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The effects of Calorie restriction and Bariatric surgery on Circulating Proneurotensin levels

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Abstract

Context: Proneurotensin (pNT) is associated with obesity and T2D, but the effects of Roux-en-Y gastric bypass (RYGB) on postprandial pNT levels are not well studied.

Objective: Assess effects of RYGB versus very low-energy diet (VLED) on pNT levels in response to mixed-meal tests (MMT), and long-term effects of RYGB on fasting pNT.

Study participants: Cohort 1: Nine normoglycemic (NG) and ten T2D patients underwent MMT before and after VLED, immediately post-RYGB and six weeks post-RYGB. Cohort 2: Ten controls with normal weight and ten patients with obesity and T2D, who underwent RYGB or vertical sleeve gastrectomy (VSG), were subjected to MMTs and GIP infusions pre-surgery and three months post-surgery. GLP-1 infusions were performed in normal weight participants. Cohort 3: Fasting pNT was assessed pre-RYGB (n=161), two months post-RYGB (n=92) and 1-year post-RYGB (n=118) in NG and T2D patients. pNT levels were measured using ELISA.

Results: Reduced fasting and postprandial pNT were evident after VLED and immediately following RYGB. Reintroduction of solid food post-RYGB increased fasting and postprandial pNT. Prior to RYGB, all patients lacked a meal response in pNT, but this was evident post-RYGB/VSG. GIP- or GLP-1 infusion had no effect on pNT levels. Fasting pNT were higher 1-year post-RYGB regardless of glycemic status.

Conclusion: RYGB causes a transient reduction in pNT as a consequence of caloric restriction. The RYGB/VSG-induced rise in postprandial pNT is independent of GIP and GLP-1 and higher fasting pNT are maintained one year post-surgically.

Introduction

Roux-en-Y gastric bypass (RYGB) is the most effective weight loss treatment for severe and complex obesity. Furthermore, the majority of people with type 2 diabetes (T2D) who undergo RYGB experience T2D remission, independently of weight loss[1]. The mechanisms through which T2D remission is achieved are not yet fully understood. Indeed, RYGB is associated with exaggerated secretion of GLP-1[1,2] as well as increased number of L-cells post-RYGB[3]. Our previous studies however suggest that the improvement in glycemia precedes the response in GLP-1 after RYGB[1]. Furthermore, the immediate impact of RYGB on the metabolome seems mainly related to caloric restriction, rather than the rerouting of the GI tract[4]. Thus, there is a need for continued investigations on RYGB-associated alterations to shed light on the mechanisms behind the effect on glycemia as well as sustained weight loss.

The gut hormone neurotensin (NT) is mainly expressed in the distal small intestine[5] and has been shown to be positively correlated with increased T2D incidence in a Nordic population-based study[6]. Furthermore, NT reduces food intake[7], gastric emptying and gastric acid secretion[8], increases blood flow to the small intestine[9], hepatic bile acid secretion[10] and uptake[11] as well as pancreatic exocrine activity[12]. Gene targeting or inhibition of NT results in reduced fat absorption as well as a protective effect against high fat diet-mediated obesity and insulin resistance[13]. It is not fully understood how NT is affected by RYGB. However, a number of

studies have tried to address this. Firstly, in a study by Jorsal *et. al.* fasting neurotensin levels were shown to be increased one week and three months after RYGB[2]. NT levels were also increased in response to a mixed-meal one week post-surgery with a reduction towards pre-surgical levels being observed three months post-surgery, although an increase in peak levels of NT was also observed at this timepoint[2]. Another study reported increased fasting proneurotensin (pNT) levels in patients subjected to RYGB compared to both age-matched patients that were either normal weight or had obesity[14], furthermore patients who underwent RYGB had a blunted response in pNT levels after a mixed-meal compared with patients that were either normal weight or had obesity. Increased pNT levels in patients with obesity has also been shown at 24 months after adjustable gastric banding or RYGB[15]. This response was shown to be affected by biliopancreatic limb length, with a longer limb resulting in higher fasting NT levels as well as higher levels in response to a mixed-meal[16]. In rats, *Nt* gene expression was increased in the alimentary limb in response to RYGB along with increased plasma NT levels[7]. It has also been shown that fasting plasma pNT levels increase more robustly after biliopancreatic diversion (BPD) than after RYGB[17]. The same study identified a positive correlation between pNT levels and insulin sensitivity, and a negative correlation with fasting glucose, suggesting positive effects of NT on glucose homeostasis[17]. In our lean porcine model of RYGB a substantial increase in duodenal NT cell density was identified post-RYGB[18]. On the other hand we have shown that NT cell density was unaffected in human jejunal samples 1-year post-RYGB[3].

To gain further insight into potential RYGB-induced alterations in pNT plasma levels we took advantage of our study design with MMTs performed with tight intervals during the RYGB treatment regimen. This design enabled a direct comparison of the effect of caloric restriction versus that of RYGB. Thus, we performed mixed-meal tests (MMT), in both patients with

normoglycemia (NG) and T2D without underlying comorbidities, before and after a very low-energy diet (VLED) regimen, acutely post-operation as well as six weeks post-surgically when solid food had been reintroduced to the patients. We used another cohort of patients subjected to RYGB or vertical sleeve gastrectomy (VSG) for replication and also studied the long-term effects of RYGB on plasma pNT levels in a third cohort. Finally, we addressed the potential influence of incretin hormones on pNT levels in infusion experiments.

Materials and Methods

Patients.

In the present study, we used samples from three previously published patient cohorts[1,19,20].

Cohort 1

The characteristics of the patients used in this study have been described previously[1]. Briefly, 19 age- and weight-matched Caucasian women subjected to RYGB were studied. Patients had a mean age of 43±1.7 years and BMI of 37.5±0.9 kg/m^2. Inclusion criteria consisted of female gender, age of 30-55 years old, body mass index (BMI) >35 and no pre-operative incretin-based medication. Exclusion criteria was prior bariatric surgery or previous surgery to the upper gastrointestinal tract. Nine patients were normoglycemic (NG; HbA1c<42.1 mmol/mol and fasting plasma glucose <6.1 mmol/L without antidiabetic intervention) while ten patients had T2D. Average duration of T2D was 3.1±0.9 years with two patients being treated solely with insulin, two patients being treated with insulin alongside metformin, two patients with only metformin, one patient with insulin, metformin and glimepiride and three patients controlling their T2D through diet and lifestyle. Antidiabetic medications were withheld the night prior to

surgery and on the first day post-operation. Patients received pre-operative counseling to try and reduce liver size by targeting 5% weight loss. Weight loss was accomplished by way of a very low-energy diet. This diet consisted of four servings of Modifast® (Modifast, Täby, Sweden) per day, totaling 858 kcal/day (110 g carbohydrate, 57 g protein and 18 g fat). All participants provided written informed consent and the study was approved by the Human Ethical Committee in Lund, Sweden and adhered to the Declaration of Helsinki.

Mixed-meal tests (MMT). MMTs were performed as previously described[1]. Patients fasted overnight prior to arrival to the clinic. Basal blood samples were collected using a cubital vein catheter. The volume of the mixed-meal was decided by the volume that was tolerable to the patients one day post-operation (MMT_{+1d}). We have previously shown this volume to be 200ml of Modifast® ingested over 20 minutes[21,22] consisting of 28 g carbohydrates, 14 g protein and 5 g fat resulting in a total of 220 kcal. The meal was ingested within 20 minutes. Blood samples were collected in chilled EDTA tubes coated with 0.1 mmol/L diprotin A and 500 KIU/ml aprotinin at -5, 0, 5, 10, 15, 30, 60 and 90 minutes post-meal ingestion. Plasma samples were stored at -80°C until analyses. Patients were given the same volume and at the same rate during all MMTs as described previously[21]. Four MMTs were performed; 1) before starting VLED four weeks before the operation (MMT_{-4w}); 2) one day prior to surgery (MMT_{-1d}); 3) the morning of the first day post-operation (MMT_{+1d}); and 4) 6 weeks after the operation (MMT_{+6w}).

Cohort 2

Patients. The characteristics of the patients used in this study have been described previously by Honka *et al*[19]. In brief, the study included ten patients with obesity and T2D who underwent

RYGB (n=5) or vertical sleeve gastrectomy (VSG; n=5), as well as ten normal weight and normoglycemic control participants. The inclusion criteria for the control population were BMI of 18–27 kg/m^2, age 18–60 years, fasting plasma glucose <6.1 mmol/L, and normal oral glucose tolerance test. All participants provided written informed consent and the Ethics Committee of the Hospital District of Southwest Finland (Turku, Finland) approved the study.

MMTs (cohort 2). Before the introduction of VLED and 69 (55–97) days after surgery patients were subjected to MMTs consisting of 250-kcal liquid meal (Nutridrink; Nutricia Advanced Medical Nutrition, Amsterdam, the Netherlands) containing 40 g carbohydrates, 6 g fat, and 9 g protein which was ingested within 10 minutes. Blood samples were taken at 0, 15, 30-, 45-, 60- and 90-minutes post-meal ingestion and handled as in cohort 1.

GIP and GLP-1 infusions (cohort 2). The GIP and GLP-1 infusions have been described previously[19,23]. GIP infusions were performed on patients with T2D who underwent RYGB (n=5) or VSG (n=5) and control patients who were normal weight (n=10). GLP-1 infusions were only performed on patients who were normal weight (n=10). Briefly, GIP_{1-42} and $GLP\text{-}1_{7-36}$ (Bachem Holding AG, Bubendorf, Switzerland) were dissolved in 2% human serum albumin in sterile water and administered via a cannula with syringe pumps into the peripheral vein. For both, a constant 75-minute infusion was performed following collection of baseline parameters. GIP_{1-42} was infused at a rate of 4.0 pmol/kg/min for 15 min and thereafter at a rate of 2.0 pmol/kg/min. This was to reproduce GIP concentrations in the circulation after a meal. Infusion was performed pre-surgery and 80 days post-surgery on average (47-92). GLP-1 was infused at a

rate of 0.75 pmol/kg/min for the duration of the test. Blood samples were collected as per the MMT for cohort 2 and handled in the same manner.

Cohort 3

This cohort has been described previously[20]. The study involved an initial cohort of 167 individuals, of which 148 qualified for RYGB during the years 2012-2019 in Sweden. All measurements were done after an overnight fast. Baseline clinical parameters and blood samples were obtained at the first visit to the clinic, before introduction of the pre-surgical VLED, which was on average two months pre-surgery. On the day of operation, only body weight was measured. Blood was sampled at baseline (n=161 out of which n=27 were diagnosed with T2D), then at two- (n=92) and 12-months post-RYGB (n=118). We classified patients as having pre-diabetes if they had a HbA1c≥39mmol/mol as per the ADA guidelines[24]. The study was approved by the Human Ethical Committee in Lund, Sweden and adhered to the standards of the Declaration of Helsinki. All patients provided written informed consent.

proNeurotensin analysis.

Due to the *in vivo* and *in vitro* instability of mature neurotensin, we measured a stable 117-amino acid fragment from the N-terminal of the pre-proneurotensin/neuromedin precursor prohormone (pNT) as previously reported[6,25]. Furthermore the production of this hormone has been proven to be in stochiometric amounts relative to mature neurotensin[26]. In order to quantify pNT levels, a chemiluminometric sandwich ELISA (SphingoTec GmbH, Hennigsdorf, Germany; **RRID:AB_3086809**) was performed as has been described previously[26].

Statistical analyses.

All data are presented as mean ± SEM or as median min to max. Statistical significance was calculated between timepoints using Student's t-test and between groups using one-way ANOVA with Dunnett's multiple comparisons test, with a single pooled variance. Pearson correlation coefficients were calculated to perform correlation analyses. Outliers were identified using ROUT. This method adopts a false discovery rate for nonlinear regression approach to identify outliers[27]. All analyses were performed using Prism 9.2.0 (GraphPad Software, San Diego, CA, USA).

Results

Impact of VLED and RYGB on fasting plasma levels of pNT (Cohort 1)

Fasting samples were collected prior to commencing MMTs in order to assess any differences in basal pNT levels (MMT_{-4w}) which may be due to VLED (MMT_{-1d}), RYGB (MMT_{+1d}) or the reintroduction of solid food (MMT_{+6w}). There were no differences in fasting pNT levels between patients who had NG or patients with T2D at any timepoints (Figure 1). VLED resulted in a 52% reduction in fasting pNT levels in patients with NG ($p<0.05$, Figure 1). RYGB (MMT_{+1d}) further reduced fasting pNT levels by 59% in patients with NG ($p<0.01$). However, at MMT_{+6w} fasting pNT levels in patients with NG were increased 7.6-fold ($p<0.001$) compared with MMT_{+1d}.

In patients with T2D, VLED resulted in a 63% reduction in fasting pNT levels ($p<0.001$). There was no significant further acute effect of RYGB on fasting levels of pNT in the patients with T2D. Fasting pNT increased 5.7-fold between MMT_{+1d} and MMT_{+6w} in patients with T2D ($p<0.001$, Figure 1). Thus, in both participants with T2D and normoglycemia, caloric restriction

reduced fasting levels of pNT, whereas pNT levels rose to above pre-surgery levels after re-introduction of solid food.

Impact of VLED and RYGB on postprandial plasma levels of pNT (Cohort 1)

In order to assess the effects of VLED, RYGB, and the reintroduction of solid food post-RYGB on postprandial pNT responses, MMTs were performed before VLED (MMT_{-4w}), the day prior to RYGB (MMT_{-1d}), the morning after RYGB (MMT_{+1d}) and six weeks post-RYGB (MMT_{+6w}). Notably, before the intervention neither of the groups showed a meal response in pNT levels (Figure 2A, C). In line with the fasting data, both patients with NG (Figure 2A-B) and T2D (Figure 2C-D) demonstrated reduced pNT levels in response to VLED (MMT_{-1d}), as well as acutely in response to RYGB (MMT_{+1d}) compared with starting levels (MMT_{-4w}).

Interestingly, acutely after RYGB (MMT_{+1d}) both groups displayed a meal-response in pNT, with 3.9-fold increases being observed at 60-90 min compared with 0 min in patients with normoglycemia ($p<0.001$; Figure 2A) and a 4.0-fold increase at 60 min and a 3.4-fold increase at 90 min compared with 0 min being observed in patients with T2D ($p<0.001$; Figure 2C). The reintroduction of solid food caused a marked increase in postprandial pNT levels and 1.6-fold higher levels were seen at (MMT_{+6w}) compared with $MMT_{+1\ d}$ in patients with NG ($p<0.01$; Figure 2B) while patients with T2D demonstrated a 1.9-fold increase at (MMT_{+6w}) compared with $MMT_{+1\ d}$ based off AUC ($p<0.001$; Figure 2D). A meal response in pNT levels was seen in both groups but reached significance only in patients with T2D at 60 mins post-meal ingestion with a 1.7-fold increase being observed ($p<0.05$; Figure 2C). There were no statistically significant differences in postprandial responses between patients with NG and T2D (Figures 2E-

H). Thus, our data show that participants with severe obesity lack a meal response in pNT, but after RYGB such a response is evident.

Replication in a T2D cohort and control participants who were normal weight (Cohort 2)

We next sought to replicate our findings in another cohort comprised of patients with both obesity and T2D who were subjected to RYGB or VSG surgery, as well as control, normal weight participants[19]. The RYGB/VSG patients were subjected to MMTs before the introduction of VLDC and within 3 months post-RYGB on average. Similar effects to that observed in cohort 1 of RYGB/VSG on fasting, as well as postprandial pNT levels, were also observed in this cohort (Figure 3A). Notably, participants with obesity pre-RYGB/VSG as well as control participants who were normal weight lacked a meal response in NT, but RYGB/VSG caused a marked meal response in pNT (Figure 3A). When analyzing RYGB and VSG patients separately, VSG had higher overall levels of pNT pre-surgery based off AUC ($p<0.05$; Figure 3C) but RYGB was found to cause a larger postprandial response in pNT than that of VSG (Figure 3B), with levels being 1.6-fold higher than those observed for VSG post-surgery ($p<0.05$; Figure 3C).

Influence of GIP or GLP-1 infusion on pNT plasma levels (Cohort 2)

As pNT levels in the post-RYGB situation were increased concurrently with the incretin hormones GIP and GLP-1[1], we next investigated the possibility that the pNT response is secondary to the rise in incretin levels. To this end we used available samples from experiments with GIP or GLP-1 infusion[19,23]; infused to reach circulating concentrations comparable with those seen postprandially after RYGB. GIP infusion had no effect on pNT levels in control participants of normal weight or in participants with obesity before or after RYGB (Fig 3D).

GLP-1 infusion, as assessed in control participants of normal weight, did not affect pNT levels (Fig 3E). Further to this, we failed to find any significant correlations (using our previously published data from Cohort 1[1]) between plasma levels of pNT and GIP or pNT and GLP-1 in any patient group at any time point (Data not shown). Thus, the observed increase in postprandial pNT seen after RYGB is unlikely secondary to the concomitant increases in GLP-1 and GIP levels.

Long-term impact of RYGB on fasting pNT plasma levels (Cohort 3)

Having established that pNT levels are affected in the short term by RYGB we next assessed long-term effects of RYGB on pNT levels. To this end we assessed fasting pNT levels in pre- and post-RYGB samples from 27 patients with T2D and 35 patients with pre-diabetes and 99 patients who were normoglycemic but also had obesity[20]. All three groups of patients had higher fasting pNT levels one year post-RYGB while patients with NG also had increased fasting pNT levels two months post-RYGB, compared with pre-RYGB (Figure 4). At two months post-surgery patients with NG demonstrated a 1.7-fold increase in fasting pNT levels ($p<0.001$) while patients classified as having pre-diabetes or T2D did not demonstrate a significant increase at this timepoint. 12 months post-RYGB, patients with NG demonstrated a 2.5-fold increase in fasting pNT ($p<0.001$) while increases of 2.0-fold ($p<0.001$) and 1.5-fold ($p<0.01$) where observed for patients classified as having pre-diabetes or T2D. Patients diagnosed with T2D pre-RYGB had 1.5-fold increased fasting pNT levels compared with patients with NG ($p<0.001$) pre-surgery.

Discussion

RYGB frequently results in immediate remission of T2D prior to weight loss[1], as well as sustained improved glycemic control several years after the operation[28,29]. Although numerous studies have been reported and mechanistic insight has been demonstrated[30,31], there are many questions remaining on the exact details underlying RYGB-induced remission of T2D.

To try and address a gap in this knowledge, we assessed RYGB-related alterations in plasma levels of the somewhat neglected gut hormone neurotensin in three cohorts of RYGB patients. Here we show that RYGB, as well as energy restriction, caused reduced fasting levels of pNT in the short term. In the longer term, patients subjected to RYGB had elevated fasting levels of pNT. Furthermore, control participants who were normal weight, as well as patients with obesity before surgery lacked a meal response in pNT. However, in patients subjected to RYGB or VSG a meal response in pNT was evident.

Our study design in cohort 1 allowed for comparisons between the effects of energy restriction versus the short-term effects of RYGB. Immediately after RYGB, fasting pNT levels were markedly reduced and this was similar to the observed effect of VLED. The period around surgery is an intense period of energy restriction in a bid to reduce pre-surgery liver size. As there were no differences observed between pNT levels following VLED and acutely post-RYGB, we propose that energy restriction explains the initial drop in fasting pNT levels. However, six weeks post-RYGB we observed a marked increase in fasting levels of pNT compared with before both VLED and RYGB. In order to strengthen this finding, we replicated these results in a second cohort which we have previously described[19]. This confirmed the observed increased fasting levels of pNT longer term post-surgery as MMTs were performed

three months post-surgery on average. Increased fasting pNT in response to RYGB was further confirmed in a third cohort, up to one-year post-RYGB. This is in agreement with a study showing RYGB to increase fasting pNT levels up to 24 months post-RYGB[15].

We have previously shown that RYGB acutely causes a number of alterations in the metabolite profile that are explained by reduced food intake[4]. Notably, these alterations could not explain the parallel increases in GLP-1 or GIP[1,4]. Thus, our data suggest a more diet-dependent behavior of pNT acutely in conjunction with RYGB compared with that of the incretin hormones. Nevertheless, in the long term, fasting pNT levels remained elevated one-year after RYGB in patients without diabetes, patients with pre-diabetes as well as patients with T2D.

Both participants with obesity planned for RYGB and control participants who were normal weight lacked a MMT response in pNT. The MMT included 5 grams of fat. Of note, the main trigger of pNT secretion is fat intake, rather than carbohydrates and proteins and in normal participants who ingested 54 grams of fat (oil or cream) there was a clear pNT response[32]. Nevertheless, we found that RYGB, and to a lesser extent, VSG triggered a mixed-meal response in pNT both acutely and longer term in both cohort 1 and cohort 2. The observation that this meal response is evident at both MMT_{+1d} when fasting levels dropped and at MMT_{+6w} when fasting pNT are increased, speak in favor of that this effect is not related to energy restriction, but rather to the re-arrangement of the GI-tract. Supporting this notion, even though VLED caused reduced fasting pNT levels, VLED had no impact on the postprandial response in pNT. This is line with what is reported in a number of other studies that have addressed the effect of bariatric surgery on NT plasma levels in both humans[2,15-17,33-39] and rats[7]. A handful of studies have also addressed the expression of NT in intestinal cells[3,40-42].

A previous study assessing biliopancreatic limb length showed that a longer biliopancreatic limb results in increased fasting and postprandial levels of pNT alongside GLP-1[16]. Meanwhile postprandial GIP levels were reduced in patients with a longer biliopancreatic limb. This could suggest a negative correlation between GIP and pNT levels postprandially. However, we found no effect of GIP infusion on pNT levels and no correlation between GIP and pNT levels in response to a mixed-meal. The work by Patricio *et. al.* may also suggest a positive correlation between GLP-1 and pNT levels[16]. Although only performed in control participants who were normal weight, our GLP-1 infusion data do not suggest any effect of GLP-1 on pNT levels. Furthermore, we could not find any correlation between pNT and GLP-1 levels. Therefore, we propose that the observed postprandial rise in pNT levels is unlikely secondary to the parallel increase in GIP or GLP-1 levels.

Another study showed that BPD resulted in a greater increase in fasting pNT levels compared with RYGB[17]. We observed no difference in fasting pNT levels between RYGB and VSG but did observe a larger increase in meal response after RYGB. This in agreement with a previous study[33]. Although the exact mechanisms remain to be established, these two studies taken in tandem suggest that bypassing the intestine clearly affects pNT levels. Further studies are needed to understand whether the observed effect of bariatric surgery on pNT levels are related to alterations in bile acids, which have been shown to regulate pNT levels[35,36], or alterations in e.g. the enteric nervous system or in food composition. It should be mentioned that previous studies in humans demonstrated increased pNT levels in response to fat[32,37] in particular long chain fatty acids[34].

In cohort 3 we found pNT levels to be higher in patients with T2D. This finding gains support from a previous study reporting higher neurotensin levels in women with gestational diabetes[43].

Nevertheless, our data does not suggest any major influence of glycemic status on fasting, or postprandial pNT levels following bariatric surgery.

Limitations: Our study design in cohort 1 allowed for comparisons of VLED and RYGB as well as the immediate effect of RYGB. The volume of the meal in the MMTs had to be limited to a volume tolerable the day after surgery. A potential limitation is that this low volume may not be enough to trigger a response preoperatively. In cohort 2, we could test the potential influence of GIP infusion on pNT levels in patients before and after RYGB, but for GLP-1 infusion we only had access to samples from control participants.

Summary/conclusion

pNT plasma were reduced by caloric restriction and immediately after RYGB, however, also likely as a consequence of caloric restriction. On the contrary, after the introduction of solid food post-RYGB, pNT plasma levels were elevated and maintained at a higher level for 12 months. RYGB and to a lesser extent VSG, triggered a meal response in pNT, that is likely independent to the rise in GIP or GLP-1. Fasting pNT levels were higher in patients with T2D preoperatively, but otherwise the responses in pNT levels were largely unaffected by glycemic status of the patients. Even though experimental studies have shown that NT has a dual effect on insulin secretion[44,45] and NT knockout mice have improved insulin sensitivity[13], it is not well known how NT affects insulin secretion or insulin sensitivity in humans. It is not inconceivable that the observed increase in pNT levels post RYGB/VSG is a consequence of weight loss and part of a compensatory mechanism trying to increase intestinal fat absorption. Thus, our study has provided detailed insight into the impact of bariatric surgery on plasma levels of pNT. Further

studies are needed to understand whether alterations in pNT levels play a role in RYGB-induced weight-loss, improved glycemia or alteration of long term cardiometabolic morbidity and mortality.

DATA AVAILABILITY STATEMENT

Original data generated and analyzed during this study are included in this published article or in the data repositories listed in References.

Figure legends

Figure 1. Fasting pNT levels 4 weeks pre-RYGB (MMT^{-4w}), immediately pre-RYGB (MMT^{-1d}), immediately post-RYGB (MMT^{+1d}) and 6 weeks post-RYGB (MMT^{+6w}) in patients with obesity and normoglycemia (NG) or Type 2 Diabetes (T2D). * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ compared with MMT^{-4w} in patients with the same glycemic status.

Figure 2. pNT levels following MMT in patients with obesity and NG (A) at MMT^{-4w}, MMT^{-1d}, MMT^{+1d} and MMT^{+6w} and corresponding AUC (B). pNT levels following MMT in patients with obesity and T2D (C) at the same timepoints with corresponding AUC (D). pNT levels in response to MMT in patients with obesity and NG versus patients with obesity and T2D at MMT^{-4w} (E), MMT^{-1d} (F), MMT^{+1d} (G) and MMT^{+6w} (H). Patients with T2D are represented with dashed lines. For A and C, * $p<0.05$ and *** $p<0.001$ compared with 0 mins for same MMT and + $p<0.05$, ++ $p<0.01$ and +++ $p<0.001$ compared with MMT^{-4w} at the same timepoint. For B and D, * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ compared with MMT^{-4w}. For G, *** $p<0.001$ for patients with NG compared with 0 mins and +++ $p<0.001$ for patients with T2D compared with

0 mins. For H, + $p<0.05$ for patients with T2D compared with 0 mins. NG – participants with normoglyceamia; T2D – participants with type 2 diabetes.

Figure 3. pNT levels in response to MMT in patients undergoing either RYGB or VSG pre- and post-surgery as well as control participants who were normal weight (A). pNT levels in response to MMT (B) in patients with obesity undergoing RYGB or VSG with corresponding AUC (C). pNT levels in response to GIP infusion (4.0 pmol/kg/min and 2.0 pmil/kg/min as indicated) in patients undergoing RYGB or VSG pre- and post-surgery as well as control participants who were normal weight (D). pNT levels in response to GLP-1 infusion (0.75 pmol/kg/min) in control patients who were normal weight (E). For A, ^^^ $p<0.001$ between post-surgery and control patients who were normal weight, + $p<0.05$ between pre-surgery values and control patients who were normal weight and ### $p<0.001$ between pre- and post-surgical values in patients with obesity. For C, * $p<0.05$ between patients undergoing RYGB v VSG at the same timepoint. For D, ^^^ $p<0.001$ between post-surgery and control patients who were normal weight, +++ $p<0.001$ between pre-surgery values and control patients who were normal weight and ### $p<0.001$ between pre- and post-surgical values in patients with obesity.

Figure 4. Pre-, 2 months post- and one-year post-RYGB fasting pNT levels in patients with obesity who had normoglycemia (non-diabetic), impaired fasting glucose (pre-diabetic) or were diagnosed with diabetes (diabetic). ** $p<0.01$ and *** $p<0.001$ compared with pre-surgical levels in patients with the same glycemic status. +++ $p<0.001$ compared with patients with normoglycemia at the same timepoint.

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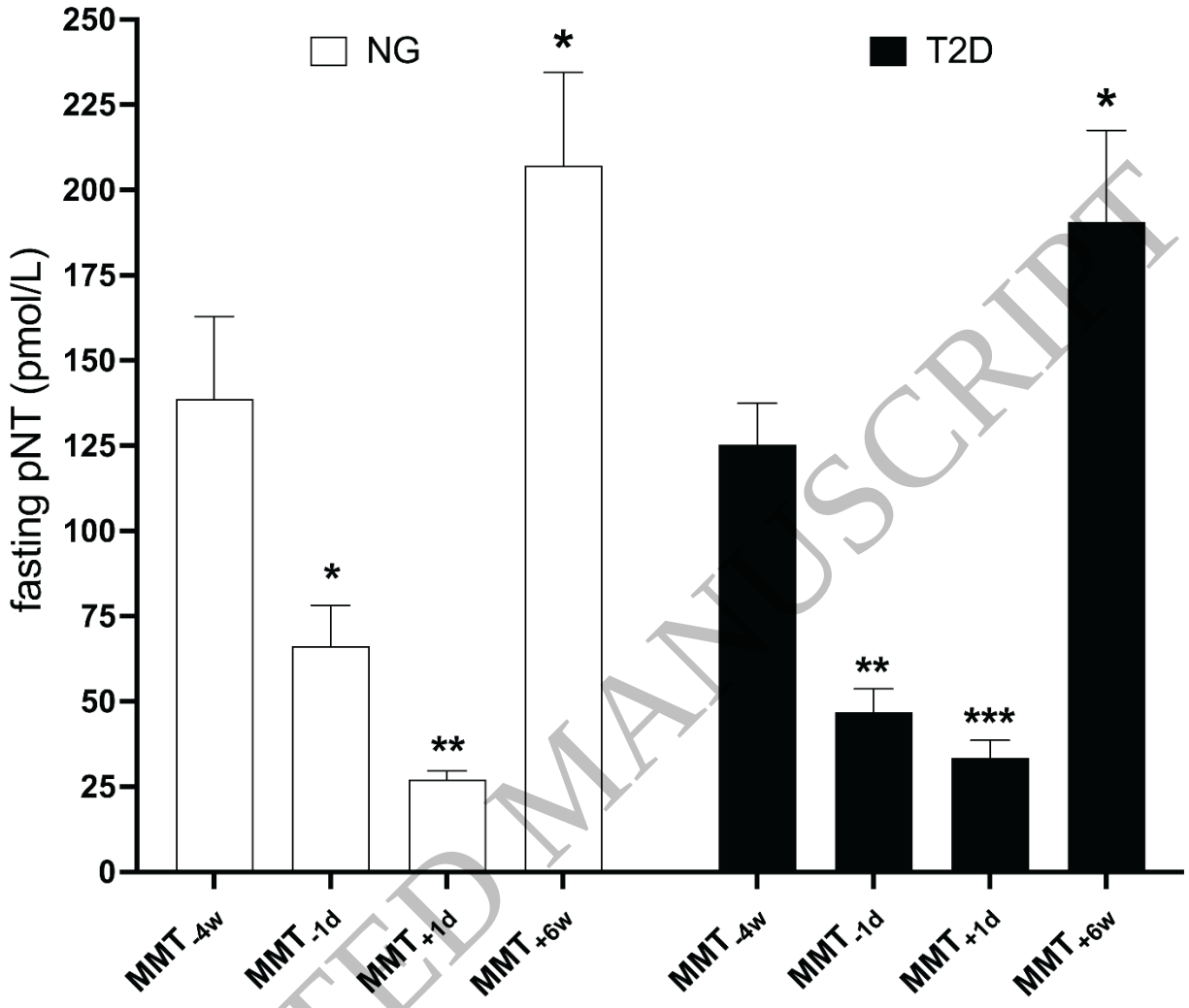

Figure 1
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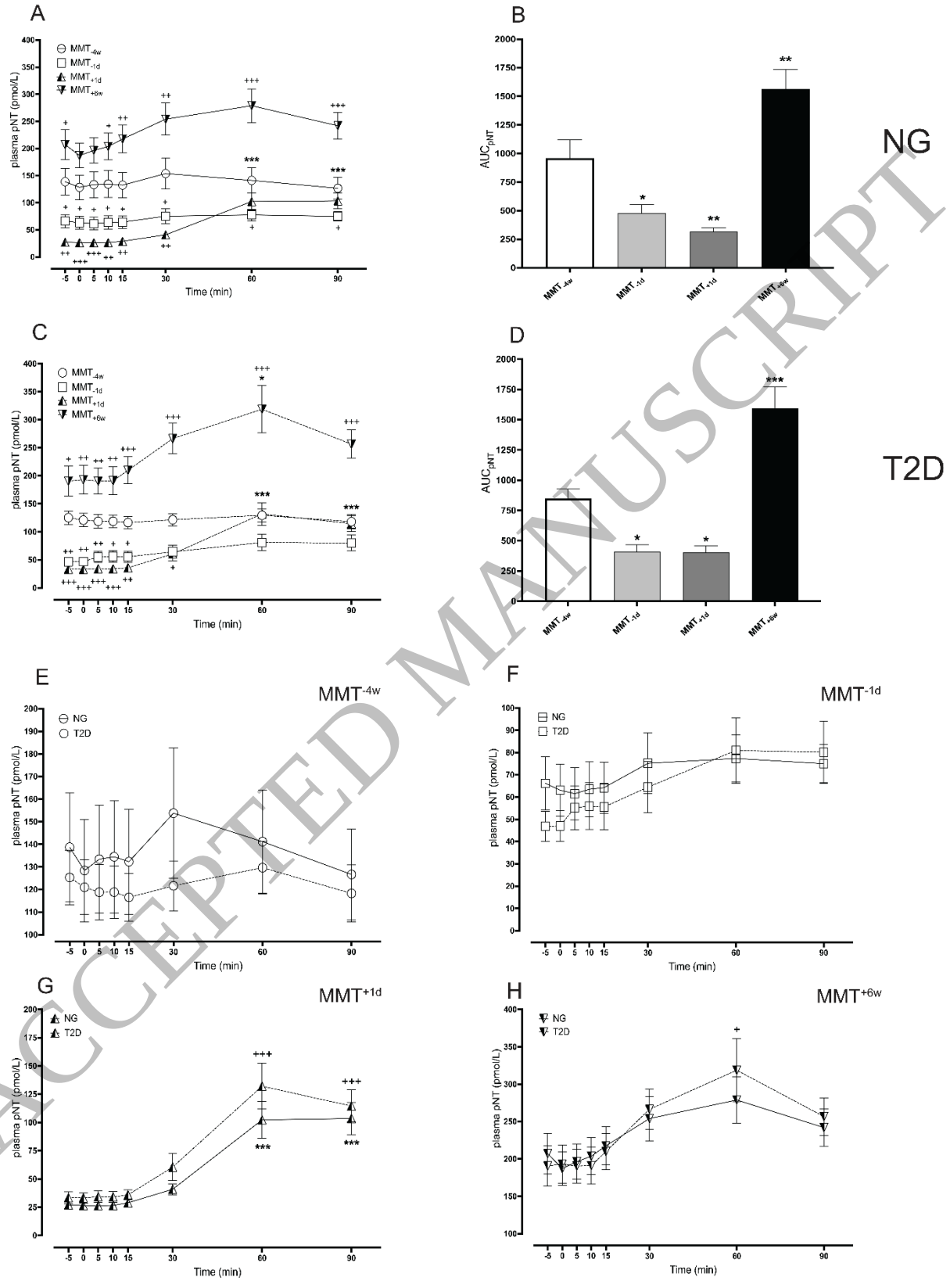

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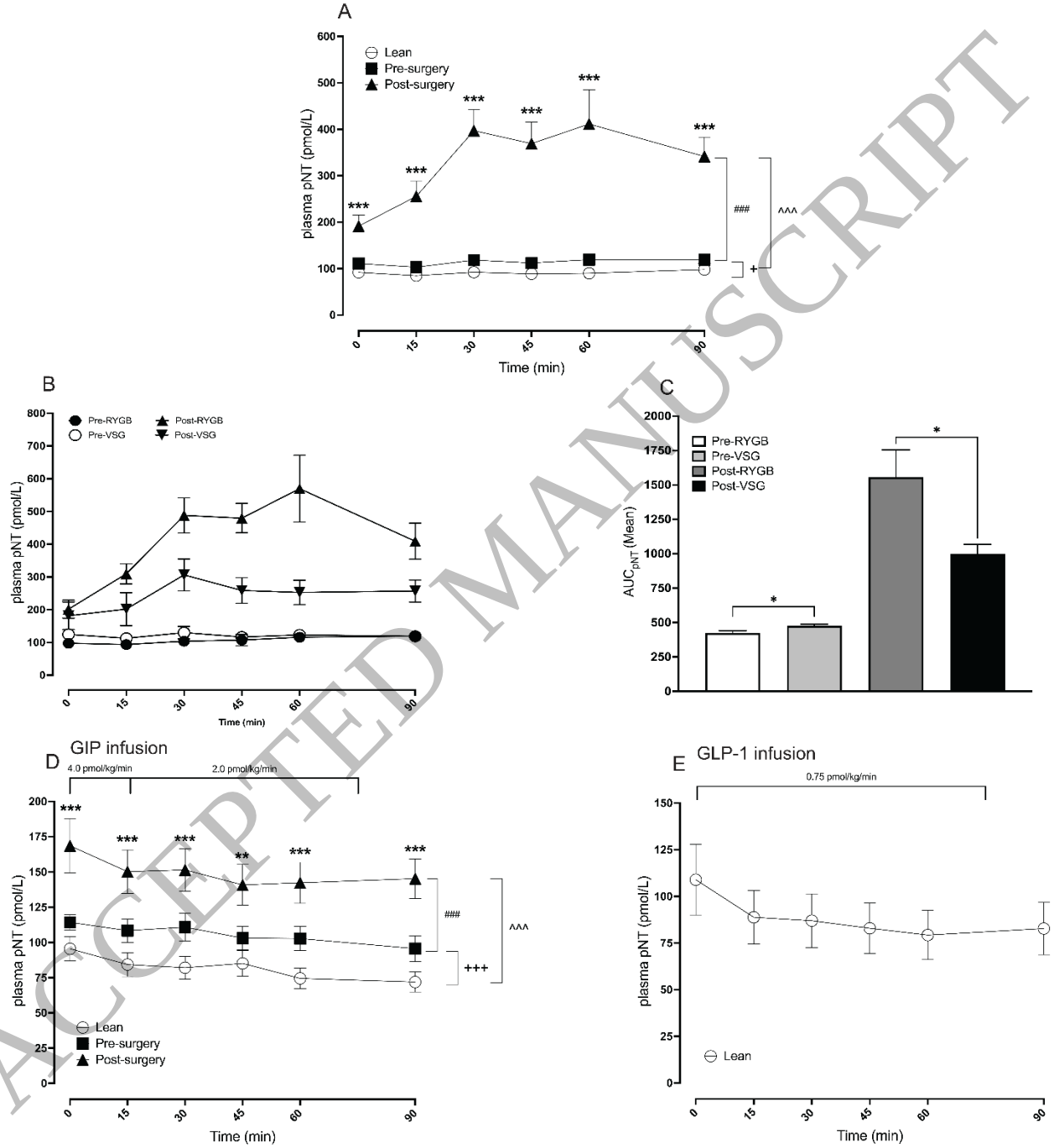

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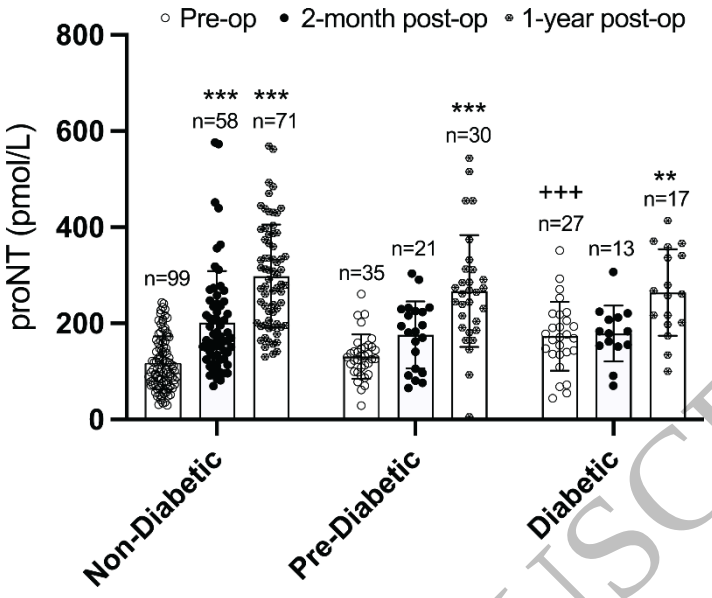

Figure 4
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