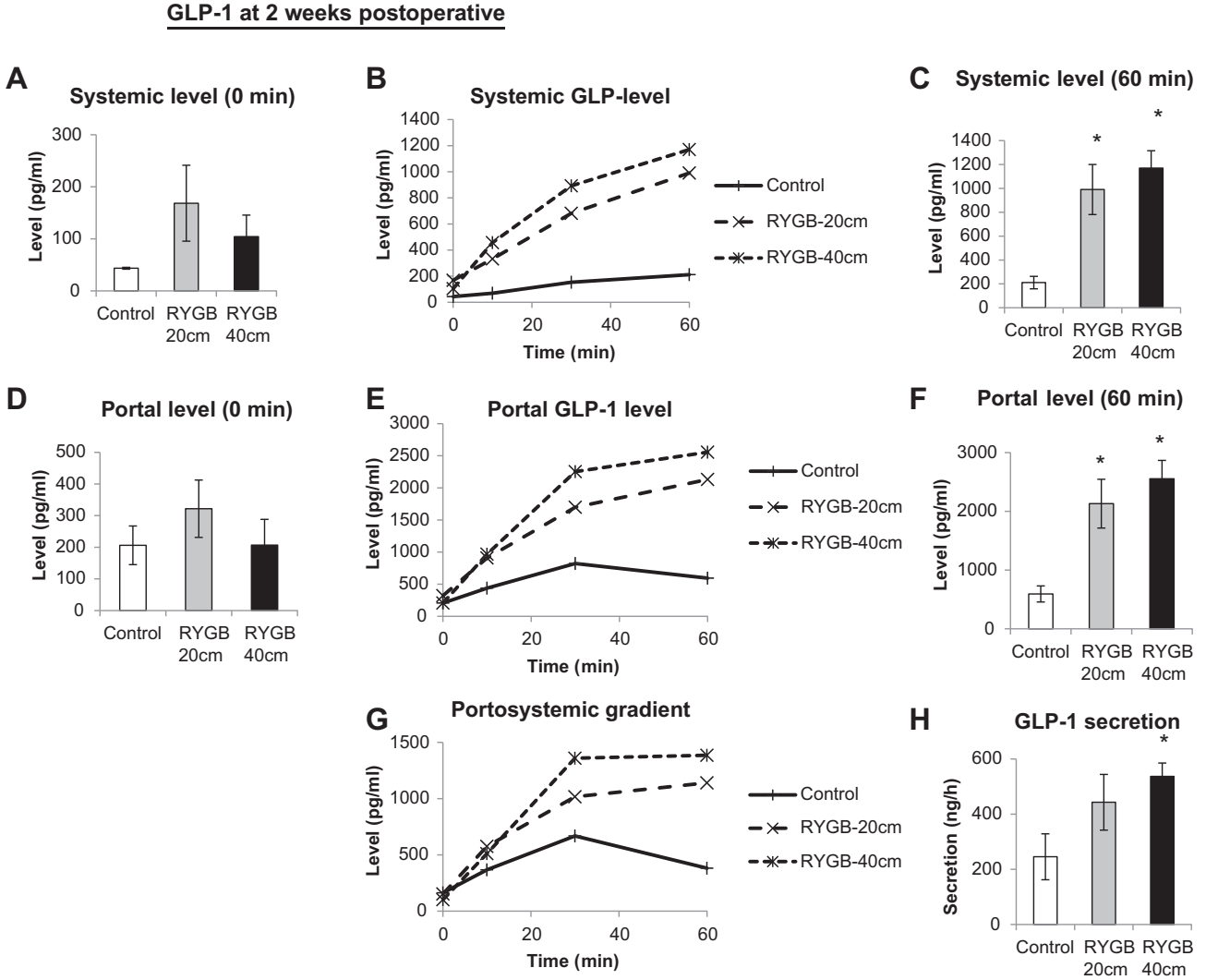

Fig. 6. Glucagon-like peptide (GLP)-1 levels and secretion at 2 wk. Systemic levels at 0 min (*A*), time course over 60 min (*B*), and at 60 min (*C*). Portal levels at 0 min (*D*), time course over 60 min (*E*), and at 60 min (*F*). *G* and *H*: portosystemic gradient (*G*) and secretion (*H*). *$P < 0.05$ vs. Control.

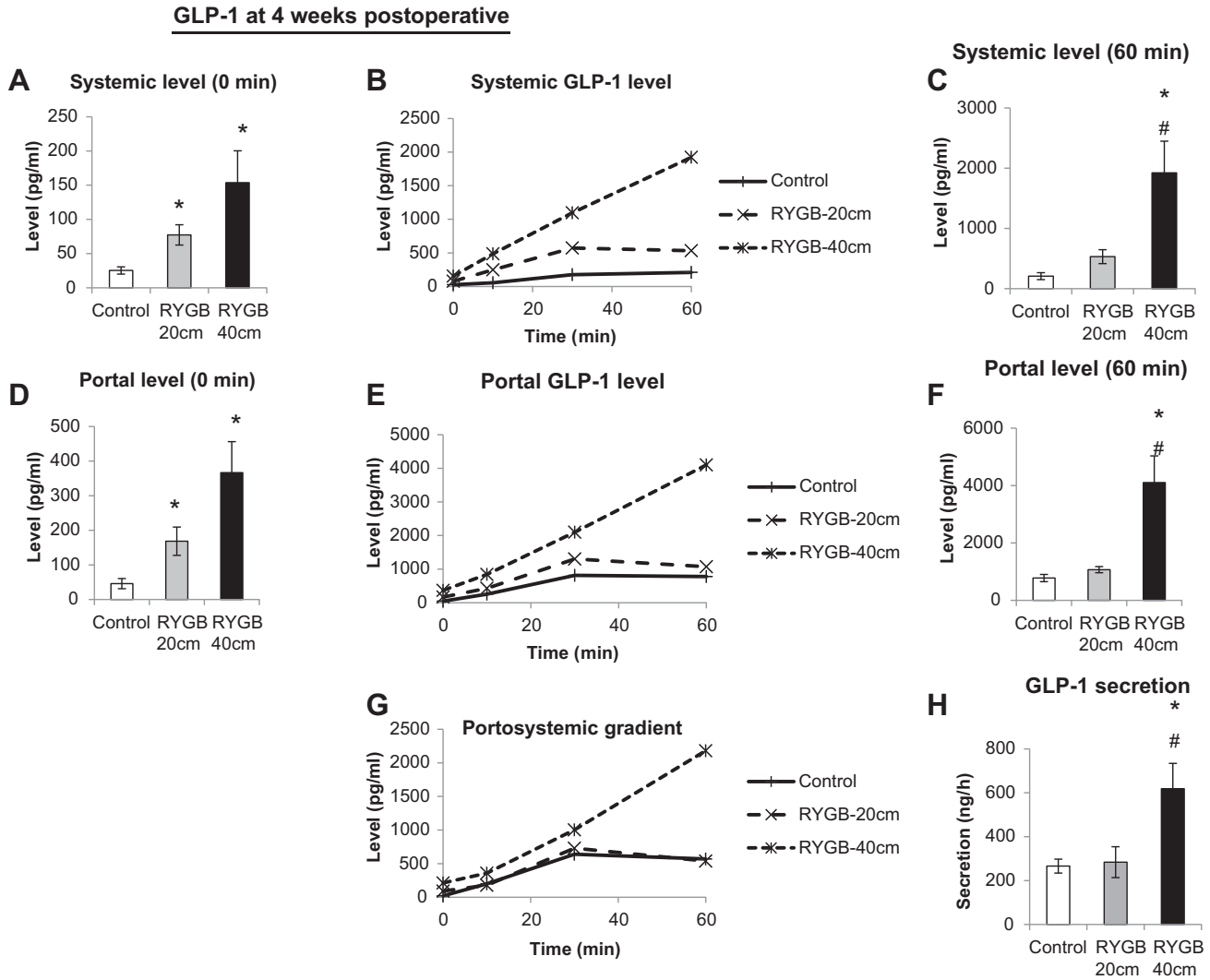

Fig. 7. GLP-1 levels and secretion at 4 wk. Systemic levels at 0 min (*A*), time course over 60 min (*B*), and at 60 min (*C*). Portal levels at 0 min (*D*), time course over 60 min (*E*), and at 60 min (*F*). *G* and *H*: portosystemic gradient (*G*) and secretion (*H*). *$P < 0.05$ vs. Control; #$P < 0.05$ vs. RYGB-20cm.

response relationship, with levels significantly higher in both RYGB groups. There was no difference in secretion.

Hepatic Gene Expression

Expression of dipeptidyl peptidase-4 (DPP4), which degrades both GLPs and GIP, showed a length-response relationship in the opposite direction to that seen in hormone levels, with expression significantly lower after RYGB-40cm (Fig. 12). Hepatic expression of ChREBP was also reduced in both RYGB groups, and expression of glycogen synthase was increased after RYGB-40cm (Fig. 12).

Multivariate Analysis

GIP level was dependent on postoperative time and type of surgery ($P < 0.05$; slope 50.1 $pg \cdot ml^{-1} \cdot wk^{-1}$ and 5.1 $pg \cdot ml^{-1} \cdot cm$ of BP $limb^{-1}$, respectively). GLP-1 secretion was dependent on type of surgery ($P < 0.01$; slope 9.7 ng/cm of BP limb). GLP-2 level was dependent on type of surgery ($P < 0.05$; slope 263 $pg \cdot ml^{-1} \cdot cm$ of BP $limb^{-1}$). The coefficient for the effect of weight was negative for all hormone levels, i.e., increased weight was associated with decreased hormone level, and this was significant for GLP-1 level ($P < 0.05$; slope -6.84 $pg \cdot ml^{-1} \cdot g^{-1}$).

DISCUSSION

Clinical studies investigating the role of varying limb length to improve weight loss and metabolic outcomes have been incomplete and inconclusive. In this study, we employed our rat model of RYGB in which we simultaneously sample portal and systemic blood. Our results reveal a mechanistic basis for varying BP limb length in RYGB to module antidiabetic effects. Specifically, we show that a longer BP limb results not only in superior weight loss, despite similar food intake, but that it also modulates several other antidiabetic mechanisms in what we have termed the length-response relationship. Furthermore, we highlight the dynamic nature of these mechanisms by showing their contribution varies throughout the postoperative period.

Intestinal glucose absorption showed a length-response relationship at 2 wk, with G_{absorp} progressively decreasing with

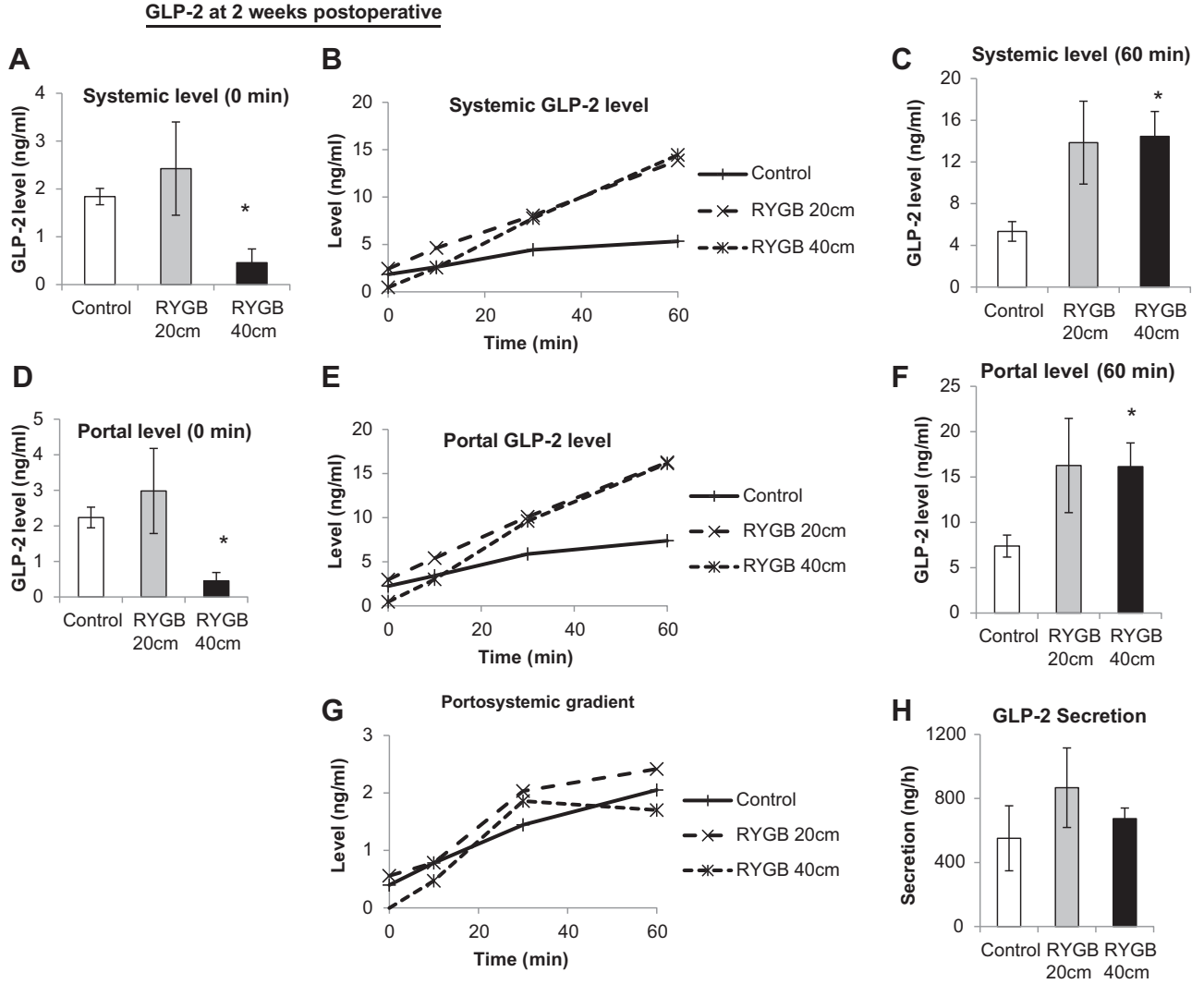

Fig. 8. GLP-2 levels and secretion at 2 wk. Systemic levels at 0 min (*A*), time course over 60 min (*B*), and at 60 min (*C*). Portal levels at 0 min (*D*), time course over 60 min (*E*), and at 60 min (*F*). *G* and *H*: portosystemic gradient (*G*) and secretion (*H*). *$P < 0.05$ vs. Control.

increasing BP limb length. At 4 wk, G_{absorp} decreased after RYGB, but the length-response relationship was less marked. This may reflect intestinal adaptation to increase glucose absorption. Previously we showed that acute exclusion of 20 cm of proximal small intestine reduced G_{absorp} by 59% (14), consistent with this study that shows that at 2 and 4 wk, G_{absorp} was reduced by 45 and 46%, respectively. A similar phenomenon may be occurring when longer lengths of small intestine are bypassed: at 2 wk RYGB-40cm reduced G_{absorp} by 71% and at 4 wk by 64%.

The mechanism underlying the reduced glucose absorption is exclusion of the proximal intestine, and specifically the glucose sensor SGLT3. We have shown that stimulation of SGLT3 in the foregut restores glucose absorption to control levels (14). Of note, SGLT3 expression was reduced in the Roux limb after RYGB with a long BP limb. This may suggest that glucose sensing has a diminished role after long BP RYGB, further contributing to the decreased proximal intestinal glucose absorption discussed above. This requires further investigation.

Intestinal glucose utilization is another important parameter that was evaluated in our studies. The Roux is known to be a major site of glucose uptake after RYGB (16). In our experiments, we did not initially show a length-response relationship at 2 wk, with G_{util} reaching a plateau with RYGB-20cm. However, at 4 wk, a length-response relationship emerged not only in G_{util}, but also in Roux limb weight, and Roux limb expression of hexokinase and preproglucagon. GLP-2, which is an intestinotrophic hormone, may explain this dynamic G_{util} response. At 2 wk, the differences in GLP-2 levels between the groups were not particularly marked. At 4 wk, baseline and 60-min levels were significantly greater in RYGB-40cm compared with RYGB-20cm, and this may be driving the Roux limb hypertrophy and increased G_{util}. We show, for the first time, that the BP limb modulates Roux limb changes in a length-response manner and elucidate the associated pathways involved. Increased hexokinase expression and decreased PEPCK expression in the Roux limb will also be contributing to increased G_{util}. Another feature of the RYGB-40cm group is that increased GLP-2 levels at 4 wk were associated with

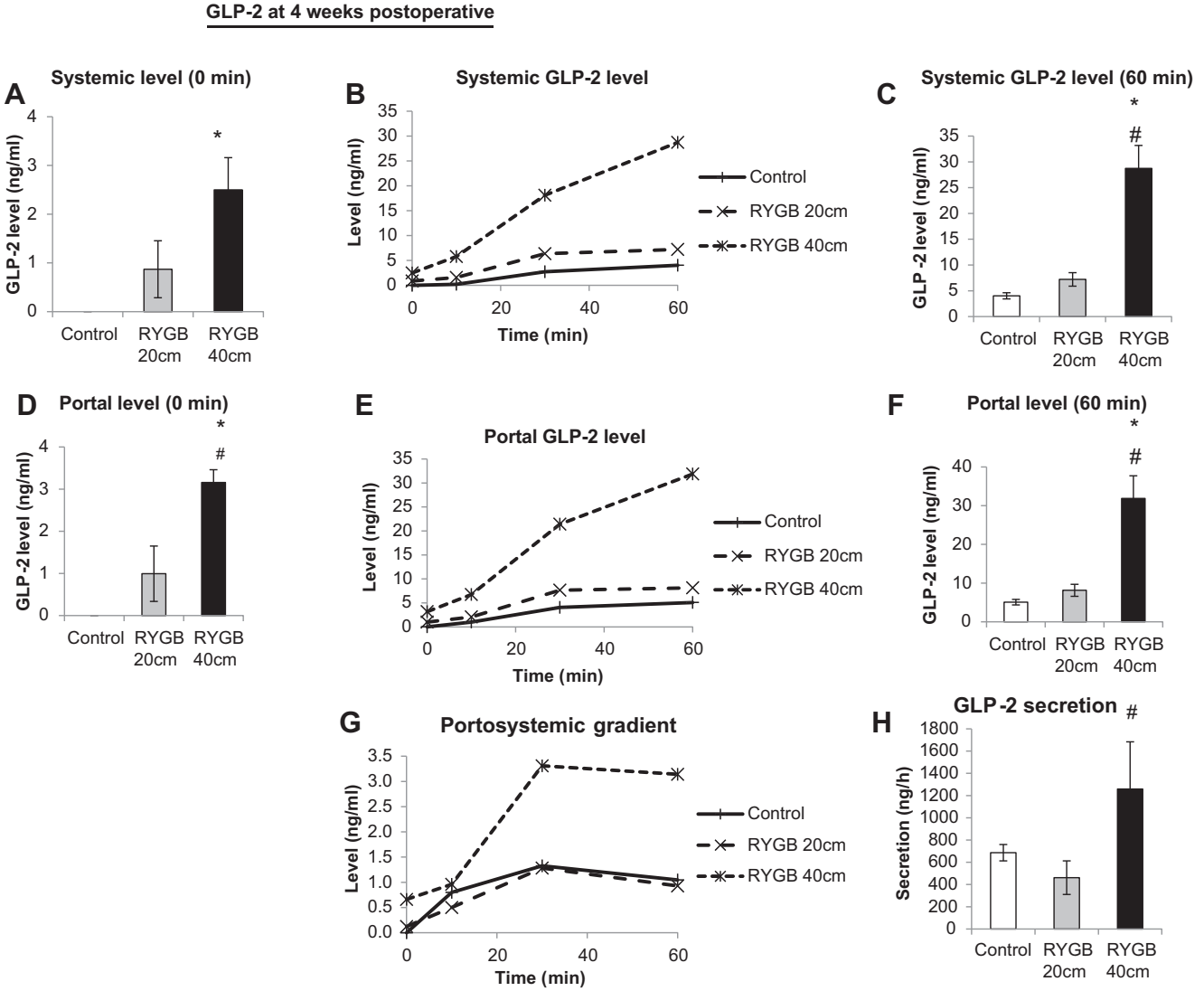

Fig. 9. GLP-2 levels and secretion at 4 wk. Systemic levels at 0 min (*A*), time course over 60 min (*B*), and at 60 min (*C*). Portal levels at 0 min (*D*), time course over 60 min (*E*), and at 60 min (*F*). *G* and *H*: portosystemic gradient (*G*) and secretion (*H*). *$P < 0.05$ vs. Control; #$P < 0.05$ vs. RYGB-20cm.

increased secretion (Fig. 9). In contrast, at 2 wk, increased GLP-2 levels occurred without a corresponding significant increase in secretion, and therefore likely through decreased degradation (discussed below).

GLP-1 levels showed a similarly dynamic response. At 2 wk, there were no significant differences between RYGB-20cm and RYGB-40cm. At 4 wk, postprandial 60-min GLP-1 levels were only elevated in RYGB-40cm, again associated with increased GLP-1 secretion, whereas there was no difference between Control and RYGB-20cm (Fig. 7). This may have clinical relevance for relapse of T2D and weight regain after RYGB, where reduced incretin levels have been implicated, and a longer BP limb may help prevent this. A similar pattern was therefore seen for both GLP-1 and GLP-2. Decreased degradation was a dominant mechanism early in the postoperative period for both RYGB groups. Increased secretion, with corresponding increase in preproglucagon expression, contributed in RYGB-40cm at 4 wk.

Although RYGB limb length did not affect GIP secretion at 2 or 4 wk, a length-response relationship in GIP levels was apparent at 2 and 4 wk. With no corresponding difference in secretion, differences in GIP degradation likely underlie this difference. GIP, like the GLPs, is also degraded by DPP4 (8). Hepatic DPP4 expression showed a length-response relationship in the opposite direction, with lowest expression after RYGB-40cm, and this may explain increased GIP levels in this group, as well as increased GLP levels at 2 wk (Fig. 12).

Hormone levels may be critical in outcomes after RYGB. Reduced incretin levels after meal stimulation were measured in patients suffering from weight regain after RYGB (17). We show that RYGB-40cm maintains higher incretin levels at 4 wk, and so a long BP limb may reduce weight regain through increased incretin levels. Multivariate analysis showed that hormone secretion and levels were positively correlated with BP limb length. Also of note, weight was negatively correlated with hormone levels, significantly so for GLP-1 level, suggesting that animals that lost less weight had lower hormone levels. This is an important avenue for further clinical study.

For the control group in this study, several options were considered. A laparotomy alone would have been technically

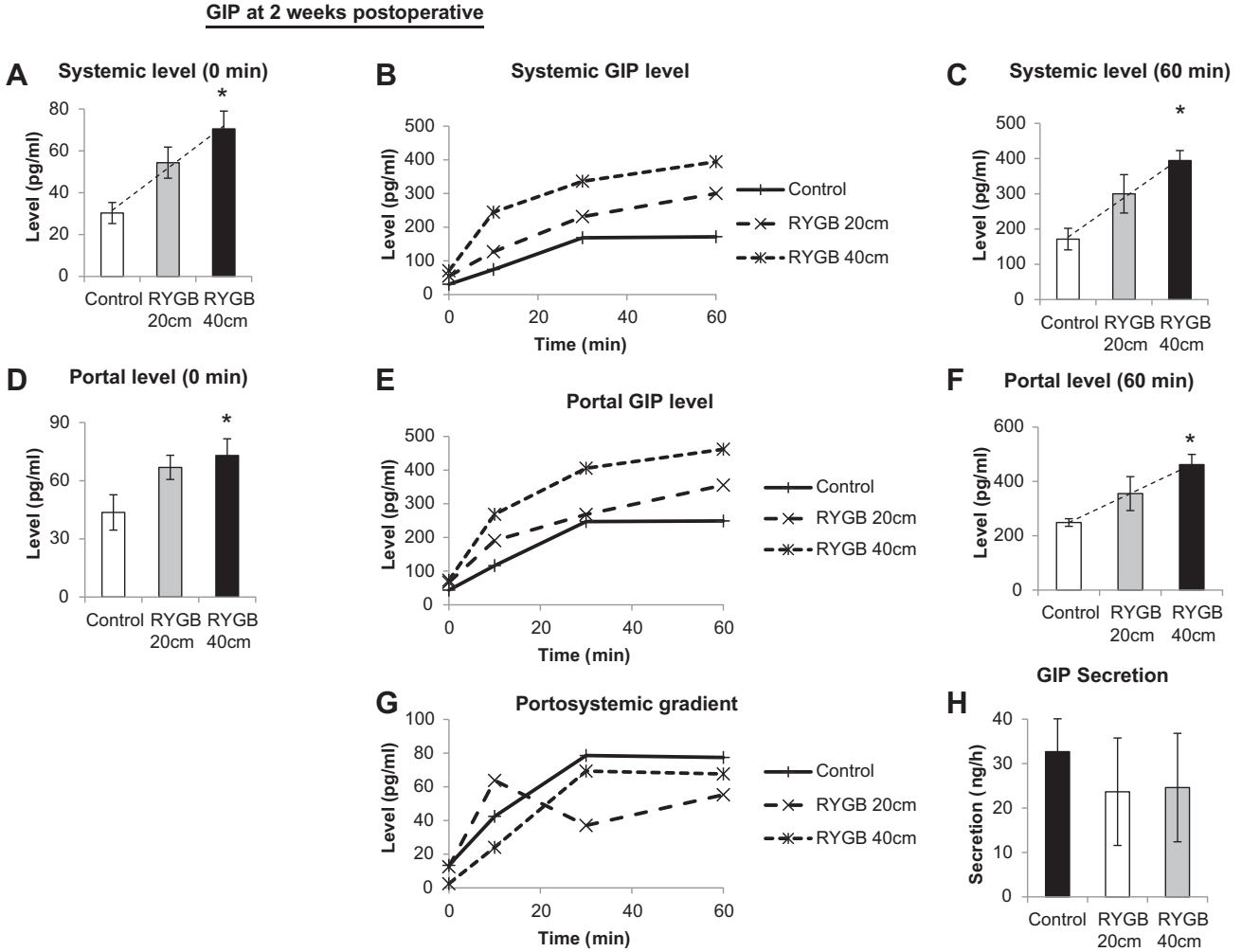

Fig. 10. Glucose-dependent insulinotropic peptide (GIP) levels and secretion at 2 wk. Systemic levels at 0 min (*A*), time course over 60 min (*B*), and at 60 min (*C*). Portal levels at 0 min (*D*), time course over 60 min (*E*), and at 60 min (*F*). *G* and *H*: portosystemic gradient (*G*) and secretion (*H*). *$P < 0.05$ vs. Control.

easier but would only have controlled for the effects of anesthesia and not the effects of intestinal anastomosis. We therefore elected to perform intestinal transection and anastomosis, which would control for the latter. In our acute studies (14), baseline portosystemic gradient was −6.4 mg/dl, corresponding to G_{util} of 32 mg/h. This contrasts with the transection-anastomosis control groups; G_{util} was 81 ($P < 0.05$) and 61 ($P < 0.05$) mg/h at 2 and 4 wk, respectively. This suggests that, in control animals, healing of the intestinal anastomosis may contribute to increased glucose utilization, especially early in the postoperative period. In the RYGB groups, additional factors are contributing to increased glucose utilization, including intestinal hypertrophy and changes in intestinal metabolism.

The effects of BP limb length on intestinal glucose and hormone fluxes will naturally affect the portal milieu because the portal vein drains the intestine. Portal glucose levels decreased and portal GLP levels increased relative to the systemic circulation, and these changes are modulated by BP limb length. This altered portal milieu will likely affect hepatic function and may influence some of the changes in hepatic gene expression we measured, including decreased ChREBP, decreased DPP4, and increased glycogen synthase. As well as modulating intestinal changes, the BP limb may therefore also be a regulator of hepatic function after RYGB. These multiple interacting mechanisms are consistent with our previous work (Pal A, Rhoads D, Tavakkoli A, unpublished observations). Other studies in a rat model have shown that resection of the BP limb after duodenojejunal bypass reversed weight loss and metabolic benefits (13), confirming its critical role.

Limited clinical data are available to establish an optimum length of intestinal limbs. A randomized controlled trial showed that longer BP and Roux limbs in T2D superobese patients led to a higher rate of T2D remission (15), but the lengths of both Roux and BP limbs were varied simultaneously, which makes it difficult to isolate the anatomic basis. A case report showed that revising a RYGB for recurrent T2D by lengthening the BP limb led to remission (12). The DUCATI trial may investigate the effect of a long Roux limb with a fixed common channel length on weight loss (9), but the BP limb length is fixed. Our current work shows that several antidiabetic mechanisms are modulated by BP limb length, thereby highlighting the importance of this segment and providing evidence for further clinical study. This may help identify the subgroup of patients that will benefit from a long BP limb length.

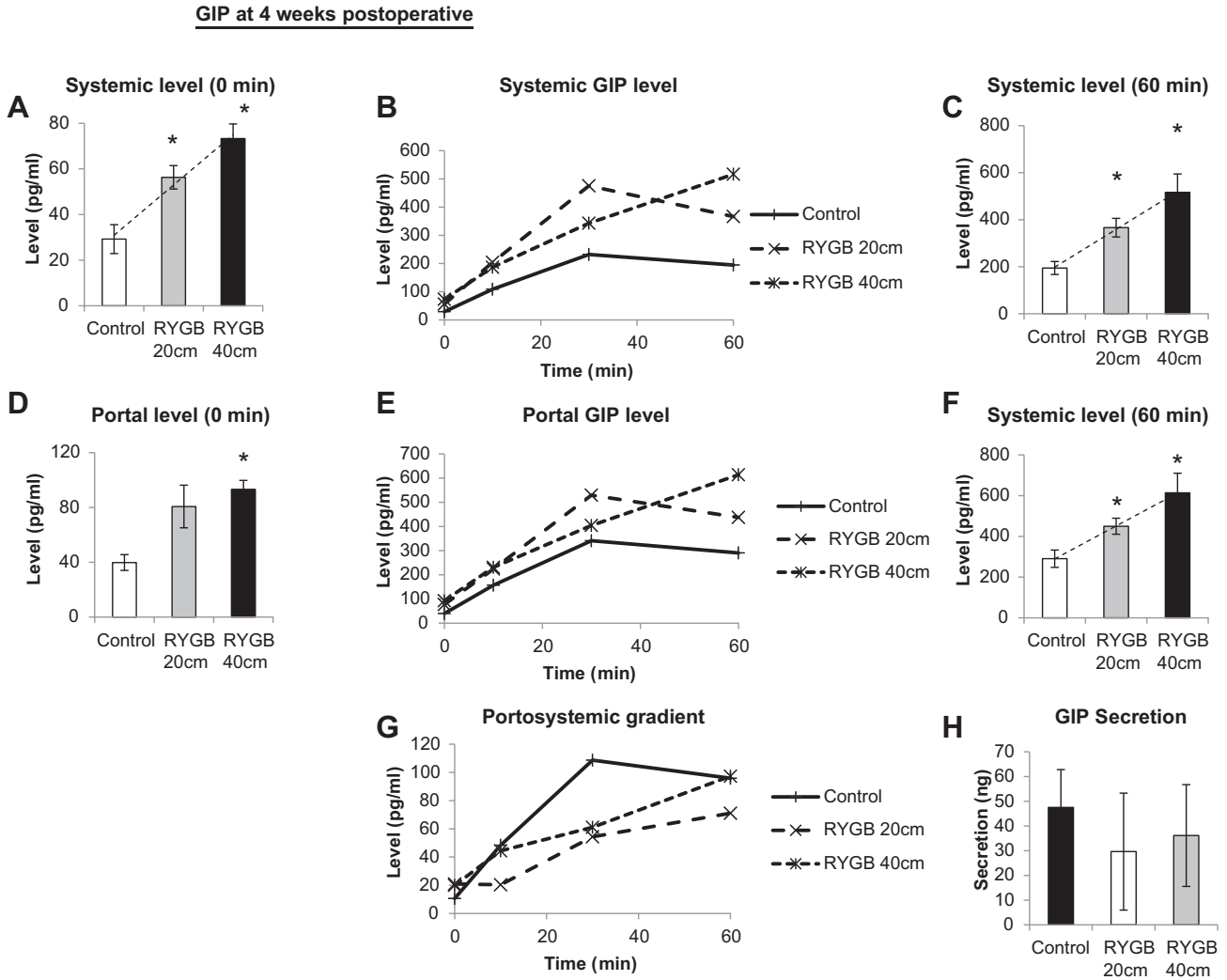

Fig. 11. GIP levels and secretion at 4 wk. Systemic levels at 0 min (*A*), time course over 60 min (*B*), and at 60 min (*C*). Portal levels at 0 min (*D*), time course over 60 min (*E*), and at 60 min (*F*). *G* and *H*: portosystemic gradient (*G*) and secretion (*H*). *$P < 0.05$ vs. Control.

In summary, BP limb length modulates multiple antidiabetic mechanisms, including intestinal function and hepatic gene expression. This is analogous to the dose-response relationship of a drug. The contribution of each mechanism varies through the postoperative period. Early post-RYGB, longer BP limb length reduces intestinal glucose absorption. Later, increased glucose utilization, Roux limb hypertrophy, and increased hormone secretion are the sustained effects of a long BP limb. The sustained high incretin levels may prevent weight regain and T2D relapse, and this requires further investigation. This

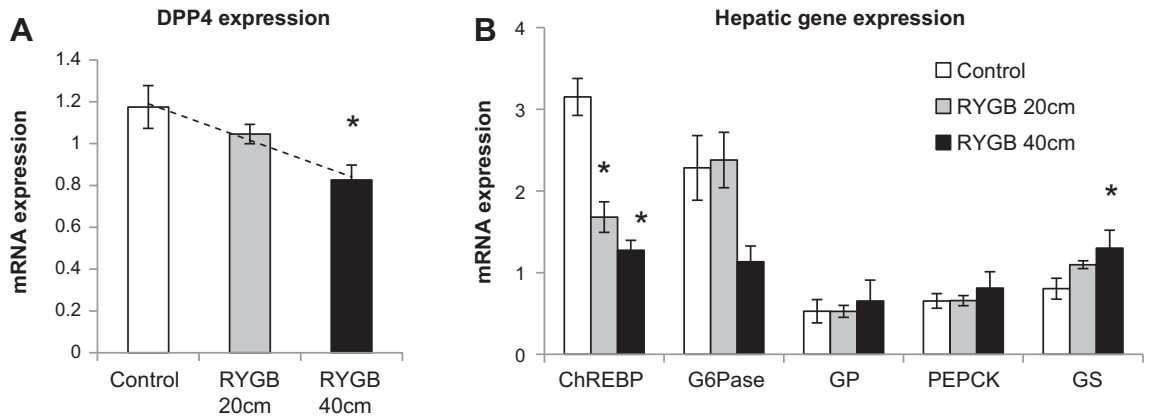

Fig. 12. Hepatic gene expression. Hepatic expression of dipeptidyl peptidase-4 (DPP4, *A*) and other genes (*B*). *$P < 0.05$ vs. Control.

study provides new insights into the mechanistic basis of customising BP limb length according to patient characteristics (e.g., T2D, metabolic syndrome, nondiabetic obesity), and desired metabolic effect.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

A.P., D.B.R., and A.T. conceived and designed research; A.P. performed experiments; A.P. analyzed data; A.P., D.B.R., and A.T. interpreted results of experiments; A.P., D.B.R., and A.T. prepared figures; A.P., D.B.R., and A.T. drafted manuscript; A.P., D.B.R., and A.T. edited and revised manuscript; A.P., D.B.R., and A.T. approved final version of manuscript.

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