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Metabolic improvement after gastric bypass correlates with changes in IGF-regulatory proteins stanniocalcin-2 and IGFBP-4

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ABSTRACT

Background: Pregnancy-associated plasma protein-A (PAPP-A) is an enzyme that increases IGF-activity through cleavage of IGF-binding proteins (IGFBPs), primarily IGFBP-4, whereby bound IGF-I becomes released as a free molecule. The enzymatic activity of PAPP-A is irreversibly suppressed by the glycoprotein stanniocalcin-2 (STC2). Pre-clinical and clinical studies suggest that the STC2 – PAPP-A – IGFBP-4 axis is important in controlling local IGF-action. STC2, PAPP-A and IGFBP-4 are expressed in adipose tissue, and as bariatric surgery markedly reduces the amount of fat, we found it relevant to study the impact of Roux-en-Y gastric bypass (RYGB) on circulating concentrations of this IGF-regulatory network.
Methods: Analysis of fasting blood samples from 20 obese subjects, hereof 10 with preoperative type 2 diabetes, investigated before RYGB, and 1 week, 3 months and 12 months post-surgery. Members of the IGF-system were analyzed by immunoassays, bioactive IGF by cell-based IGF-I receptor activation assay. We compared changes in IGF-system components with changes in fasting plasma insulin and glucose, and HbA1c.
Results: PAPP-A remained unchanged, but STC2 decreased following RYGB ($p < 0.05$). The PAPP-A substrate IGFBP-4 declined ($p < 0.01$), whereas levels of PAPP-A specific IGFBP-4 fragments increased ($p < 0.05$), indicating an increased PAPP-A enzymatic activity post-RYGB. Further, the reduction in intact IGFBP-4 correlated with increased levels of bioactive IGF ($p < 0.05$). In multivariable regression analyses, an improved glucose metabolism correlated with reductions in STC2 and IGFBP-4, and with increases in bioactive IGF and IGF-I ($p < 0.05$).
Conclusion: After 12 months, RYGB caused reduced serum concentrations of intact IGFBP-4 and STC2, whereas serum PAPP-A remained at pre-operative levels. However, concentrations of PAPP-A generated IGFBP-4 fragments increased, pointing to an overall increased PAPP-A enzymatic activity following RYGB. Notably, reductions in intact IGFBP-4 and STC2 associated with improvements in glucose metabolism. Therefore, we propose that STC2 and IGFBP-4 are involved in the metabolic improvement that follows RYGB.

1. Introduction

Bariatric surgery constitutes an effective and durable treatment for obesity-related insulin resistance and type 2 diabetes (T2D) [1,2]. In addition, bariatric surgery alters several nutritionally regulated endocrine systems, hereunder the growth hormone (GH)/insulin-like growth factor I (IGF-I) axis [3]. The secretion of GH is functionally suppressed in obesity and increases following bariatric surgery [3]. As GH is a potent stimulant of IGF-I synthesis [4], increases in plasma IGF-I may accompany the improved GH secretion. However, increases in IGF-I appears to a less prominent feature of bariatric surgery than increases in GH secretion, most likely due to the negative energy balance induced by bariatric surgery [3,4].

Besides being regulated by GH at the level of synthesis, IGF-I action is regulated by enzymes that control the liberation of IGF-I from its high-affinity IGF-binding proteins (IGFBPs), which impede IGF-I from activating the IGF-I receptor (IGF-IR) [5]. In particularly pregnancy-associated plasma protein-A (PAPP-A) has received attention, because it appears to operate predominantly in the tissues, increasing IGF-I local action without altering circulating insulin, GH or IGF-I concentrations [6–8]. PAPP-A increases local IGF action by cleaving IGF-binding proteins (IGFBPs) into fragments with reduced IGF-binding affinity,

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whereby bound IGF becomes released as a free molecule, available for IGF-IR activation [7]. The principal substrate for PAPP-A is IGFBP-4, which is cleaved when carrying an IGF molecule [7,8].

Experiments in mice have shown that genetic silencing of PAPP-A increases health- and life-span [9]. Although the role of PAPP-A in human health remains to be fully established, pre-clinical findings have been supported by clinical studies indicating that increased PAPP-A activity is associated with pre-diabetes and T2D [10], cardiovascular disease (CVD) [11–13] and breast cancer relapse [14].

PAPP-A and its principal substrate IGFBP-4 are expressed [15] and secreted from adipose tissue [16], and the same is true for Stanniocalcin-2 (STC2) [17], an irreversible inhibitor of PAPP-A enzymatic activity [18]. Given that bariatric surgery reduces adipose tissue volume, induces beneficial alterations in adipocyte function and changes the expression of numerous genes [19–21], we found it of interest to investigate the role of Roux-en-Y gastric bypass (RYGB) on the STC2 – PAPP-A – IGFBP-4 – IGF-I network. We hypothesized that RYGB reduced the activity of PAPP-A, thereby potentially contributing to some of the beneficial effects of RYGB on mortality, CVD and cancer [22]. To this end, we examined serum and plasma from an existing cohort of obese subjects with and without preoperative T2D, who were investigated before RYGB and 1 week, 3 months and 12 months after surgery [23], focusing on RYGB-induced changes in the circulating STC2 – PAPP-A – IGFBP-4 – IGF-I network.

2. Design and methods

2.1. Subjects and study design

The study included 10 obese subjects with normal glucose tolerance (NGT group, age 40.1 ± 2.8 years (mean ± SEM) and 10 obese patients with T2D (T2D group, median diabetes duration: 2.5 years [range 1–11], age 43.6 ± 3.4 years), who were scheduled for laparoscopic RYGB at Hvidovre Hospital, Denmark. Prior to surgery, all subjects had to undergo a mandatory, diet-induced weight loss of at least 8% (9.2 ± 1.2% pre-surgery) as required by health authorities. Patients with T2D were controlled with metformin alone (n = 4), metformin in combination with liraglutide (n = 2), NPH insulin (n = 2) or diet alone (n = 2). Liraglutide was discontinued ≥10 days before the preoperative study day, and all other anti-diabetic agents were discontinued ≥3 days before. All anti-diabetic agents were discontinued at the time of surgery, and only one patient required metformin postoperatively. Patients were investigated before surgery (baseline), and 1 week, 3 months and 12 months, postoperatively. All patients completed the preoperative and 3-month visits, whereas four subjects did not complete the 1 week visit because of postoperative complications. Two subjects did not wish to participate at the 12-month visit.

Written informed consent was obtained from all patients prior to enrolment, and the study was approved by the Ethics Committee of Copenhagen and complied with the guidelines of the Helsinki Declaration and the Danish Data Protection Agency. Details of the study design, patients characteristics and metabolic changes have previously been reported (Clinical Trial no. NCT 01202526) [23,24].

2.2. Laboratory measurements

2.2.1. Routine methods

Serum and EDTA-plasma were collected and stored at −80 °C until analysis. Plasma glucose and serum insulin were analyzed as previously reported [23].

2.2.1.1. Circulating IGF-I, IGF-II, IGFBP-1, IGFBP-2 and IGFBP-3. In general, all samples from the same individual were analyzed in the same run. IGF-I and IGFBP-3 were measured by an IDS-iSYS Multi-Discipline Automated Analyzer (Immunodiagnostic Systems Nordic SA, Denmark), as previously published [25,26]. IGF-II, IGFBP-1 and IGFBP-2 were measured by in-house assays, with intra-assay CVs and inter-CVs of 5% and 10%, 7% and 8%, and 5% and 12% respectively [27,28].

2.2.2. Circulating IGF bioactivity

The ability of serum IGF-I and IGF-II to activate the IGF-IR in vitro was determined by an in-house kinase receptor activation (KIRA) assay as originally described [29], using a commercial phospho-IGF-IR ELISA kit from R&D Systems (Cat# DYC 1770E; Abingdon, UK). The assay measures the ability of IGF-I and IGF-II to phosphorylate the IGF-IR in vitro, using cultures of human embryonic kidney cells transfected with the human IGF-IR cDNA. A serial dilution of rhIGF-I (WHO 02/254) served as calibrator. As the IGF-IR may be activated by both IGFs, we refer to the assay signal as "IGF bioactivity". Limit of detection was <0.08 μg/L. Intra- and inter-assay CVs averaged 12% and 20%, respectively.

2.2.3. Circulating intact and PAPP-A fragmented IGFBP-4, PAPP-A and STC2

EDTA plasma levels of IGFBP-4 and the two PAPP-A generated IGFBP-4 fragments, CT-IGFBP-4 and NT-IGFBP-4, were determined by in-house assays based on monoclonal antibodies (MAb) and corresponding recombinant human (rh) calibrators, generously provided by HyTest Ltd. (Turku, Finland). The assays were performed as recently described [11]. In all three assays, intra- and inter-assay CVs were less than 10% and 15%, respectively.

Serum PAPP-A and STC2 levels were determined by commercial ELISAs according to instructions by the manufacturer (AnshLabs, Webster, Texas, USA).

2.3. Statistical analysis

Baseline data have previously been described [23]. Data with a non-parametric distribution were transformed using the natural logarithm prior to analysis (IGF-I, IGF-II, IGF bioactivity, IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-4, CT- and NT-IGFBP-4, PAPP-A, and STC2). Postoperative changes were evaluated by ANOVA in a linear mixed-effects model using time from surgery and group as fixed categorical effects and between-patient variability as random effect. If the ANOVA was significant, main effects of time and significant interactions were evaluated by Tukey post hoc test and compared to pre-surgery values. Post hoc comparisons of group differences at a given time point were based on unpaired *t*-tests. To investigate the overall change from baseline to 12 months in IGF system parameters and metabolic variables, delta values were calculated for each subject (concentration at 12 months minus concentration at baseline). Correlations were investigated using Pearson or Spearman correlation coefficients as appropriate. Multiple regression analyses were performed with age, sex and BMI at baseline as independent co-variables. Data are reported as mean ± SEM for normally distributed variables and median (25;75 percentiles) for non-normally distributed variables. Level of significance was $p < 0.05$. Statistical analyses were performed using Stata 13.1.

3. Results

Data on weight loss, glycemic control and insulin sensitivity have previously been described [23,24]. In brief, RYGB resulted in a weight loss of 28.5 ± 2.9% and 22.1 ± 2.1% in the NGT and T2D group, respectively (both $p < 0.005$). Reductions in HbA1c were significant in the T2D group ($p = 0.001$). Fasting glucose and insulin were significantly lower 12 months postoperatively in both groups (all comparisons $p < 0.01$), with the largest reductions observed in patients with T2D (Table 1).

3.1.1. RYGB-induced changes in the STC2 – PAPP-A – IGFBP-4 network

No group or group × time differences in the STC2 – PAPP-A – IGFBP-4 network were detected (Fig. 1A) and accordingly, we merged the groups (Table 2). Intact IGFBP-4 declined, whereas the concentration of the two PAPP-A generated IGFBP-4 fragments increased ($p < 0.05$)

Table 1
Effects of RYGB on anthropometry and metabolism in obese subjects with NGT or T2D.

	NGT				T2D				Mixed effect model ANOVA		
	Before	1 week	3 months	12 months	Before	1 week	3 months	12 months	Time	Group	Time × group
Subjects (males/females) (n)	10 (3/7)	8 (3/5)	10 (3/7)	9 (3/6)	10 (4/6)	8 (4/4)	10 (4/6)	9 (4/5)	–	–	–
BMI (kg/m^2)	40.2 ± 0.8	37.9 ± 0.9	33.2 ± 1.1**	28.5 ± 1.5**	38.9 ± 1.6	37.3 ± 1.7	33.1 ± 1.5**	30.8 ± 1.7**	<0.01	0.52	<0.01
Weight (kg)	116.9 ± 4.9	112.3 ± 6.2	96.6 ± 4.8**	82.6 ± 4.8**	121.5 ± 8.9	118.1 ± 9.5*	103.3 ± 7.8**	96.0 ± 8.2**	<0.01	0.52	0.06
Fat free mass (kg)	64.4 ± 4.1	–	58.2 ± 3.7**	56.3 ± 3.5**	73.3 ± 6.9	–	66.8 ± 6.0**	64.9 ± 6.4**	<0.01	0.25	0.97
HbA1c (mmol/mol)	36 ± 1.1	–	34 ± 1.1	34 ± 1.1	53 ± 3.3††	–	41 ± 2.2**,†	39 ± 2.2**,†	<0.01	<0.01	<0.01
Fasting glucose (mmol/L)	5.1 ± 0.1	4.8 ± 0.1	4.6 ± 0.1*	4.7 ± 0.1*	8.3 ± 0.6††	6.6 ± 0.4**,††	5.7 ± 0.2**,††	5.6 ± 0.2**,††	<0.01	<0.01	<0.01
Fasting insulin (pmol/L)	77 ± 9	57 ± 6*	30 ± 3**	30 ± 3**	97 ± 13	89 ± 18*	51 ± 8**	41 ± 6**	<0.01	0.12	0.32

Data have been published previously by [23,24].
*$p < 0.05$, **$p < 0.01$ for the change from preoperative level within the group (post-hoc estimates from mixed effect model). †$p < 0.05$, ††$p < 0.01$ for differences between the groups at a given study session (post-hoc unpaired t-test). Values are mean ± SEM. BMI, body mass index; HbA1c, glycosylated hemoglobin A1c; NGT, normal glucose tolerance; RYGB, Roux-en-Y gastric bypass; T2D, type 2 diabetes.

(Table 2). Overall, this reduced the ratios of intact IGFBP-4/CT-IGFBP-4, and intact IGFBP-4/NT-IGFBP-4, respectively ($p < 0.01$). PAPP-A declined at week 1 ($p < 0.001$), but returned to baseline at 3 months. STC2 continuously decreased after RYGB, being reduced by 17% at 12 months ($p < 0.001$).

3.1.2. RYGB-induced changes in IGF-I, IGF-II, bioactive IGF and IGFBP-1 to -3

At baseline, bioactive IGF, IGF-I, IGFBP-1 and IGFBP-2 were similar in the two groups, whereas IGF-II and IGFBP-3 were highest in patients with T2D ($p < 0.01$) (Fig. 1B). However, as both IGF-II and IGFBP-3 declined following RYGB, we decided to pool data from the two groups (Table 3). IGF-I declined at 1 week and 3 months, but returned to baseline concentrations at 12 months. Bioactive IGF did not change significantly during the study, whereas we observed increases in IGFBP-1 and IGFBP-2 ($p < 0.001$), and decreases in IGF-II and IGFBP-3 ($p < 0.001$).

3.1.3. Correlations between the IGFBP-4 – PAPP-A – STC2 axis and metabolic parameters in all patients

At baseline, intact IGFBP-4 correlated positively with CT-IGFBP-4 and NT-IGFBP-4 ($r = 0.45$, $p = 0.042$ and $r = 0.53$, $p = 0.017$, respectively). In addition, CT-IGFBP-4 and NT-IGFBP-4 correlated positively ($r = 0.53$, $p = 0.018$). Intact IGFBP-4 was negatively associated with IGF bioactivity ($r = -0.48$, $p = 0.045$) and positively associated with STC2 levels ($r = 0.63$, $p < 0.005$). At 12 months, intact IGFBP-4 remained correlated with CT-IGFBP-4 and NT-IGFBP-4 ($r = 0.57$, $p = 0.013$ and $r = 0.53$, $p = 0.024$, respectively). CT-IGFBP-4 was highly correlated to NT-IGFBP-4 ($r = 0.85$, $p < 0.001$). Intact IGFBP-4 was negatively associated with IGF bioactivity ($r = -0.57$, $p = 0.16$) and PAPP-A ($r = -0.81$, $p < 0.001$).

Metabolically, baseline levels of IGFBP-4 ($r = 0.51$, $p = 0.022$), CT-IGFBP-4 ($r = -0.48$, $p = 0.034$) and bioactive IGF ($r = -0.73$, $p < 0.001$) all associated with fasting insulin concentrations, whereas BMI associated with STC2 ($r = 0.62$, $p = 0.005$) and intact IGFBP-4 ($r = 0.50$, $p = 0.028$).

To investigate individual changes from baseline of IGF system components, we calculated differences (delta values) between protein levels at baseline and 12 months. Changes in intact IGFBP-4 correlated negatively with PAPP-A ($r = -0.48$, $p = 0.046$), suggesting that increased PAPP-A results in more degraded IGFBP-4. Conversely, changes in intact IGFBP-4 correlated positively with STC2 ($r = 0.56$, $p = 0.023$), suggesting that increased STC2 reduces the ability of PAPP-A to cleave IGFBP-4. Furthermore, changes in intact IGFBP-4 and IGF bioactivity were negatively correlated ($r = 0.57$, $p = 0.022$), suggesting decreased IGF bioavailability in response to increased concentrations of intact IGFBP-4.

Metabolically, changes in IGF-I, and IGF bioactivity correlated negatively with changes in HbA1c ($r = -0.72$, $p = 0.001$ and $r = -0.52$, $p = 0.032$, respectively), fasting glucose ($r = -0.61$, $p = 0.009$ and $r = -0.61$, $p = 0.009$, respectively), and fasting insulin concentrations ($r = -0.51$, $p = 0.041$ and $r = -0.48$, $p = 0.048$, respectively (Fig. 2)). Changes in intact IGFBP-4 correlated positively with changes in HbA1c ($r = 0.67$, $p = 0.004$), fasting glucose ($r = 0.53$, $p = 0.029$), and fasting insulin concentrations ($r = 0.57$, $p = 0.021$). Similarly, changes in STC2 correlated positively with changes in HbA1c ($r = 0.64$, $p = 0.007$), fasting glucose ($r = 0.45$, $p = 0.048$) and fasting insulin ($r = 0.55$, $p = 0.027$). Thus, patients demonstrating the most pronounced metabolic improvements also exhibited the largest reductions in intact IGFBP-4 and STC2. Changes in PAPP-A did not associate with changes in HbA1c, glucose or insulin levels.

Finally, we performed univariable and multivariable regression analyses, whereof the latter included age and sex at baseline and delta BMI from baseline to 12 months as independent co-variables (Table 4). Delta values of IGF-I, IGF bioactivity, IGFBP-4 and STC2 served as dependent variables. As shown in Table 4, changes in HbA1c, fasting glucose, and fasting insulin associated with changes in IGFBP-4 and STC2, whereas changes in fasting glucose and HbA1c associated with changes in IGF-I and IGF bioactivity. Thus, reductions in concentrations of IGFBP-4 and STC2 and increases in concentrations of IGF bioactivity and IGF-I associated with an improved glucose metabolism.

4. Discussion

STC2, PAPP-A and IGFBP-4 have emerged as functionally related proteins that in concert serve to modulate IGF-I action [7,9,18]. STC2, PAPP-A and IGFBP-4 are produced in numerous tissues, including adipose tissue [7,30,31] and therefore, we investigated 12-month changes in the circulating STC2 – PAPP-A – IGFBP-4 – IGF-network in obese subjects undergoing RYGB. Our data demonstrate that RYGB reduced concentrations of STC2 and IGFBP-4, whereas PAPP-A remained unchanged. However, PAPP-A enzymatic activity appeared to increase, as evidenced by a reduction in the PAPP-A inhibitor STC2, and the relative increases of the two PAPP-A generated IGFBP-4 fragments. Thus, if anything, RYGB appeared to augment the PAPP-A – IGFBP-4 axis via a reduction of STC2. Accordingly, our study cannot link RYGB with beneficial reductions in PAPP-A. On the other hand, our data suggest that changes in STC2 and IGFBP-4, but not PAPP-A, are associated with RYGB-related improvements in glucose homeostasis. Finally, there seemed to be little difference in the response of the STC2 – PAPP-A – IGFBP-4 – IGF axis in obese patients with and without T2D.

PAPP-A is ubiquitously expressed, but in particular its release from adipose tissue has attracted attention [9]. Human studies have shown that subcutaneous and intra-abdominal adipose tissue fragments secrete large amounts of enzymatically active PAPP-A when cultured in vitro [16]. Thus, we expected the massive loss of adipose tissue following RYGB to cause lower circulating PAPP-A levels. However, circulating PAPP-A remained unchanged post RYGB, staying within the range previously observed in lean subjects and obese patients with

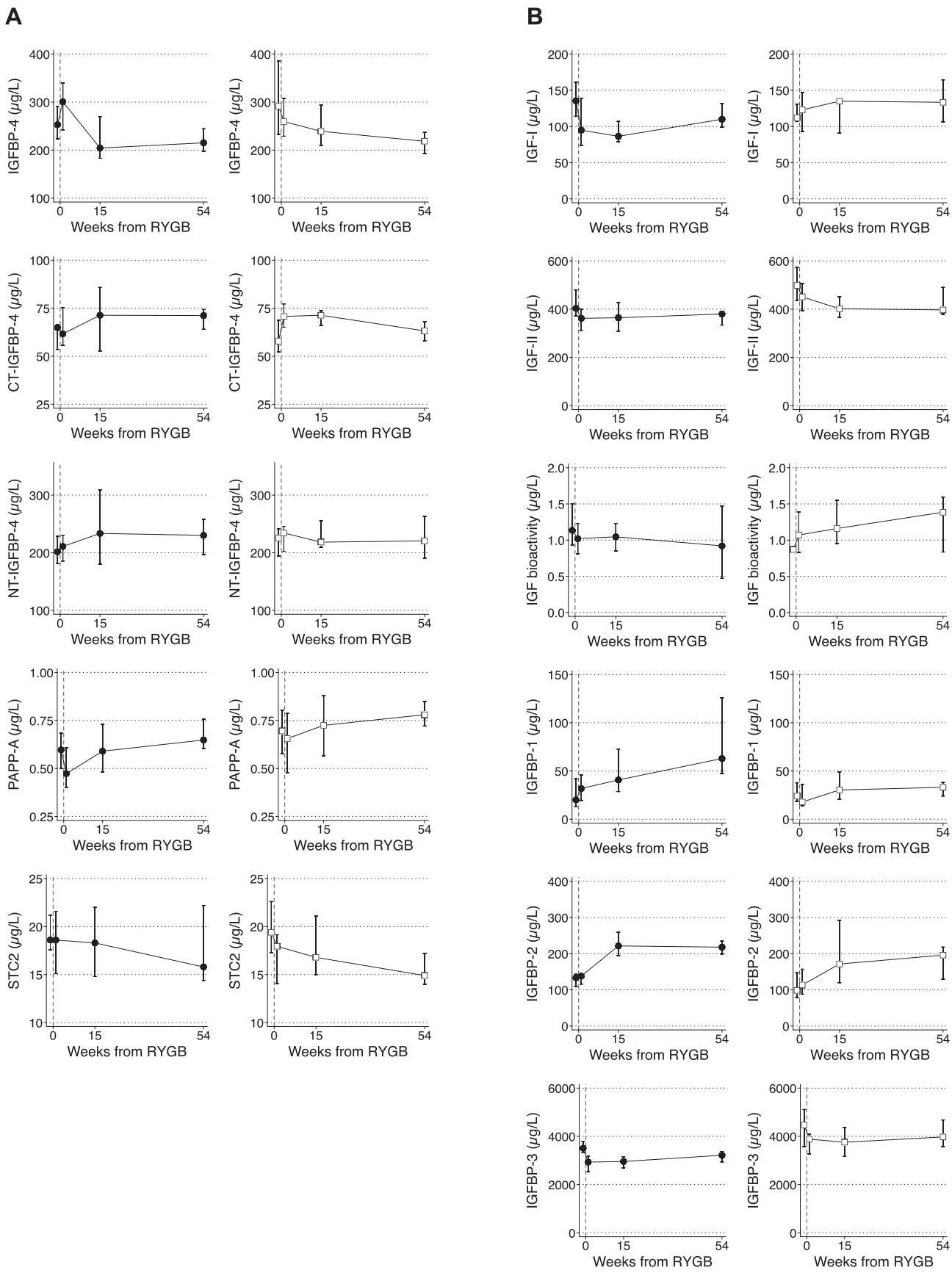

Fig. 1. Changes in IGF system protein levels in patients with NGT (left columns, closed circles) and T2D (right columns, open squares) before, 1 week, 3 months, and 1 year after RYGB. Values of A) IGFBP-4, IGFBP-4 fragments, PAPP-A, and STC2 and B) IGF-I, IGF-II, IGF bioactivity, and IGFBP-1 to -3 are shown as median (25;75 percentiles).
CT, C-terminal; IGF, insulin-like growth factor; IGFBP, IGF binding protein; NGT, normal glucose tolerance; NT, N-terminal; PAPP-A, pregnancy-associated plasma protein-A; RYGB, Roux-en-Y gastric bypass; STC2, Stanniocalcin-2; T2D, type 2 diabetes.

Table 2
Effects of RYGB on the circulating STC2 – PAPP-A – IGFBP-4 axis.

	Before	1 week	3 months	12 months	Repeated measures ANOVA
Intact IGFBP-4 (μg/L)	271 (228;315)	274 (235;331)	226 (199;282)*	218 (193;239)**	<0.01
CT-IGFBP-4 (μg/L)	61 (52;68)	69 (58;77)*	71 (65;83)**	67 (58;74)*	0.031
NT-IGFBP-4 (μg/L)	211 (185;240)	228 (187;243)	219 (196;274)**	226 (192;263)*	0.040
Intact IGFBP-4 to CT-IGFBP-4 ratio	4.59 (3.69;5.60)	4.06 (3.53;4.60)	3.38 (2.60;4.33)**	3.41 (2.83;3.98)**	<0.01
Intact IGFBP-4 to NT-IGFBP-4 ratio	1.31 (1.13;1.45)	1.24 (1.11;1.44)	1.02 (0.83;1.22)**	0.97 (0.86;1.12)**	<0.01
PAPP-A (μg/L)	0.66 (0.50;0.78)	0.58 (0.40;0.74)**	0.64 (0.52;0.77)	0.75 (0.60;0.85)	<0.01
STC2 (μg/L)	19.2 (17.3;21.9)	18.2 (14.3;20.1)*	17.2 (14.9;21.3)*	15.2 (14.0;19.9)**	<0.01
STC2/PAPP-A ratio	28.7 (22.9;37.2)	29.2 (22.9;48.9)	23.4 (21.9;35.8)	23.1 (19;30.6)*	0.022

*$p < 0.05$, **$p < 0.01$ for the change from preoperative level. Values are median (25;75 percentile).
CT, C-terminal; IGFBP-4, insulin-like growth factor binding protein-4; NT, N-terminal; PAPP-A, pregnancy-associated plasma protein-A; RYGB, Roux-en-Y gastric bypass; STC2, stanniocalcin-2.

T2D [32]. The most obvious explanation is that the circulating PAPP-A pool is independent of the secretion of PAPP-A from adipose tissue. In this context one must bear in mind that PAPP-A concentrations are markedly higher in tissue fluids than in the circulation [33], and that PAPP-A is believed to act primarily locally within the tissues [8]. Thus, we cannot exclude the possibility that although circulating PAPP-A concentrations were unaffected by RYGB, local adipose tissue levels become reduced. However, this idea requires further studies. Still, even though circulating PAPP-A concentrations were unaffected by the anthropometric and metabolic changes that followed RYGB, this was not the case for STC2, which is an irreversible inhibitor of PAPP-A enzymatic activity [18].

Studies in genetically modified mice have shown that neither STC2 [34] nor PAPP-A knock-out [6] alters serum IGF-I concentrations, despite clear effects on growth. Therefore, it is believed that PAPP-A and STC2 primarily affect IGF-I activity within the tissues [8,33]. From the present study, we have indications that systemic changes in concentrations of STC2, PAPP-A enzymatic activity and IGFBP-4 may indeed alter circulating IGF-activity, albeit to a minor degree. Changes in circulating intact IGFBP-4 correlated negatively with PAPP-A, and positively with STC2. This observation fits with the fact that PAPP-A stimulates IGFBP-4 cleavage, whereas STC-2 inhibits PAPP-A, and thereby preserves IGFBP-4 in its intact form [18]. Furthermore, changes in intact IGFBP-4 correlated negatively with changes in IGF bioactivity. In summary, we have now human data supporting previous experimental data demonstrating that PAPP-A and STC2 in conjunction regulate IGFBP-4 cleavage and thereby IGF-activity. However, the physiological implication of this observation needs further evaluation.

STC2 may also be involved in regulating appetite and body weight [35]. A recent human cross-sectional study observed a significant positive correlation between serum STC2 and the percentage of body fat [36], which aligns with our observation that STC2 declined following RYGB. As STC2 is expressed in adipose tissue [17], the reduction in fat mass following RYGB provides a simple explanation for the observed decline in serum STC2. In this context, it is interesting that STC2 administration to mice has anorexic effects through activation of the hypothalamic STAT3 pathway. In both normal mice and leptin-deficient hyperphagic ob/ob-mice, systemic STC2 administration reduced food intake and body weight, primarily by reducing the amount of adipose tissue without altering lean body mass or energy expenditure. STC2 also improved glucose homeostasis in obese mice [35]. If we assume that SCT2 also in humans exerts anorexic effects, then our data suggest that the reduction in STC2 is a consequence rather than a cause of the reduced food intake that follows RYGB.

Recent in vitro studies show that STC2 increases glucose uptake in cultures of white adipose tissue from fed but not fasted rats [37]. If these in vitro effects of STC2 are also valid in humans, then it might make sense to down-regulate STC2 activity to avoid hypoglycemia after RYGB. We believe our observation that the reduction in STC2 correlated positively with changes in HbA1c, fasting glucose and insulin is interesting, but it requires caution. Correlations were based on a limited number of participants and in particular one subject, who responded very well to RYGB, contributed to some of the observed significances. Furthermore, given the descriptive nature of our data, we are limited from making any causal conclusions. Finally, a recent cross sectional study of 122 healthy subjects failed to observe significant relationships between plasma STC2 concentrations and different indices of glucose metabolism [36]. Therefore, we speculate that STC2 rather than reflecting glucose metabolism plays a hitherto unrecognized orchestrating role as regards appetite, adiposity and glucose metabolism. Thus, we advocate for further metabolic studies of STC2 aiming to identify the clinical potential of SCT2 as a predictor of improvements in glucose homeostasis following RYGB.

As recently reviewed, the metabolic role of IGFBP-4 remains elusive. Some, but far from all, studies have observed associations between IGFBP-4 serum levels and BMI, insulin and measures of insulin resistance [30]. These discrepancies most likely relate to the well-known issues with IGFBP measurements: that most assays are unable to discriminate between intact and degraded IGFBP. We have circumvented these problems by utilizing assays specific for intact and PAPP-A generated IGFBP-4 fragments, respectively, and hereby we demonstrate that reductions in intact IGFBP-4 display an association with reductions in HbA1c, plasma glucose and insulin. However, in mice, IGFBP-4 silencing [38] does not appear to cause major metabolic aberrations. Thus, it remains an open question whether the reduction in intact IGFBP-4 is metabolically relevant. The association may reflect the RYGB-related loss of fat mass, as IGFBP-4 is produced in adipose tissue, among others [16].

Table 3
Effects of RYGB on the circulating concentrations of IGF-I, IGF-II, bioactive IGF and IGFBP-1 to -3.

	Before	1 week	3 months	12 months	Repeated measures ANOVA
IGF-I (μg/L)	119 (107;155)	113 (92;139)**	106 (79;135)**	119 (104;104)	0.017
IGF-II (μg/L)	440 (399;502)	400 (362;497)**	384 (356;448)**	385 (353;486)**	<0.001
IGF bioactivity (μg/L)	0.93 (0.85;1.37)	1.05 (0.83;1.23)	1.10 (0.95;1.55)	1.28 (0.78;1.49)	0.743
IGFBP-1 (μg/L)	21 (14;39)	21 (16;37)	36 (26;56)**	43 (27;63)**	<0.001
IGFBP-2 (μg/L)	114 (93;145)	138 (91;152)	206 (143;282)**	207 (145;231)**	<0.001
IGFBP-3 (μg/L)	3789 (3454;4474)	3260 (2873;4053)**	3160 (2848;3978)**	3472 (3025;4006)**	<0.001

*$p < 0.05$, **$p < 0.01$ for the change from preoperative level within the group (post-hoc estimates from mixed effect model). Values are median (25;75 percentile). IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein. At baseline, concentrations of IGF-II and IGFBP-3 were significantly higher in patients with than without T2D. However, IGF-II and IGFBP-3 declined in both groups following RYGB. IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; RYGB, Roux-en-Y gastric bypass.

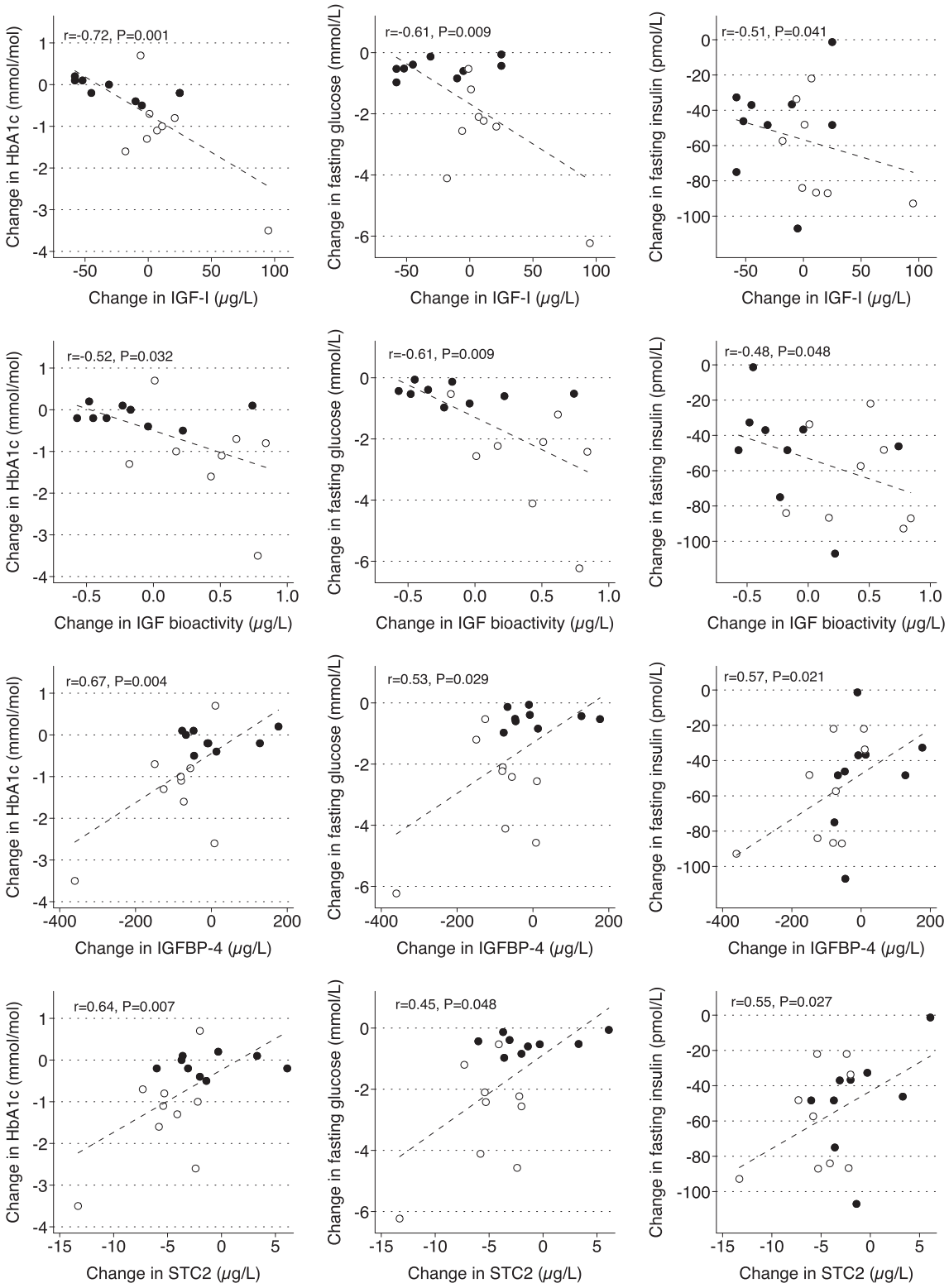

Fig. 2. The delta value (change) in IGF-I (top panel), bioactive IGF (2nd panel top), intact IGFBP-4 (3rd panel top) and STC2 (bottom panel) vs. HbA1c (left columns), fasting glucose (middle column) and fasting insulin (right column). Subjects with NGT are depicted with closed circles, patients with T2D with open circles. Correlation coefficient r values and *p* values are shown. HbA1c, glycosylated hemoglobin A1c; IGF, insulin-like growth factor; IGFBP-4, insulin-like growth factor binding protein-4; NGT, normal glucose tolerance; RYGB, Roux-en-Y gastric bypass; r, correlation coefficient; STC2, stanniocalcin-2; T2D, type 2 diabetes.

The synthesis of IGFBP-1 and IGFBP-2 is inhibited by insulin, and therefore, it was expected that both proteins increased following RYGB [39,40]. Despite these changes, bioactive IGF remained stable 12 months after RYGB, in agreement with our recent study [39]. As regards 12 months post-surgical changes in serum IGF-I, increased concentrations have been observed in some [41], but not all studies [39,42]. In addition, in some studies, the increase in serum IGF-I related to the chosen surgical procedure [43], the pre-operative presence of T2D [44], or the presence of post-surgical hypoglycemia, with elevated serum IGF-I before and after surgery in patients prone to develop

Table 4
Univariable and multivariable regression analyses of the association between IGF proteins and various parameters of glucose metabolism.
The delta change (Δ) in each variable from before to 12 months after RYGB served as an estimate of the true effect of surgery, and thus, was used as dependent and independent variables. The multivariable regression analyses were adjusted for age and sex at baseline as well as delta BMI from baseline to 12 months. Values are given as regression analysis coefficient and 95% CI.
HbA1c, glycosylated hemoglobin A1c; IGF, insulin-like growth factor; IGFBP-4, insulin-like growth factor binding protein-4, RYGB, Roux-en-Y gastric bypass; STC2, stanniocalcin-2.

Coefficient (95% CI) and *p* value	Δ Fasting glucose (mmol/L)		Δ Fasting insulin (pmol/L)		Δ HbA1c (mmol/mol)	
	Univariable	Multivariable	Univariable	Multivariable	Univariable	Multivariable
Δ IGF-I (μg/L)	−14 (−24;−4.1) $p = 0.009$	−15 (−26;−3.1) $p = 0.017$	−0.34 (−1.05:0.36) $p = 0.273$	−0.90 (−1.93:0.14) $p = 0.084$	−29 (−44;14) $p = 0.001$	−29 (−46;−12) $p = 0.003$
Δ IGF bioactivity (μg/L)	−0.18 (−0.30;−0.05) $p = 0.009$	−0.18 (−0.33;−0.04) $p = 0.015$	−0.006 (−0.015;0.002) $p = 0.132$	−0.012 (−0.025;0.000) $p = 0.063$	−0.26 (−0.49;−0.25) $p = 0.032$	−0.27 (−0.52;−0.03) $p = 0.038$
Δ IGFBP-4 (μg/L)	34 (5.3;63) $p = 0.023$	38 (8.4;67) $p = 0.016$	1.90 (0.12;3.68) $p = 0.038$	2.49 (0.042;4.95) $p = 0.047$	69 (25;113) $p = 0.005$	67 (19;115) $p = 0.010$
Δ STC2 (μg/L)	1.40 (0.39;2.41) $p = 0.010$	1.53 (0.49;2.58) $p = 0.007$	0.087 (0.001;0.172) $p = 0.047$	0.094 (0.003;0.186) $p = 0.045$	2.36 (0.67;4.06) $p = 0.009$	2.43 (0.60;4.26) $p = 0.013$

hypoglycemia [42]. Thus, numerous factors may influence the post-surgical course of serum IGF-I.

Even though neither IGF nor bioactive IGF changed significantly during the 12 months of study, intra-individual increases in IGF-I concentrations and IGF bioactivity associated with reductions in fasting glucose and HbA1c in both unadjusted and adjusted analyses. This fits with the concept that signaling through the IGF-IR promotes insulin sensitivity [4]. On the other hand, it is also clear that neither IGF-I nor bioactive IGF are clinically useful as markers of the improvement in glucose homeostasis that follows gastric surgery; a conclusion agreeing with previous findings [41,45].

Obesity is associated with increased serum IGF-II concentrations, which decline after diet-induced weight loss [46], and we now confirm that levels also decline after RYGB [39]. As adipose tissue secretes considerably more IGF-II than IGF-I in vitro, we have hypothesized that a significant fraction of circulating IGF-II originates from adipose tissue. This idea readily explains why serum IGF-II is elevated in obesity and declines following loss of fat mass [16]. Given that IGF-II is suspected to promote malignancy [47], and that obesity is associated with increased cancer incidence [48], we hypothesize that the reduction in serum IGF-II is beneficial, but this notion has to await further studies.

Our study has some limitations. To be elective for RYGB, all patients had to obtain an 8% weight loss prior to RYGB, and this may have affected our baseline levels. Furthermore, we included a limited number of subjects and clearly, this may have compromised our ability to identify differences between subjects with NGT and T2D. Lastly, because we did not include adipose tissue, we are unable to determine whether the observed changes in the circulating IGF-network (STC2 in particular) following RYGB are causally linked to changes in the gene expression in adipose tissue.

5. Conclusion

Subjects undergoing RYGB demonstrate reductions in serum STC2 and IGFBP-4, which both associate with variables reflecting an improved glucose metabolism. As STC2 inhibits the ability of PAPP-A to cleave IGFBP-4, the association between intact IGFBP-4 and an improved glucose metabolism is likely to be secondary to changes in STC2. If our assumption is correct, we may have identified a new metabolically responsive pathway linking glucose metabolism and the STC2 – PAPP-A – IGFBP-4 network, which is well-recognized for its ability to modify IGF-activity in vivo [9]. As STC2 is highly expressed in adipose tissue [17] and exerts anorexic effects in the hypothalamus [35], we speculate that the STC2 – PAPP-A – IGFBP-4 network is reflecting the metabolic and nutritional adaptation that occurs following RYGB, but this hypothesis requires further consolidation.

CRediT authorship contribution statement

Conception and design of the study (KBM, SM, JF), acquisition of data (RH, JF, CO, MS, JF), analysis and interpretation of data, (RH, JF, KBM), drafting the article (RH, SM, JF), revising it critically for important intellectual content (all authors), final approval of the version to be submitted (all authors).

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Declaration of competing interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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