



Differential Effects of Laparoscopic Sleeve Gastrectomy and Laparoscopic Gastric Bypass on Appetite, Circulating Acyl-ghrelin, Peptide YY3-36 and Active GLP-1 Levels in Non-diabetic Humans

Ahmed Yousseif · Julian Emmanuel · Efthimia Karra · Queensta Millet · Mohamed Elkalaawy · Andrew D. Jenkinson · Majid Hashemi · Marco Adamo · Nicholas Finer · Alberic G. Fiennes · Dominic J. Withers · Rachel L. Batterham

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Abstract Laparoscopic Roux-en-Y gastric bypass (LRYGBP) reduces appetite and induces significant and sustainable weight loss. Circulating gut hormones changes engendered by LRYGBP are implicated in mediating these beneficial effects. Laparoscopic sleeve gastrectomy (LSG) is advocated as an alternative to LRYGBP, with comparable short-term weight loss and metabolic outcomes. LRYGBP and LSG are anatomically

distinct procedures causing differential entero-endocrine cell nutrient exposure and thus potentially different gut hormone changes. Studies reporting the comparative effects of LRYGBP and LSG on appetite and circulating gut hormones are controversial, with no data to date on the effects of LSG on circulating peptide YY3-36 (PYY3-36) levels, the specific PYY anorectic isoform. In this study, we prospectively investigated appetite and gut hormone changes in response to LRYGBP and LSG in adiposity-matched non-diabetic patients. Anthropometric indices, leptin, fasted and nutrient-stimulated acyl-ghrelin, active glucagon-like peptide-1 (GLP-1), PYY3-36 levels and appetite were determined pre-operatively and at 6 and 12 weeks post-operatively in obese, non-diabetic females, with ten undergoing LRYGBP and eight adiposity-matched females undergoing LSG. LRYGBP and LSG comparably reduced adiposity. LSG decreased fasting and post-prandial plasma acyl-ghrelin compared to pre-surgery and to LRYGBP. Nutrient-stimulated PYY3-36 and active GLP-1 concentrations increased post-operatively in both groups. However, LRYGBP induced greater, more sustained PYY3-36 and active GLP-1 increments compared to LSG. LRYGBP suppressed fasting hunger compared to LSG. A similar increase in post-prandial fullness was observed post-surgery following both procedures. LRYGBP and LSG produced comparable enhanced satiety and weight loss. However, LSG and LRYGBP differentially altered gut hormone profiles.

Ahmed Yousseif and Julian Emmanuel contributed equally.

A. Yousseif · J. Emmanuel · E. Karra · Q. Millet · R. L. Batterham (✉)

Centre for Obesity Research Department of Medicine, University College London, Rayne Institute, 5, University Street, WC1E 6JJ London, UK
e-mail: r.batterham@ucl.ac.uk

A. Yousseif · M. Elkalaawy · A. D. Jenkinson · M. Hashemi · M. Adamo · N. Finer · R. L. Batterham

Centre for Weight Loss, Metabolic and Endocrine Surgery, University College London Hospitals, 250 Euston Road, NW1 2BU London, UK

M. Elkalaawy
Clinical and Experimental Surgery Department, Medical Research Institute, University of Alexandria, Hadara, Alexandria, Egypt

A. G. Fiennes
Surrey Weight Loss Centre, St Anthony's Hospital North, Cheam SM3 9DW, UK

D. J. Withers
Metabolic Signalling Group, Medical Research Council Clinical Sciences Centre, Imperial College, W12 0NN London, UK

R. L. Batterham
Biomedical Research Centre, National Institute of Health Research University College London Hospitals, W1T 7DN London, UK

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Introduction

Obesity is a leading cause of morbidity and mortality, placing growing demands on healthcare systems. Bariatric surgery induces significant long-lasting weight loss, ameliorates obesity-associated co-morbidities and reduces mortality [1]. Laparoscopic Roux-en-Y gastric bypass (LRYGBP) is the ‘gold standard’ procedure, resulting in 65–80 % excess bodyweight loss, decreased appetite, and rapid weight-independent amelioration of type-2 diabetes mellitus (T2DM) [2–5]. LRYGBP is cost-effective, but technically challenging with associated mortality—albeit low ~0.09 % [6]—and micronutrient deficiencies risks, necessitating lifelong follow-up. Laparoscopic sleeve gastrectomy (LSG) (originally undertaken as a first-step in super-obese patients with subsequent conversion to a hybrid *restrictive–malabsorptive* procedure) is technically less complex, with lower complications and nutritional deficiencies rates than LRYGBP [2]. In light of reports of comparable weight loss and metabolic outcomes to LRYGBP, LSG is increasingly undertaken as a stand-alone procedure [7–10]. However, its long-term efficacy for weight loss and metabolic benefit remains unclear [8, 10].

Understanding the mechanisms mediating the weight loss and metabolic effects of bariatric surgery is key for developing less invasive procedures and medical obesity treatments. Post-operative changes in circulating gut hormones, including ghrelin, peptide YY (PYY) and glucagon-like peptide-1 (GLP-1), are thought to play a key role to the beneficial outcomes of bariatric surgery [2].

Ghrelin is produced by *X/A*-like cells predominantly located in the stomach fundus and proximal small intestine. Its circulating levels increase with fasting and decrease post-prandially. Acyl-ghrelin, the bioactive form, exerts orexigenic properties and is produced by ghrelin octanoylation in serine-3 mediated by *ghrelin-O-acyl transferase* (GOAT). Acyl-ghrelin is rapidly converted by endogenous esterases to the main circulating form, des-acyl-ghrelin [11]. Moreover, ghrelin is labile, with highest stability in acidic states [12]. Thus, plasma acyl-ghrelin assessment requires specific sample processing by addition of esterase inhibitor and plasma acidification [13].

GLP-1 and PYY are released post-prandially by distal gut entero-endocrine L-cells [2]. Bioactive forms of GLP-1 augment glucose-dependent insulin release and decrease appetite [2]. Active GLP-1 is rapidly inactivated by the protease dipeptidyl-peptidase-4 (DPP-4). PYY3-36 reduces appetite, bodyweight and adiposity and is produced by truncation of the full-length PYY1-36 by DPP-4 [14]. The effects of PYY1-36 on appetite and feeding are less clear. In rodents, central PYY1-36 administration stimulates feeding [15], whereas equipotent or weaker anorexigenic effects to PYY3-36 have been attributed to peripheral PYY1-36 administration [16, 17]. However, to date, there is no evidence that systemically

administered PYY1-36 alters human appetite [18]. Accurate assessment of plasma active GLP-1 and PYY3-36 requires rapid addition of DPP4-inhibitor to samples [13]. Diet-induced weight loss increases ghrelin and reduces PYY, but has little effect on GLP-1 [4, 19, 20], whilst in post-LRYGBP despite the weight loss, ghrelin decreases and marked, weight-independent, increments in nutrient-stimulated plasma PYY and GLP-1 occur [2–5, 21–24].

LRYGBP and LSG differentially alter gastrointestinal anatomy. LRYGBP reduces stomach volume and bypasses the majority of the stomach, duodenum and proximal jejunum, with direct nutrient delivery to the distal gut [2]. In LSG, the gastric fundus (the major source of ghrelin source) is excised, and this accelerates gastric emptying and intestinal transit post-operatively resulting in rapid nutrient delivery to the duodenum and hindgut [25–27]. Thus, LSG and LRYGBP produce differential nutrient exposure of entero-endocrine cells, and as such would be anticipated to differentially alter circulating gut hormones. Most studies comparing the two procedures report larger decreases in circulating fasting and/or meal-stimulated ghrelin after LSG versus LRYGBP [21, 28–30]; however, findings of their effects on hindgut hormones have been inconsistent, reporting either comparable increases in GLP-1 and/or total-PYY [8, 21, 28, 29, 31]; or superior total-PYY [32] and GLP-1 [30] increases post-LRYGBP versus LSG. Technical procedural variations, differences in hormonal isoforms assessed, time-lapse from surgery, subjects’ HOMA-IR and glycaemic status, and differences in subject standardization and sample processing may account for these discrepancies.

Several studies have measured total-ghrelin, total-GLP-1 and/or total-PYY post-surgery, which depict hormone production, but may not necessarily reflect circulating levels of their respective bioactive forms. In support of this, DPP-4 activity declines after LRYGBP [33], whereas *GOAT* expression is altered by caloric restriction and plasma *GOAT* is BMI-dependent and thus may change post-bariatric surgery [34–36]. Moreover, the effects of LSG on the anorectic PYY-isoform, PYY3-36, are unknown. Therefore, we prospectively compared the effects of LSG and LRYGBP on anthropometric indices, leptin, acyl-ghrelin, active GLP-1, PYY3-36, and appetite in non-diabetic patients using our established subject standardisation and stringent sample-processing protocols.

Materials and Methods

Subjects

Ten obese non-diabetic females undergoing LRYGBP (age = 46.8 ± 1.5 years, body mass index [BMI] = 45 ± 1.5 kg/m²) and eight adiposity-matched non-diabetic females undergoing

LSG (age=41.4±3.4 years, BMI=44±1.6 kg/m²) were recruited. Inclusion criteria were female sex (for purpose of sex matching), BMI=40–50 kg/m², aged 18–60 years, and undergoing their first bariatric procedure. Exclusion criteria were T2DM, smoking, alcohol consumption >20units/week and intra-operative/ early post-operative complications. Subjects gave written consent, and approval was obtained from University College London Hospitals Ethics Committee (project 09/H0715/65).

Surgical Procedures

LRYGBP included an antecolic–antegastric Roux-en-Y construction with 120-cm alimentary/80-cm biliopancreatic limbs and a small vertical gastric pouch. LSG was performed as previously described [37] and calibrated tightly with a 32-Ch bougie with stapling commenced 5 cm from the pylorus.

Study Protocol

Patients attended for a 500-kcal test meal (43 % carbohydrate/18 % protein/39 % fat) within 2 weeks pre-surgery and at 6 and 12 weeks (6w and 12w) post-operatively. Subjects maintained similar food intake, refrained from alcohol 24 h prior to each study day, fasted from 9 p.m. and drank only water. At ~9 a.m. on study days, a peripheral cannula was inserted, and 1 h was allowed for recovery/habituation [13]. At t0 min, a baseline visual analogue scale (VAS) [13] and a blood sample were collected. Subjects consumed the test meal (250 ml Resource2.0+fibre, Nestle Nutrition, Croydon, UK) within 15 min, with blood drawn at t15, t30 and every 30 min thereafter until t180 min post-meal. Coincident with blood sampling, subjects completed appetite VAS [13]. Samples were processed by previously described stringent protocols [13].

Anthropometric Phenotyping

Height was determined by a wall-mounted stadiometer (242 Measuring Rod, Seca, UK), and body composition by multi-frequency bioimpedance (InBody_720, Biospace, Derwent Healthcare, UK). Excess weight (EW) was calculated by subtracting ideal bodyweight (based on BMI=25 kg/m²) from pre-operative bodyweight. Percent excess weight loss (%EWL) was calculated as: $[100 \times (\text{bodyweight pre-surgery} - \text{current bodyweight}) / \text{excess weight}]$.

Hormone Assays

To limit inter-assays' variability, samples were processed simultaneously. Plasma insulin, PYY3-36 and acyl-ghrelin were assayed by radioimmunoassay (assays' characteristics, respectively: sensitivity 2 μU/mL, 20 pg/mL, 7.8 pg/ml; inter-assay

variability, 3.8, 6.6, and 6.8 %; intra-assay variability, 2.6, 4.5, and 5 %). Plasma leptin and active GLP-1 were assayed by ELISA (respectively: sensitivity 0.5 ng/mL, 2 pM; inter-assay variability N/A, 7.5 %; intra-assay variability, 3.2 and 5.2 %) (radioimmunoassays and ELISAs by Millipore, Watford, UK). Plasma glucose was determined by enzymatic colorimetric assay, Infinity™ Glucose Oxidase Reagent (sensitivity 3.3 mmol/L; ThermoFisher, Loughborough, UK). Insulin resistance was calculated using the homeostasis model assessment for insulin resistance, HOMA-IR [38].

Statistical Analysis

Results are expressed as mean ± standard error of the mean. Normal distribution was assessed by D'Agostino-Pearson omnibus normality test. Integrated area-under-the-curve (AUC) t0–t180 for appetite and hormone concentrations versus time was calculated using the trapezoid rule. Non-paired student's *t* test was used for between-groups comparisons, and paired student's *t* test and repeated measures one-way ANOVA with Bonferroni post-hoc for within-groups' analysis. *p* < 0.05 was considered significant.

Results

Preoperative Patient Characteristics

The two groups had comparable age, BMI and adiposity and exhibited similar circulating fasting leptin, acyl-ghrelin, PYY3-36, active GLP-1, glucose, insulin levels and HOMA-IR at baseline (Fig. 1, Tables 1 and 2). In response to the test meal, we observed decreases in hunger and acyl-ghrelin and increases in perceived satiety and in plasma PYY3-36, active GLP-1, glucose, and insulin, with no between-groups differences (Fig. 1, Tables 1 and 2).

Post-surgery Changes in Adiposity and Plasma Leptin

Both procedures induced comparable, marked reductions in BMI, fat mass, visceral fat area (VFA) and fasting leptin, with similar BMI and %EWL observed at 6w and 12w post-surgery (Tables 1 and 2).

Post-operative Fasting and Nutrient-Stimulated Plasma Acyl-ghrelin Levels

Post-LRYGBP fasting acyl-ghrelin non-significantly decreased at 6w (*p*=0.06), but rose towards pre-surgery values by 12w (*p*=0.74). Post-meal, plasma acyl-ghrelin levels were reduced compared to pre-surgery at t30 at 6w and 12w post-operatively, whereas acyl-ghrelinAUC₀₋₁₈₀ remained unaltered (Fig. 2, Table 2). At both post-LSG visits, lower fasting acyl-

ghrelin levels and nutrient-stimulated levels at t30 and t60 were observed, with a trend towards decreased acyl-ghrelinAUC₀₋₁₈₀ ($p < 0.1$) compared to pre-surgery (Table 2). Fasting acyl-ghrelin and acyl-ghrelinAUC₀₋₁₈₀ were significantly lower post-LSG versus post-LRYGBP (Fig. 2, Table 2).

Post-operative Fasting and Nutrient-Stimulated Plasma PYY3-36 and Active GLP-1 Levels

Fasting PYY3-36 and active GLP-1 did not significantly change following either procedure (Fig. 3, Table 2). However, marked augmentation of nutrient-stimulated PYY3-36 and active GLP-1 occurred post-operatively (Fig. 3) (LRYGBP: PYY3-36 6w and 12w: t15–t180, active GLP-1 6w: t15–t180, 12w: t15–t150; LSG: PYY3-36 6w: t15–t180, 12w: t15–t150, active GLP-1 6w: t15–t90, 12w: t30–t120), with associated increases in PYY3-36AUC₀₋₁₈₀ and active GLP-1AUC₀₋₁₈₀ (Fig. 3, Table 2) (LRYGBP: PYY3-36AUC₀₋₁₈₀ 6w=↑2.5±0.2-fold, 12w=↑2.3±0.3-fold, active GLP-1AUC₀₋₁₈₀: 6w&12w=↑5.1±0.8-fold; LSG: PYY3-36AUC₀₋₁₈₀ 6w and 12w=↑1.7±0.2-fold, active GLP-1AUC₀₋₁₈₀: 6w=↑3.1±0.4-fold, 12w=↑2.4±0.3-fold). The LRYGBP group exhibited significantly higher PYY3-36AUC₀₋₁₈₀ and active GLP-1AUC₀₋₁₈₀, with higher meal-stimulated levels versus LSG (PYY3-36, 6w: t90–t150, 12w: t120–150; active GLP-1 6w: t15–t30, 12w: t30–t180) (Fig. 3, Table 2).

Post-operative Subjective Hunger and Satiety Scores

Fasting hunger significantly decreased post-LRYGBP, but remained unchanged post-LSG. Both procedures comparably reduced hungerAUC₀₋₁₈₀ in response to the standard test meal (Fig. 4, Table 2). At 12w, fasting hunger was lower post-LRYGBP *versus* LSG, with no other between-groups differences in hunger (Fig. 4, Table 2).

Fasting fullness non-significantly declined at 6w ($p = 0.06$), but returned to baseline by 12w post-LRYGBP, whereas remained unchanged post-LSG. Both procedures comparably enhanced postprandial fullness (Fig. 4, Table 2).

Discussion

LRYGBP and LSG reduced BMI, excess weight, adiposity and plasma leptin at 6w and 12w post-operatively to a similar extent. Hence, the observed differences in appetite and gut hormones were not attributable to differences in weight loss and more likely reflect differences in the surgical procedures per se.

Our study reports the first comparison of plasma acyl-ghrelin in non-diabetic patients' post-LRYGBP and LSG. These procedures produce differential nutrient contact with ghrelin-producing *X/A*-like cells. Post-LRYGBP, *X/A*-like

cells in the gastric fundus and duodenum remain in situ but are excluded from nutrient contact. Diet-induced weight loss increases ghrelin [19]. Yet, despite marked weight loss, post-LRYGBP fasting acyl-ghrelin non-significantly decreased at 6w but rose towards baseline values by 12w, whereas at t30 post-meal declined from pre-surgery at 6w and 12w. Our results suggest that stomach fundus and duodenal ghrelin-producing cells contribute to circulating ghrelin post-LRYGBP despite their exclusion from nutrient contact and that mechanisms independent from *X/A*-like cell nutrient-sensing, for example the vagus nerve [39], may signal meal-induced ghrelin suppression. Chronaiou et al. provided direct evidence that fundus ghrelin-producing cells remain active post-LRYGBP by demonstrating superior decreases in ghrelin post-LRYGBP+fundus resection versus LRYGBP with fundus preservation [23]. Interestingly Barazzoni et al. reported no change in total ghrelin, but significantly increased acyl-ghrelin at 1, 3, 6 and 12 months post-LRYGBP [24]. Substantial differences in sample handling by addition of esterase inhibitor and plasma acidification in our study may underlie these discrepant findings.

LSG removes gastric fundus ghrelin-producing cells and accelerates nutrient delivery to duodenal ghrelin-producing cells by increasing gastric emptying [25–27]. After LSG, 40–50 % decreases in fasting total-ghrelin have been reported, sustained for up to 5 years post-surgery, with reductions in post-meal circulating ghrelin levels [21, 28, 40]. We also observed reductions in fasting acyl-ghrelin and acyl-ghrelinAUC₀₋₁₈₀ post-LSG. Moreover, as anticipated in view of the stomach fundus excision, LSG induced superior acyl-ghrelin reductions than LRYGBP. Interestingly, despite removing the majority of the gastric fundus, fasting acyl-ghrelin and acyl-ghrelinAUC₀₋₁₈₀ declined by only ~20–30 % post-LSG. A possible explanation is that although the majority of ghrelin-producing cells are in the stomach fundus, circulating acyl-ghrelin may primarily originate from the duodenum. Alternatively, plasma acyl-ghrelin is highly regulated, and compensatory up-regulation of duodenal ghrelin-production may occur.

PYY and GLP-1 are released from distal gut L-cells. Post-meal their levels rapidly rise, implicating a yet unknown neural and/or humoral mechanism to this initial release. Subsequent PYY and GLP-1 release results from L-cell nutrient-contact. LRYGBP and LSG increase gastric emptying [25–27, 41] but have different effects on gut nutrient-passage. LRYGBP excludes nutrients from foregut contact and expedites nutrient delivery to distal gut L-cells, which is suggested to augment hindgut hormone release; *the* 'hindgut theory'. LSG accelerates gastric emptying, reduces acid production and rapidly transits nutrients into the duodenum and proximal intestine, enhancing foregut stimulation [2]. Few studies have examined the effects of LRYGBP on circulating PYY3-36, the anorectic PYY-isoform. This is the

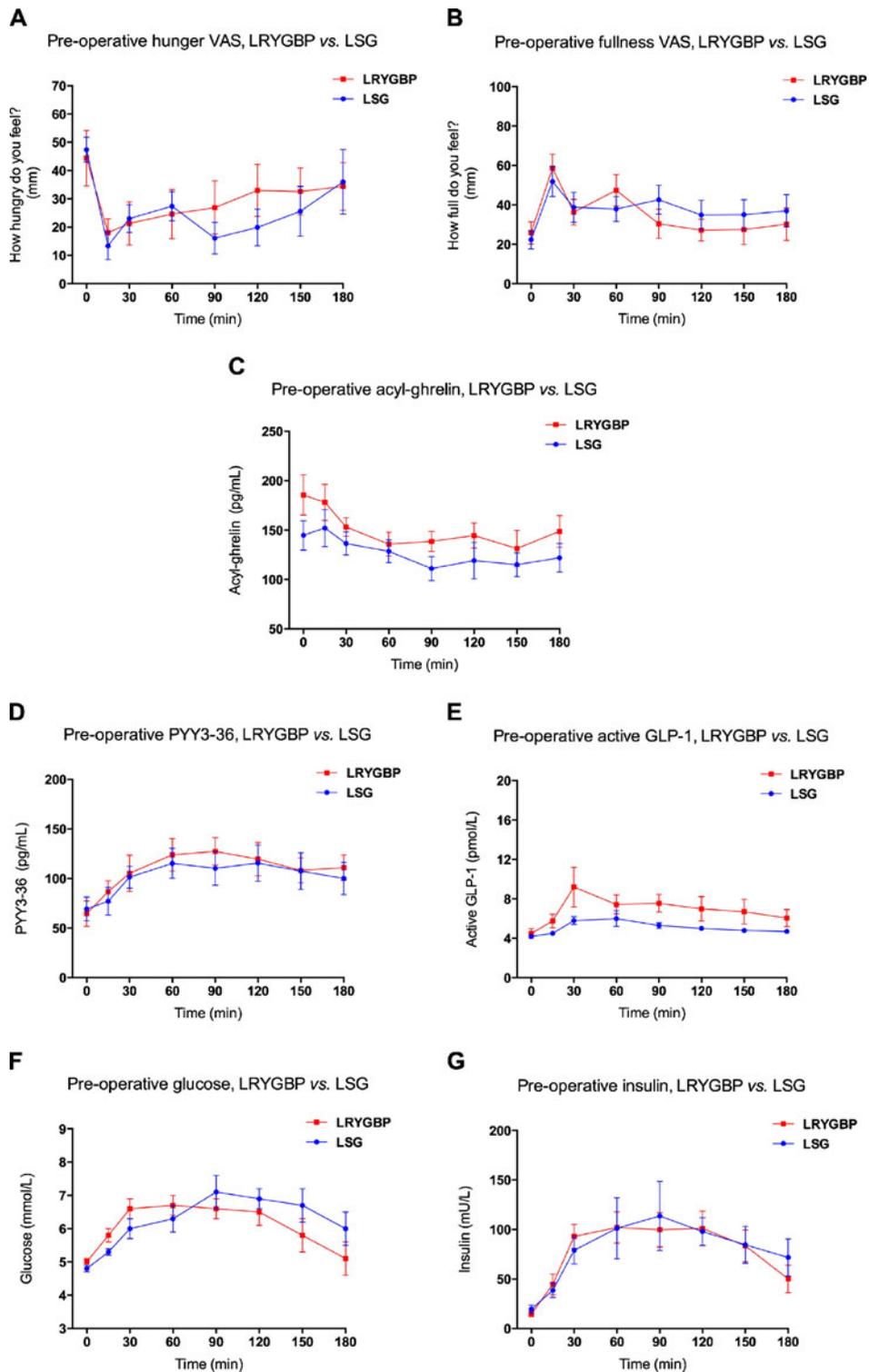


Fig. 1 Fasting and nutrient-stimulated appetite, gut hormones, glucose and insulin for the LRYGBP and LSG groups pre-operatively. **(A)** Temporal hunger visual analogue scales (VAS) ratings for the LRYGBP (red, filled squares) and LSG (blue, filled circles). **(B)** Temporal fullness VAS for the LRYGBP (red, filled squares) and LSG (blue, filled circles) groups. **(C)** Plasma acyl-ghrelin temporal profile for the LRYGBP (red, filled squares) and LSG (blue, filled circles) groups. **(D)** Plasma PYY3-

36 temporal profile for the LRYGBP (red, filled squares) and LSG (blue, filled circles) groups. **(E)** Plasma active GLP-1 temporal profile for the LRYGBP (red, filled squares) and LSG (blue, filled circles) groups. **(F)** Plasma glucose temporal profile for the LRYGBP (red, filled squares) and LSG (blue, filled circles) groups. **(G)** Plasma insulin temporal profile for the LRYGBP (red, filled squares) and LSG (blue, filled circles) groups. Results are expressed as mean±SEM

Table 1 Anthropometric characteristics and appetite VAS for the LRYGBP and LSG groups pre-operatively and at 6 and 12 weeks post-surgery (mean ± SEM)

	LRYGBP				LSG				P (between groups)								
	Pre-operatively		6 Weeks		12 Weeks		Pre-operatively		6 Weeks		12 Weeks		p ^c	p ^d	p ^e	p ^f	p ^g
	Mean±SEM	Mean±SEM	p ^a	Mean±SEM	p ^b	Mean±SEM	p ^b	Mean±SEM	p ^c	Mean±SEM	p ^d	Mean±SEM					
Age	46.8±1.5 (40–53)				41.4±3.4 (28–57)				0.13								
Anthropometrics																	
BMI (kg/m ²)	45±1.5	40.2±1.7	<0.0001	37.9±1.6	<0.0001	44±1.6	<0.0001	39.6±1.7	<0.0001	37.4±1.7	<0.0001	0.66	0.81	0.84			
Weight loss (kg)	N/A	12.6±0.8	N/A	18.7±0.8	N/A	N/A	13.9±2.2	N/A	19.9±3.0	N/A	N/A	N/A	0.56	0.67			
%EWL (%)	N/A	27.1±3.6	N/A	39.4±3.8	N/A	N/A	28.9±5.1	N/A	37.8±5.3	N/A	N/A	N/A	0.77	0.81			
WHR	1.13±0.02	1.08±0.02	<0.0001	1.05±0.02	<0.0001	1.10±0.02	<0.0001	1.05±0.02	<0.01	1.02±0.0	<0.0001	0.30	0.37	0.22			
Total fat mass (kg)	62±3.3	53.7±3.1	<0.0001	49.3±3.2	<0.0001	62.1±3.0	<0.0001	54±3.3	<0.0001	49±3.2	<0.0001	0.99	0.95	0.95			
VFA (cm ²)	245±12.1	207±10.9	<0.0001	188±11	<0.0001	231±11.8	<0.0001	195±13.6	<0.0001	180±13	<0.0001	0.45	0.49	0.63			
Appetite VAS																	
Fasting hunger (mm)	44.4±9.8	13.4±3.5	0.008	19.1±8.3	0.009	47.4±4.4	0.40	34±11.7	0.40	53.1±12.3	0.70	0.80	0.08	0.03			
Hunger AUC0-180 (mm × min)	5,109±1,335	1,385±328	<0.05	2,588±1033	0.07	4,282±914	<0.05	1,818±287	<0.05	2,645±388	0.06	0.63	0.35	0.96			
Fasting fullness (mm)	26±5.6	47±9.6	0.06	30±9.6	0.7	22±5.1	0.47	34±11.6	0.47	25±9.0	0.81	0.66	0.40	0.72			
Fullness AUC0-180 (mm × min)	6,315±729	12,592±1050	<0.01	1,102±1,677	<0.05	6,883±1,214	<0.01	13,917±546	<0.01	13,116±546	<0.001	0.68	0.32	0.31			

LRYGBP proximal gastric bypass, LSG sleeve gastrectomy, BMI body mass index, EWL excess weight loss, WHR waist to hip ratio, VFA visceral fat area, AUC area-under-the-curve

^a Signifies LRYGBP 6w post-surgery versus pre-operatively

^b LRYGBP 12w post-surgery versus pre-operatively

^c Signifies LSG 6w post-surgery versus pre-operatively

^d LSG 12w post-surgery versus pre-operatively

^e Signifies LRYGBP versus LSG at pre-surgery

^f LRYGBP versus LSG at 6w post-surgery

^g LRYGBP versus LSG at 12w post-surgery

Table 2 Gut hormones and glycaemia indices for the LRYGBP and LSG groups (mean ± SEM)

	LRYGBP			LSG			p (between groups)					
	Pre-operatively		12 weeks	Pre-operatively		6 weeks	12 weeks		p ^e	p ^f	p ^g	
	Mean±SEM	p ^a	Mean ± SEM	p ^b	Mean ± SEM	p ^c	Mean ± SEM	p ^d				
Fasting leptin (ng/mL)	63.3±5.6	<0.01	33.4±3.6	<0.001	62.4±6.9	38.9±5.0	<0.01	33.4 ±4.9	<0.01	0.92	0.93	0.93
Acyl-ghrelin												
Fasting acyl-ghrelin (pg/mL)	186±20.2	0.06	179±16.6	0.74	145±14.9	110±6.3	<0.05	100±6.5	<0.01	0.14	0.02	0.001
Acyl-ghrelin AUC0-180 (pg × min/mL)	25,853±2,242	0.38	25,144±1,457	0.72	22,485±2,231	18,725±1,275	0.08	18,234±1,087	0.09	0.31	0.008	0.002
PYY3-36												
Fasting PYY3-36 (pg/mL)	64.6±12.8	0.11	90.2±9.2	0.11	69.4±12	74.2±11.3	0.57	87.8±10.7	0.09	0.79	0.34	0.87
PYY3-36 AUC0-180 (pg × min/mL)	19,794±2,273	<0.0001	42,177±3,709	<0.001	18,917±2,656	30,771±3,528	<0.01	30,186±4,233	<0.05	0.80	0.007	0.05
Active GLP-1												
Fasting active GLP-1 (pmol/L)	4.5±0.5	0.18	5.8±1.2	0.21	4.2±0.1	4.2±0.1	0.97	4.2±0.1	0.88	0.62	0.20	0.25
Active GLP-1 AUC0-180 (pmol × min/L)	1,279±188	<0.0001	5,832±583	<0.0001	933±40	2,804±443	<0.01	2,255±328	<0.05	0.13	0.005	0.0001
Glucose												
Fasting glucose (mmol/L)	5±0.1				4.8±0.1					0.26		
Glucose AUC0-180 (mmol × min/L)	1,116±44.6				1,152±45.9					0.58		
Insulin												
Fasting Insulin (mU/L)	14.8±2.7				19.6±4.3					0.34		
Insulin AUC0-180 (mU × min/L)	15,221±2,206				15,508±3,324					0.94		
HOMA-IR	3.3±0.6				4.3±1					0.41		

LRYGBP proximal gastric bypass, LSG sleeve gastrectomy, PYY peptide YY, GLP-1 glucagon-like peptide-1, AUC area-under-the-curve

^a Signifies LRYGBP 6w post-surgery versus pre-operatively

^b LRYGBP 12w post-surgery versus pre-operatively

^c Signifies LSG 6w post-surgery versus pre-operatively

^d LSG 12w post-surgery versus pre-operatively

^e Signifies LRYGBP versus LSG at pre-surgery

^f LRYGBP versus LSG at 6w post-surgery

^g LRYGBP versus LSG at 12w post-surgery

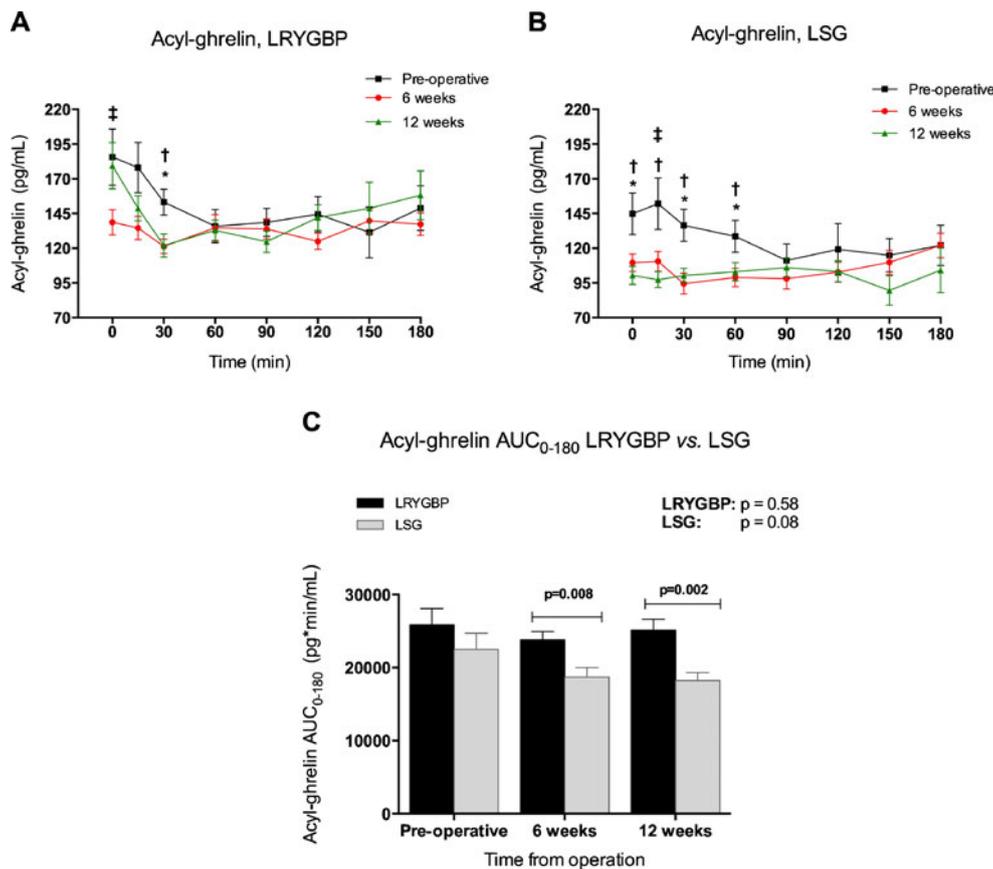


Fig. 2 The effects of LRYGBP and LSG on plasma fasting, meal-stimulated acyl-ghrelin and acyl-ghrelinAUC₀₋₁₈₀. Plasma acyl-ghrelin temporal profile in response to the test-meal for LRYGBP (**a**) and LSG (**b**) groups at pre-surgery (*black, solid squares*), and at 6w and 12w post-operatively (*red, solid circles and green, solid triangles*, respectively). **c** Acyl-ghrelinAUC₀₋₁₈₀ for LRYGBP (*black, solid columns*) and LSG groups (*grey, solid columns*) at pre-surgery and at 6w and 12w post-

operatively. Results are expressed as mean±SEM. * $p < 0.05$, within-group at 6w post-operatively compared to pre-surgery; † $p < 0.05$, within-group comparisons at 12w post-operatively versus pre-surgery; ‡ $p < 0.05$, within-group 6w versus 12w comparison. The p values at the *right upper corner* of **c** indicate one-way ANOVA within-group analysis. Within-group Bonferroni post hoc and between-group t test significance is indicated over the corresponding bars

first report of plasma PYY3-36 post-LSG. LRYGBP and LSG markedly enhanced nutrient-stimulated PYY3-36, with, however, greater, more sustained post-prandial PYY3-36 release post-LRYGBP. These findings are in keeping with the procedural anatomical differences and suggest that factors originating from foregut and hindgut regulate PYY3-36 levels. Our findings of superior PYY3-36 enhancement post-LRYGBP versus LSG are in accord with those of Valderas et al. for total-PYY [32]. However, they are at odds with reports of comparable post-prandial total-PYY following LRYGBP and LSG [21, 29, 31]. Differences in PYY-isoforms assessed, sampling time-points, subject standardization, sample handling and in subjects' HOMA-IR pre-surgery may account for these discrepancies.

Similarly to PYY3-36, both procedures augmented nutrient-stimulated active GLP-1 levels, with again greater, more sustained release observed post-LRYGBP. These

findings are at odds with reports by Chambers et al. of similar increases in nutrient-stimulated active GLP-1 post-sleeve gastrectomy and gastric bypass in rats [8]. These discrepancies may be accounted for by structural inter-species rodent-human stomach differences, which potentially affect gastric emptying post-surgery and hence gut hormone responses. Moreover, Chambers and colleagues measured active GLP-1 5 months post-surgery in weight-stable rats, whilst our studies were undertaken during the acute weight-loss phase. Peterli et al. have reported greater meal-stimulated active GLP-1 responses at 1 week and non-significant increases 12 weeks post-LRYGBP versus LSG [21]. In another study, they showed non-significantly greater active GLP-1 peak and active GLP-1AUC following LRYGBP [29]. Again, methodological and subject-related differences may underlie these discrepancies.

Despite greater reductions in the 'hunger hormone' acyl-ghrelin post-LSG versus post-LRYGBP, paradoxically the

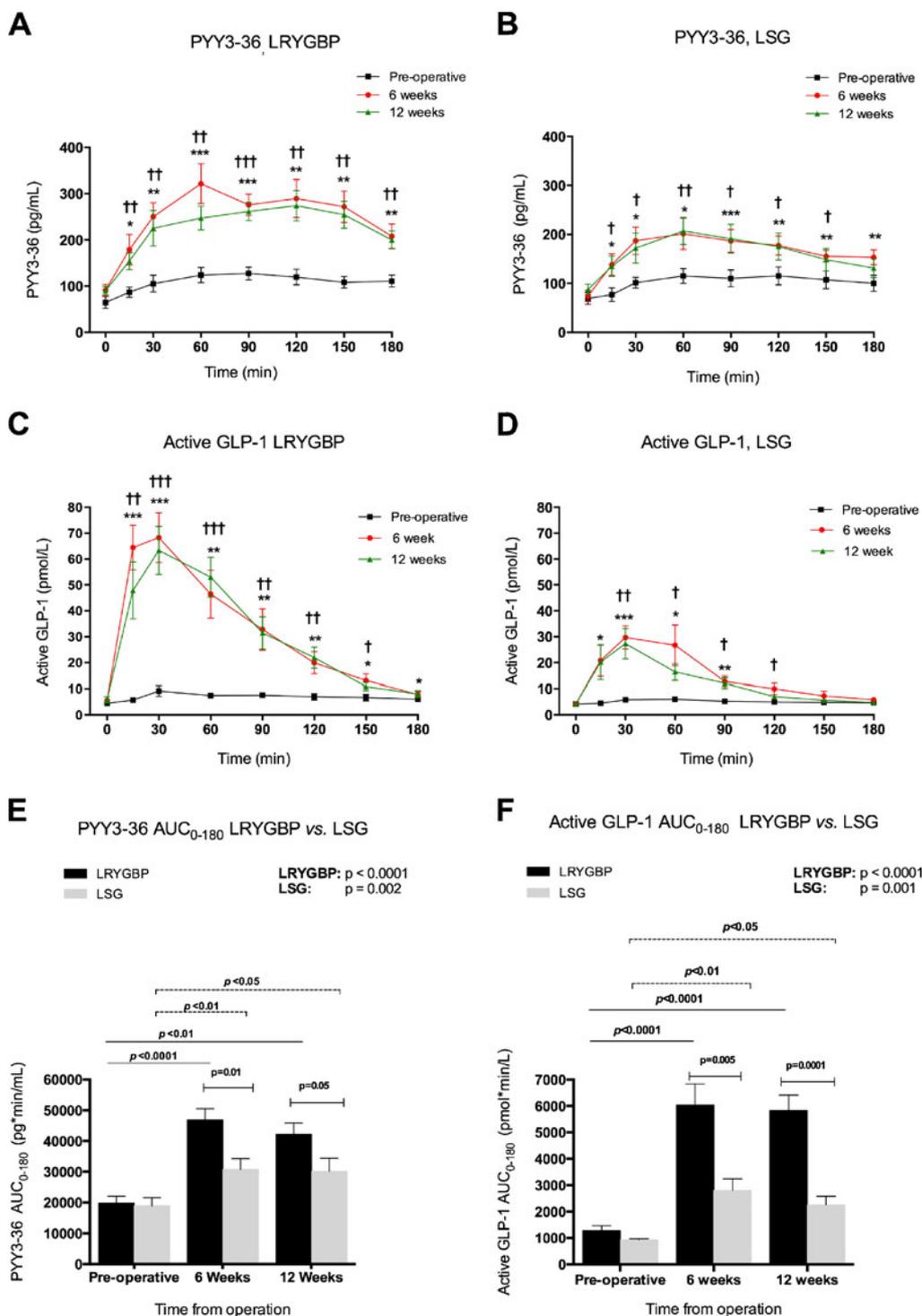


Fig. 3 The effects of LRYGBP and LSG on fasting, meal-stimulated plasma concentrations and area-under-the curve (AUC₀₋₁₈₀) for PYY₃₋₃₆ and active GLP-1. Plasma PYY₃₋₃₆ temporal profile in response to the test-meal for LRYGBP (a) and LSG (b) groups at pre-surgery (black, solid squares) and at 6w and 12w post-operatively (red, solid circles and green, solid triangles, respectively). Plasma active GLP-1 temporal profile in response to the test-meal for LRYGBP (c) and LSG (d) groups at pre-surgery (black, solid squares) and at 6w and 12w post-operatively (red, solid circles and green, solid triangles, respectively). PYY₃₋

₃₆AUC₀₋₁₈₀ (e) and active GLP-1AUC₀₋₁₈₀ (f) for LRYGBP (black, solid columns) and LSG groups (grey, solid columns) at pre-surgery and at 6w and 12w post-operatively. Results are expressed as mean±SEM. **p* < 0.05, ***p* < 0.01 and ****p* < 0.001 within-group at 6w post-operatively compared to pre-surgery. †*p* < 0.05, ††*p* < 0.01 and †††*p* < 0.001 for within-group comparisons at 12w post-operatively versus pre-surgery. The *p* values at the right upper corner of e and f indicate one-way ANOVA within-group analysis. Within-group Bonferroni post hoc and between-group *t* test significance is indicated over the corresponding bars

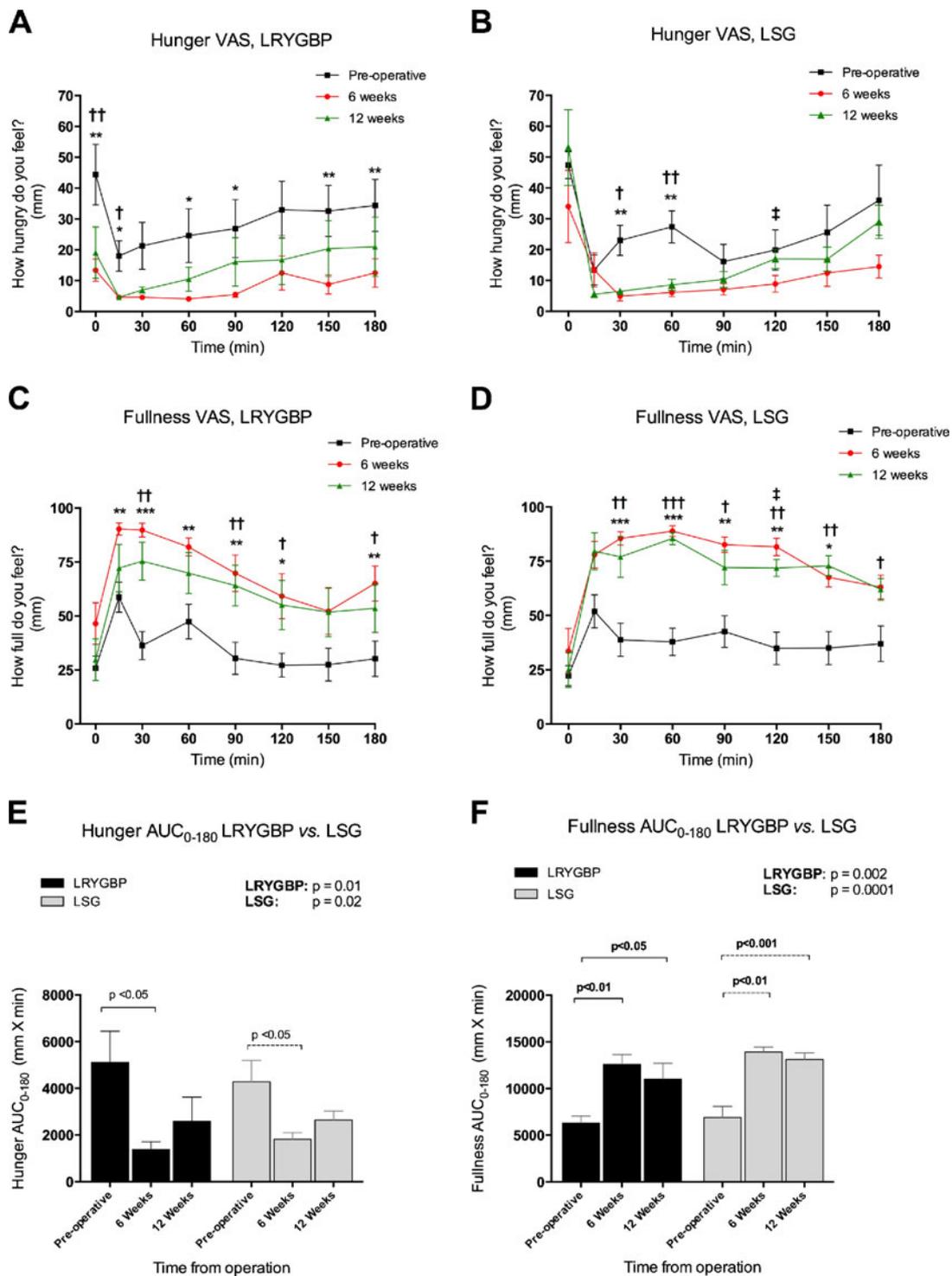


Fig. 4 The effects of LRYGBP and LSG on fasting, meal-stimulated and area-under the curve (AUC₀₋₁₈₀) for subjective hunger and fullness VAS ratings. Hunger VAS temporal profile in response to the test-meal for LRYGBP (a) and LSG (b) groups at pre-surgery (black, solid squares), and at 6w and 12w post-operatively (red, solid circles and green, solid triangles, respectively). Fullness VAS temporal profile in response to the test-meal for LRYGBP (c) and LSG (d) groups at pre-surgery (black, solid squares) and at 6w and 12w post-operatively (red, solid circles and green, solid triangles, respectively). HungerAUC₀₋₁₈₀ (e) and

fullnessAUC₀₋₁₈₀ (f) for LRYGBP (black, solid columns) and LSG groups (grey, solid columns) at pre-surgery and at 6w and 12w post-operatively. Results are expressed as mean±sem. **p*<0.05, ***p*<0.01 and ****p*<0.001 within-group at 6w post-operatively compared to pre-surgery. †*p*<0.05, ††*p*<0.01 and †††*p*<0.001 for within-group comparisons at 12w post-operatively versus pre-surgery. The *p* values at the right upper corner e and f indicate one-way ANOVA within-group analysis. Within-group Bonferroni post hoc and between-group *t* test significance is indicated over the corresponding bars

LRYGBP group exhibited lower fasting hunger; post-prandial hunger was reduced comparably by both procedures. We also observed similar post-operative increments in nutrient-stimulated fullness perception in both groups. These findings again are slightly at odds with the accepted notion that PYY3-36 and active GLP-1 mediate satiety, as LRYGBP induced superior increases of these anorectic peptides and thus would be expected to result in greater satiety perception. These findings highlight that additional factors to active GLP-1 and PYY3-36 regulate satiety.

The novel finding of our study is the characterisation for the first time of the effects of LSG on circulating levels of the anorectic PYY-isoform, PYY3-36. Moreover, our study is the first to simultaneously measure bioactive forms of ghrelin, PYY and GLP-1 in the same patient cohort, while concurrently undertaking parallel appetite assessment. The main strength of our study is the use of validated subject-standardisation protocols, stringent sample processing [13], and tight group-matching pre-operatively for the gut hormone confounders age [42], sex [43] and adiposity [44]. Furthermore, we studied patients without T2DM, dissecting out confounding effects of T2DM on the incretin effect [45], circulating PYY3-36 [5] and ghrelin [46]. The limitations of our study are our small sample sizes, non-randomization and limited follow-up. We studied females only as these represent the majority of patients undergoing bariatric surgical procedures in the UK, and future studies are needed to assess whether gender differences exist in post-operative gut hormone changes. Moreover, studies in patients with T2DM are required to examine whether their inferior weight-loss outcome post-bariatric surgery [47] results from altered/aberrant gut hormone responses. Future larger, randomised studies with longitudinal assessment of gut hormones, intestinal transit, glycaemic and anthropometric indices are required to further elucidate the mechanisms underlying the beneficial effects of LRYGBP and LSG in an attempt to develop novel, less invasive surgical and non-surgical T2DM and obesity treatments.

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Conflict of Interest All authors declare no conflict of interest.

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