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Relationships among tonic and episodic aspects of motivation to eat, gut peptides and body weight before and after bariatric surgery

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Running title: *Motivation to eat in bariatric patients*

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Abstract

Background The interaction between motivation to eat, eating behaviour traits and gut peptides following Roux-en-Y gastric bypass (RYGB) surgery are not fully understood.

Setting Danderyd Hospital, Karolinska Institutet, Stockholm, Sweden.

5 **Method** Appetite and hormone responses to a fixed liquid pre-load were assessed in 12 obese (BMI 45 ± 1.9 kg/m²) participants immediately before, 3 days, 2 months, and 1 year following RYGB surgery. Subjective appetite and plasma levels of ghrelin, leptin, insulin and GLP-1 were measured for a 3-hour postprandial period. Eating behaviour traits were also measured using the Three Factor eating Questionnaire 18 (TFEQR18).

10 **Results** There was a decrease in TFEQ Emotional Eating (EE) and Uncontrolled Eating (UE) from pre to 1-year post-surgery, but no significant change in Restraint. In addition, there was a reduction in subjective appetite ratings, and alterations in appetite peptides favouring an anorectic response. Pre-surgery EE was significantly related to fasting and AUC ghrelin; UE was associated with AUC desire to eat while there was a significant association between
15 fasting desire to eat and ghrelin (fasting and AUC). 1 year post-surgery, UE was positively related to fasting insulin and Restraint was negatively associated with GLP-1. UE and subjective hunger were positively correlated, while the relationship between desire to eat and ghrelin remained.

Conclusion The relationships amongst subjective appetite ratings, eating behaviour traits and
20 appetite peptides in obese patients both before and at one-year post RYGB surgery contribute to the reduction in a propensity to over-eat and weight loss.

Keywords: eating behaviour, TFEQR18, appetite peptides, weight loss, gastric by-pass surgery

25 **Introduction**

Currently bariatric surgery is becoming routinely integrated into the management of morbidly obese patients ¹. The intended outcome of bariatric surgery is a reduction in excess weight and improvement of co-morbidities. The average long term excess weight reduction following surgery is 50% ². The achievement of such a success requires dramatic
30 modification of behaviour and associated eating patterns ³. Roux-en-Y gastric bypass (RYGB) alters levels of circulating appetite peptides ^{4, 5}, but the association of such physiological changes with the subsequent expression of motivations to eat and eating behaviour traits is less well known.

35 It is expected that RYGB surgery will force a change in eating behaviour, leading to weight loss. This change is expected to occur within short-term, episodic eating behaviour patterns (within a meal and over a day) as well as affecting eating patterns over a longer time frame (eating behaviour traits). Indeed, alternations in levels of circulating peptides known to be involved in episodic (glucagon-like peptide-1 (GLP-1), ghrelin) and tonic (leptin) control of
40 appetite are seen. For example, Falkén et al., ⁵ found that circulating levels of GLP-1 levels increased as early as three days following surgery, and were associated with a decreased subjective hunger rating. Furthermore, a decrease in levels of ghrelin post-surgery has been shown to be associated with lower hunger ratings ⁶. On the other hand, leptin levels decrease following surgery ⁷ which in turn could affect the tonic processes involved in appetite
45 control. These changes in appetite peptides (except for leptin) favour an anorectic response, which is reflected in the patients' subjective appetite sensations. These effects occur immediately following surgery and persist for at least 12 months.

In addition, a change in eating behaviour trait profiles has also been found to occur in response to gastric surgery as measured by the Three Factor Eating Questionnaire 18 (TFEQ18)⁸. The TFEQ measures Cognitive Restraint (CR: limiting food intake in order to control weight), Uncontrolled Eating (UE: eating in response to external cues) and Emotional Eating (EE: eating in response to negative affect). A reduction in UE and EE, but no change in CR has been reported in response to RYGB surgery⁹.

55

Gut peptide data and effects on glucose homeostasis from this study have been published previously⁵. The aim of this study was to assess the relationship between TFEQ18, weight loss, motivation to eat and appetite peptides in patients undergoing RYGB and if these changes are associated with alternations in levels of tonic (leptin) and episodic appetite peptides (ghrelin, insulin and GLP-1).

60

Method

Participants

Fourteen participants were recruited to take part in this study. Two participants were removed from the analysis due to incomplete data. Therefore twelve, non-diabetic obese participants (BMI 45.3 ± 1.9 kg/m², mean age 36 ± 2 years, 3 men) completed the study. All participants gave oral and written consent. Participants received standard dietary advice before surgery and were seen by the dietician 6 months after surgery. The study was approved by the regional Ethics Committee of Stockholm.

70

Design

The patients were studied prospectively before and at 3 days, 2 months and one year
75 following a standard laparoscopic, RYGB with a 1-metre Roux-limb. On test days,
participants consumed a fixed liquid pre-load (200ml, ProvideXtra, Fresenius Kabi AB,
Uppsala, Sweden, 300 kcal, fat (0%), protein (11%) and carbohydrates (89%)) following an
overnight fast. Peptides and appetite were measured immediately before the pre-load, and
periodically (10, 20, 30, 60, 90, 120 and 180 min) after. The TFEQR18 was also completed
80 on the morning of each study day.

Peptides

Blood samples were collected in pre-chilled EDTA tubes. Samples were centrifuged at 4° C
for 10 minutes at 3000 rpm. Plasma samples were collected and stored at -20°C until analysis
85 in a single run. Detailed description of assays can be found in Falkén et al (5).

Insulin was measured with a human insulin specific radioimmunoassay (RIA) kit (HI-14HK,
Millipore, Billerica, MA, USA). The sensitivity and detection limit of the RIA was 2 µU/L
and coefficient of variation below 4% (100 µU/L).

90

Leptin was measured with the human leptin specific RIA kit (EZHL-80SK, Millipore,
Billerica, MA, USA). The detection limit and sensitivity of the assay is 0.5 ng/mL with a
variation coefficient of less than 4.6% (22.1 ng/mL).

95 Ghrelin (total) was measured with ELISA (EZGRT-89K, Millipore, Billerica, MA, USA), which was standardized in our laboratory. As calibrator for the assay a lyophilized ghrelin standard was used. The detection limit of the assay was 27 pmol/L and variation coefficient 10% (300 pmol/L) and 4% (900 pmol/L).

100 GLP-1 was measured using RIA against a standard of synthetic GLP-1(7-36) amide using antiserum code no. 89390, specific for the amidated C-terminus of GLP-1. This assay displays equal avidity to intact GLP-1 and GLP-1(9-36) amide, its primary metabolite. The sensitivity and detection limit were below 1 pmol/L and intra-assay coefficient of variation below 6% at 20 pmol/L ¹⁰.

105

Appetite ratings

Subjective appetite sensations were measured using 100 mm visual analogue scales (VAS) anchored with “not at all” and “extremely”. Appetite sensations were assessed by questions relating to hunger, fullness and desire to eat.

110

TFEQR18

The TFEQR18 ⁸ is a modified version of the 51-item TFEQ ¹¹. The 18-item scale measures three factors; Restraint (cognitive restriction of food intake to control body weight), Uncontrolled Eating (UE: excessive appetite and external eating) and Emotional Eating (EE: eating in response to negative affect). Seventeen items are answered on a four point Likert
115 scale anchored with “definitely true” to “definitely false”, while one item uses an eight point

Likert scale. Scores are summed and transformed to a scale of 1-100. The TFEQR18 has acceptable criterion and discriminant validity and internal consistency^{8, 12}.

120 **Statistical analysis**

Data were analysed via repeated measures ANOVA to discern any change in motivation to eat, TFEQR18 factors and peptide levels (fasting, intra-meal change and AUC). Intra-meal (meal induced change) values were calculated by subtracting pre-meal measures from 10 minutes post-meal values and AUC calculated as the mean of all time points. Pearson's
125 partial correlation, controlling for BMI, was used to elicit any relationship between peptides, motivation to eat and TFEQR18. As there was very little change in measures from pre-surgery to 3 days post-surgery, the correlation analysis was conducted on data from pre-surgery, 2 months and 1 year post-surgery measures. The statistical analysis was conducted using PASW 17.

130

Results

Changes from pre to one-year post surgery

There was a significant decrease in body weight ($F_{(3, 33)} = 255.33, p < 0.001$) and BMI ($F_{(3, 33)} = 278.78, p < 0.001$; see Table 1). There was also a significant decrease in EE ($F_{(3, 24)} = 3.71, p = 0.025$) and UE ($F_{(3, 24)} = 12.73, p < 0.001$). There was no significant change in CR.
135

Table 1 about here

There was no change in AUC ghrelin or insulin one year after surgery. However there was a
140 reduction in AUC leptin ($F_{(3,33)} = 37.21$, $p < 0.001$) and an increase in AUC GLP-1 ($F_{(3,33)} =$
13.35, $p < 0.001$) (see Table 2). An increase in fasting ghrelin ($F_{(3,30)} = 3.94$, $p = 0.18$), and
decreases in fasting leptin ($F_{(3,33)} = 2.59$, $p < 0.001$) and insulin ($F_{(3,33)} = 4.77$, $p = 0.007$) were
observed. However, there was no significant change in fasting GLP-1 (see Table 3 for peptide
profiles). There were no significant changes in intra-meal levels of ghrelin, however, there
145 was a significant increase in intra-meal insulin ($F_{(3,33)} = 27.21$, $p < 0.001$) and GLP-1 ($F_{(3,33)} =$
7.98, $p < 0.001$: see Table 2).

Table 2 about here

A significant decrease in AUC desire to eat ($F_{(3,33)} = 3.43$, $p = 0.028$), an increase in AUC
150 fullness ($F_{(3,33)} = 7.45$, $p = 0.001$) and a decrease in AUC hunger ($F_{(3,33)} = 4.37$, $p = 0.011$)
one year after surgery was found (see Table 2). There were no significant changes in fasting
ratings of desire to eat, fullness or hunger or changes in intra-meal appetite ratings from pre-
to one year post surgery.

155 Table 3 about here

Relationship amongst the variables at baseline

Baseline body weight was not associated with any baseline TFEQR18 factors, ratings of
motivation to eat or appetite peptides, with the exception of insulin. Both fasting and AUC
160 insulin were associated with baseline weight ($r = 0.89$, $p < 0.001$; $r = 0.66$, $p = 0.021$,

respectively). Upon examination of the associations between pre-surgery TFEQR18 and peptide levels, there was a correlation between EE and fasting ghrelin ($r = .84$, $p = 0.005$) and a marginal association between UE and fasting leptin ($r = .65$, $p = 0.056$). In addition, there was an association between EE and ghrelin AUC ($r = .83$, $p = 0.006$).

165

In terms of the relationship between motivation to eat and TFEQR18 factors, a significant association between desire to eat AUC and UE ($r = .67$, $p = 0.047$) was found. Two months following surgery there was a negative correlation between AUC desire to eat and CR ($r = -.67$, $p = 0.023$) and UE ($r = -.74$, $p = 0.009$). In addition, UE was positively associated with AUC fullness ($r = .77$, $p = 0.005$) and negatively associated with AUC hunger ($r = -.69$, $p = 0.019$). When considering the relationships between appetite peptides and motivation to eat, there were correlations between fasting desire to eat and fasting ($r = .68$, $p = 0.021$) and AUC ghrelin ($r = .79$, $p = 0.004$).

175 **Relationships amongst variables from pre- to one year post-surgery**

Following surgery body weight was positively associated with CR ($r = 0.71$, $p = 0.014$) and fasting insulin ($r = 0.88$, $p < 0.001$), and negatively with AUC GLP-1 ($r = -0.74$, $p = 0.006$). Investigation of the relationship between TFEQR18 and peptides revealed that at 2 months post-surgery, there was an association between UE and fasting ($r = .64$, $p = 0.035$) and ghrelin AUC ($r = .68$, $p = 0.022$). One year following surgery, there was a correlation between UE and fasting insulin ($r = 0.64$, $p = 0.04$). There was also negative relationship between Restraint and GLP-1 AUC ($r = -0.76$, $p = 0.01$).

180

At two months following surgery, the relationships between TFEQR18 and ratings of
185 motivation to eat showed that desire to eat was negatively associated with CR ($r = -0.67$, $p = 0.023$) and UE ($r = -0.74$, $p = 0.009$). Fullness was positively related to UE ($r = 0.77$, $p = 0.005$) and marginally related to CR ($r = 0.59$, $p = 0.058$) and EE ($r = 0.59$, $p = 0.057$). Following one year there was a significant association between UE and hunger AUC ($r = 0.63$, $p = 0.05$).

190

The correlations between peptides and motivation to eat, found that two-months following surgery a relationship between AUC ghrelin and AUC hunger ($r = -.61$, $p = 0.045$) and AUC fullness ($r = 0.72$, $p = 0.013$) existed. AUC GLP-1 was negatively associated with fasting hunger levels ($r = -.64$, $p = 0.035$), whereas fasting GLP-1 was negatively related to fasting
195 desire to eat ($r = -.63$, $p = 0.037$) and positively related to fullness ($r = .62$, $p = 0.041$) and AUC hunger ($r = .69$, $p = 0.018$). At one year post-surgery, there was a positive relationship between fasting desire to eat and fasting ($r = 0.68$, $p = 0.21$) and ghrelin AUC ($r = 0.79$, $p = 0.004$).

200 **Relationship of changes in variables from pre to one year post-surgery**

Change in weight was not associated with any changes in TFEQR18 factors, change in AUC or change in intra-meal peptide or motivation to eat ratings. Significant, positive correlations were found between change in CR and change in AUC and fasting ghrelin ($r = 0.68$, $p = 0.031$; $r = 0.77$, $p = 0.01$, respectively). In addition a positive relationship was found between
205 change in EE and change in fasting GLP-1 ($r = 0.69$, $p = 0.028$). No significant associations

between change in AUC or intra-meal subjective appetite ratings and change in peptides were found.

Discussion

210 This study has demonstrated that TFEQR18 factors of EE and UE significantly decrease following gastric by-pass surgery, but that Restraint remains stable. These changes are congruous with alterations, which occurred in subjective appetite ratings and in levels of appetite peptides. To our knowledge, this is the first paper to assess relationships amongst the TFEQR18 factors, subjective appetite and appetite peptides in obese patients undergoing
215 RYGB surgery.

Previous research has used the original TFEQ to assess eating behaviour traits pre- and post-gastric weight loss surgery. This research demonstrated that in response to weight loss induced by gastric surgery (both gastric band and gastric bypass), a reduction in Disinhibition and Hunger and increase in Restraint are seen¹³⁻¹⁷. Within this study, a significant reduction in UE and EE was seen over a year following gastric by-pass surgery, with no change in Restraint observed, which is in line with findings from Laurenus et al.⁹ The factors of UE and EE are comprised of Hunger and Disinhibition items, thus the decrease is in line with previous findings and reflect a reduction in susceptibility to overeat. What is interesting is the
225 lack of change in Restraint. This pattern was also observed by Karlsson et al.,¹⁷ and Laurenus et al.,⁹ who found a return towards baseline values of TFEQ factors after gastric surgery. However, despite movement towards baseline levels, the change in TFEQR18 traits from pre- to post-surgery, do show an eating behaviour trait profile, which promotes weight regulation.

230

The mechanism behind the change in eating behaviour traits remains to be elucidated. However, a relationship between appetite peptides and eating behaviour traits, both pre- and post-operatively was established. The finding in this study of a relationship between ghrelin (fasting and AUC) and UE and EE, independently of BMI, supports previous findings of a significant association between fasting ghrelin and TFEQ Disinhibition¹⁸ and TFEQ Hunger⁶. As ghrelin is associated with a hyperphagic response¹⁹ it is congruous that it is associated with UE and EE which both represent a propensity to overeat^{8, 20}. This is an important finding as it supports the notion that psychological eating behaviour traits are, at least in part, underpinned by appetite peptides.

240

The changes in eating behaviour traits represent a tonic response of subjective appetite regulation following RYGB. The more episodic subjective response was assessed using VAS. The regulation of appetite in response to the liquid meal from pre- to post-surgery showed an improvement in appetite profiles with a reduced hunger and desire to eat and increased fullness following surgery. Desire to eat was positively associated with ghrelin (fasting and AUC), while fasting GLP-1 was negatively associated with desire to eat and positively with fullness. The relationship between peptides and subjective appetite response could at least in part explain the mechanism behind the sustained weight loss following bariatric surgery.

250 Furthermore, the episodic subjective appetite ratings are also related to the TFEQR18 factors. UE was related to desire to eat pre-surgery and hunger post-surgery. It is plausible that the association of UE with heightened appetite ratings could be the expression of the tonic UE

trait in the short term. In line with this, EE has been associated with increased consumption of sweet foods²¹, and both UE and EE have been associated with obesity and lower levels of self-control²⁰, supporting the relationship between heightened episodic appetite responses to more enduring eating behaviour traits.

In conclusion, this study has demonstrated that TFEQR18 factors of EE and UE significantly decrease following RYGB surgery, but that Restraint remains stable. Although these changes were not significantly associated with weight loss, they were significantly associated with appetite peptides and subjective appetite ratings. This study suggests that there are biological mechanisms underpinning the expression of tonic and episodic subjective appetite sensations. In addition, it is apparent that these peptide changes can, at least partially, explain the changes seen in eating behaviour traits following RYGB surgery. There are several limitations to this study however. The sample size was small and the participants were only followed for one-year.

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Conflict of Interest

The authors report no conflict of interest.

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Table 1: Body weight, BMI and TFEQR18 factor scores (mean \pm SD) before and following surgery

	Pre surgery	3 days post surgery	2 months following surgery	1 year following surgery	Change
Body weight (kg)	137.79 (32.42)		117.33 (29.36)	92.63 (27.67)	-45.16***
BMI (kg/m²)	45.50 (1.90)		38.60 (1.80)	30.30 (1.80)	-15.20***
Cognitive Restraint	38.33 (15.59)	34.26(21.17)	52.78 (16.15)	39.39 (20.10)	5.55
Uncontrolled Eating	43.70 (29.20)	43.21 (19.23)	19.75 (12.06)* ¹	20.88 (10.90)* ^{1,2}	-25.51***
Emotional Eating	58.89 (33.15)	61.11 (31.25)	37.04 (24.77)	37.37 (24.48)	-24.69*

*p<0.05, *** p<0.001

¹ significantly different to pre-surgery scores

² Significantly different to scores 2 months following surgery

BMI – body mass index

TFEQR18 – Three Factor Eating Questionnaire R18

SD – standard deviation

Table 2 – AUC and intra-meal (mean ±SD) peptides and appetite ratings from pre-surgery to one year post-surgery

		Pre surgery	3 days post surgery	2 months following surgery	1 year following surgery	Change
AUC	Ghrelin (pmol/L)	642.33 (267.99)	433.23 (106.62)	616.64 (280.74)	708.27 (306.66)* ³	65.94
	Leptin (ng/mL)	46.00 (15.40)	50.53 (22.52)	24.66 (10.50)** ^{1,2}	13.42 (9.11)*** ^{1,2,3}	- 32.58***
	Insulin (µU/L)	58.67 (22.63)	66.88 (29.60)	72.07 (21.79)	66.25 (18.31)	7.58
	GLP-1 (pmol/L)	12.75 (6.97)	43.69 (39.72)	67.31 (52.65)* ¹	77.58 (35.19)* ^{1,2}	64.83***
	Desire to Eat (mm)	26.00 (22.02)	11.74 (12.82)	11.44 (7.75)	13.83 (7.93)	-12.17*
	Hunger (mm)	28.25 (22.08)	10.49 (13.18)	14.03 (9.33)	15.67 (8.25)	-12.58**
	Fullness (mm)	49.08 (29.16)	81.58 (19.11)* ¹	80.12 (13.53)* ¹	74.08 (19.36)	25.00***
Intra-meal	Ghrelin (pmol/L)	13.50 (91.26)	-122.83 (118.58)	22.92 (251.08)	-4.64 (176.15)	-18.14
	Insulin (µU/L)	20.82 (21.28)	92.93 (53.67)** ¹	110.76 (35.84)*** ¹	109.31 (45.87)*** ¹	88.49***
	GLP-1 (pmol/L)	4.00 (4.00)	82.83 (106.83)	113.08 (112.45)* ¹	102.08 (99.72)* ¹	98.08***
	Desire to Eat (mm)	-35.08 (35.10)	-24.83 (36.32)	-24.25 (31.83)	-35.08 (35.10)	0
	Hunger (mm)	-22.08 (27.13)	-20.92 (27.12)	-26.75 (36.71)	-40.25 (33.41)	-18.17
	Fullness (mm)	26.25 (24.36)	42.25 (42.27)	36.92 (43.05)	57.08 (30.16)* ¹	30.83

*p<0.05, **p<0.01, ***p<0.001

¹ significantly different to pre-surgery

² significantly different to 3 days post surgery

³ significantly different to 2 months post surgery

AUC – area under the curve

SD – standard deviation

GLP-1 – glucagon-like peptide-1

pmol/L – picomoles per litre

ng/mL – nanograms per litre

μU/L – microunits per litre

mm - millimeters

Table 3 – Profiles (mean \pm SD) of peptides and appetite ratings following a fixed liquid meal, pre and one year post-surgery

		-10 mins	0 mins	10 mins	20 mins	30 mins	60 mins	90 mins	120 mins	180 mins
Ghrelin (pmol/L)	pre-surgery	678.92 (284.88)	707.42 (293.29)	692.42 (341.57)	640.92 (270.82)	638.83 (279.96)	583.75 (241.96)	576.75 (239.57)	621.42 (276.93)	641.08 (272.38)
	1 year post-surgery	778.36 (376.46)	820.42 (591.60)	738.67 (304.20)	654.08 (212.68)	615.50 (231.37)	589.42 (188.31)	586.67 (190.81)	602.75 (327.72)	781.92 (608.77)
Leptin (ng/mL)	pre-surgery	46.90 (16.88)	51.35 (20.75)	46.28 (17.46)	50.53 (17.57)	44.64 (14.60)	46.74 (16.70)	42.73 (13.73)	43.84 (16.66)	44.68 (16.04)
	1 year post-surgery	14.23 (9.49)	13.28 (9.12)	13.03 (9.04)	12.75 (8.64)	12.67 (8.92)	13.23 (9.15)	13.13 (9.50)	13.36 (8.96)	13.84 (9.16)
Insulin (μU/L)	pre-surgery	26.26 (20.10)	28.58 (23.16)	47.07 (13.89)	93.87 (41.82)	93.93 (73.74)	97.52 (48.54)	61.01 (38.65)	51.51 (48.96)	34.54 (52.88)
	1 year post-surgery	12.37 (6.48)	40.33 (25.88)	121.69 (45.25)	158.62 (36.60)	158.31 (40.33)	53.67 (50.56)	25.21 (22.59)	14.51 (7.15)	10.56 (5.93)
GLP-1 (pmol/L)	pre surgery	8.50 (6.04)	8.67 (5.61)	12.50 (7.47)	18.27 (8.66)	17.75 (10.50)	14.83 (8.32)	13.08 (8.86)	13.50 (9.51)	9.00 (4.99)
	1 year post-surgery	12.71 (8.11)	50.33 (87.50)	114.25 (100.09)	233.17 (106.69)	189.92 (127.63)	44.00 (32.91)	22.50 (12.08)	19.33 (8.15)	12.08 (6.58)
Desire	pre-surgery	30.67	15.83	13.33	19.64	17.75	23.17	28.67	33.75	51.00

to eat (mm)		(33.26)	(28.03)	(20.02)	(20.00)	(20.21)	(28.57)	(24.14)	(28.63)	(37.84)
	1 year post- surgery	35.17 (35.15)	0.08 (0.29)	0.08 (0.29)	0.00 (0.00)	1.17 (2.33)	3.83 (6.04)	9.83 (15.22)	23.42 (24.60)	50.58 (26.60)
Fullness (mm)	pre-surgery	39.33 (39.08)	63.83 (31.52)	68.58 (28.71)	59.36 (37.06)	59.42 (37.18)	45.42 (35.91)	38.75 (34.31)	53.83 (49.21)	22.58 (25.87)
	1 year post- surgery	34.33 (33.46)	85.67 (23.95)	91.42 (17.94)	90.58 (24.48)	92.25 (24.10)	90.08 (22.88)	85.16 (26.54)	67.00 (27.75)	30.42 (32.68)
Hunger (mm)	pre-surgery	33.00 (34.86)	15.00 (25.21)	10.92 (14.54)	21.27 (23.45)	15.75 (19.60)	30.08 (30.10)	34.25 (26.38)	36.42 (30.13)	59.08 (32.23)
	1 year post- surgery	44.75 (36.90)	9.42 (17.14)	4.50 (8.65)	2.42 (5.65)	2.00 (4.16)	3.17 (5.27)	5.08 (6.61)	21.58 (20.34)	49.25 (26.45)

SD – standard deviation

GLP-1 – glucagon-like peptide-1

pmol/L – picomoles per litre

ng/mL – nanograms per litre

μU/L – microunits per litre

mm - milimeters