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ORIGINAL CONTRIBUTIONS

# The IGF-Axis and Diabetic Retinopathy Before and After Gastric Bypass Surgery

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**Abstract**
*Background* Laparoscopic gastric bypass (LGB) abruptly causes remission of type 2 diabetes (T2D). Such dramatic metabolic changes have previously been found to cause worsening of diabetic retinopathy (DR) and circulating insulin-like growth factor I (IGF-I) has been suggested as a causal mediator. We aimed to evaluate baseline imbalances in the circulating IGF-system and changes after LGB in patients with T2D.
*Methods* Prospective ocular examinations and measurement of the IGF-axis before and 3 and 12 months after LGB. IGF-bioactivity was measured by cell-based IGF-I receptor (IGF-IR) kinase activation assay (bioactive IGF). Total IGF-I, IGF-II and IGF binding protein (IGFBP) 1 and 3 were determined by immunoassays.
*Results* At baseline, 18 of 36 patients presented with DR. These patients had higher levels of bioactive IGF ($p = 0.03$) than patients without DR and this association was strengthened in multivariate analysis ($p = 0.006$). Three patients had worsening of DR, unrelated to other markers. In univariate analysis, bioactive IGF increased at 3 months ($p = 0.05$) but this change became insignificant in multivariate analysis ($p = 0.11$). IGFBP-1 increased whereas IGFBP-3 and total IGF-II decreased at the two postoperative visits ($p \leq 0.001$). Total IGF-I showed no significant changes. HbA1c, glucose, HOMA-IR and lipids improved after surgery. Two patients did not complete the 12-month visit.
*Conclusions* In obese T2D patients, bioactive IGF is a potential biomarker for DR and levels tended to increase 3 months after bariatric surgery. IGFBP-1 increased while IGFBP-3 and total IGF-II decreased postoperatively, but these changes were unassociated with the development of DR. Markers of the metabolic syndrome improved.

**Keywords** Bariatric surgery · Gastric bypass surgery · Bioactive IGF · IGF-I · IGF-II · IGFBP-1 · IGFBP-3 · Diabetic retinopathy · Diabetes

## Introduction

Gastric bypass surgery acutely reverses hyperglycemia in patients with type 2 diabetes (T2D). This dramatic metabolic change has raised ophthalmologic concern for a detrimental development of diabetic retinopathy. Previous studies have established that an abrupt lowering of $HbA_{1c}$ can lead to a paradoxical worsening of diabetic retinopathy [1, 2]. The basis for these ocular changes has never been elucidated. One hypothesis proposes that lowering of blood glucose leads to a surge in circulating insulin-like growth factor I (IGF-I) that crosses the blood-retina barrier and in patients with pre-existing diabetic retinopathy stimulates the local release of vascular endothelial growth factor (VEGF) [3]. VEGF stimulates leakage and proliferation and is established as a pivotal cytokine in the development of both proliferative diabetic retinopathy and diabetic macular edema [4]. Supportive of the IGF-I

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hypothesis, vitreous levels of IGF-I are consistently elevated in patients with diabetic retinopathy [1, 2]. Furthermore, systemic IGF-I and IGF binding protein-3 (IGFBP-3) have been associated with diabetic retinopathy albeit with some discrepancy in the results [5]. Approximately 99 % of the circulating IGF-I pool is bound to the IGFBPs. Total IGF-I can be measured by removal or neutralization of the IGFBPs, and this methodology constitutes the traditional way of measuring total IGF-I in clinical samples. However, such measurement of total IGF-I ignores the ability of the IGFBPs and IGFBP-proteases to modify the in vivo action of IGF-I [6]. IGFBP-3 is the dominant IGFBP, binding as much as 75 % of serum IGF-I [7], and therefore, the molar ratio between total IGF-I and IGFBP-3 has been suggested to serve as a surrogate for free IGF-I. An alternative approach is to measure bioactive IGF using an IGF-I kinase receptor activation (KIRA) assay that measures the ability of serum to phosphorylate (activate) the IGF-I receptor (IGF-IR) in transfected cell cultures [6].

A high degree of remission of diabetes after bariatric surgery is well established [8] and includes improvements in insulin sensitivity as determined by the homeostatic model assessment for insulin resistance (HOMA-IR) [9]. However, postoperative changes in the IGF-system, which may also affect insulin sensitivity [7], are poorly characterized after gastric bypass surgery.

We have previously reported that diabetic retinopathy is clinically stable after gastric bypass surgery [10]. In this study, we aimed to compare baseline characteristics and circulating biomarkers in obese patients with T2D, with and without diabetic retinopathy, before and after gastric bypass surgery. Our outcome variables were bioactive IGF, total IGF-I and -II as well as IGFBP-3 and the molar IGF-I to IGFBP-3 ratio which has been suggested to reflect free IGF-I [11]. We also included IGFBP-1, which reflects the hepatic insulin sensitivity [12]. The major task was to investigate whether the outcome variables were associated with changes in diabetic retinopathy postoperatively.

## Methods

### Patients

The recruitment of patients has recently been described [10]. Briefly, all patients were referred for examination after having achieved a preoperative weight loss of 8 % of their BMI. Examinations and blood samples were repeated 3 and 12 months postoperatively. We included 46 patients with a history of or newly diagnosed T2D and excluded patients who were either converted to gastric sleeve gastrectomy ($n$ = 3), were suspected of improper fasting ($n$ = 1) or were lost to follow-up after the baseline visit ($n$ = 6). Two patients did not complete the 12-month visit, but were included in the three-month analysis.

### Blood Samples and Assays

After an overnight fast, patients had blood samples drawn from an antecubital vein. $HbA_{1c}$, glucose, LDL, HDL, and triglycerides were measured in freshly collected blood samples at the hospital laboratory. The remaining blood was centrifuged and transferred to microtubes and stored at −80 °C in a surveilled freezer until being analyzed in duplicates en bloc. Bioactive IGF was determined by an in-house IGF-I KIRA assay based on human embryonic renal cells (EBNA 293) transfected with the human IGF-IR gene [6], with modification as previously described [13]. Total IGF-I [14] and IGFBP-3 [15] were assayed using chemilumiscence in an IDS-iSYS multidiscipline automated analyzer (Immunodiagnostic Systems Ltd., Tyne & Wear, England). Serum insulin was measured by time-resolved immunofluorometric assay (TR-IFMA; AutoDELFIA insulin; PerkinElmer Life Sciences, Waltham, USA). Serum IGF-II was determined by an in-house assay as described previously [16]. IGFBP-1 was determined by an in-house TR-IFMA as previously described [6] with a minor modification, as the detection antibody has been changed to a new polyclonal antibody (catalog no MAB 675, Bio-Techne, Abingdon, UK). HOMA-IR was calculated using the HOMA2 calculator for Microsoft Excel [17]. The IGF-I to IGFBP-3 molar ratio was calculated using the molecular weights of IGF-I and IGFBP-3 of 7.65 and 30.5 kDa, [15].

### Other Examinations

Blood pressure was measured two times after 10 min rest (Omron M5–1, Omron Healthcare Co. Ltd., Kyoto, Japan). Body weight was measured with clothes but without shoes (ADE M301020, ADE Gmbh, Hamburg, Germany). Seven-field fundus ETDRS standard images were taken (Visucam Pro NM, Carl Zeiss Meditec, Jena, Germany) after pharmacologic dilation of the pupil.

### Grading of Diabetic Retinopathy

Images were graded according to the Wisconsin Epidemiologic Study of Diabetic Retinopathy scale [18] by two independent graders masked to patient identity, contralateral eye status, and visit number. All grades of 21/10 (at least one microaneurysm in one eye) or above were interpreted as the presence of diabetic retinopathy. The scale has 15 steps and an increase of at least two steps was considered as clinically meaningful worsening [19].

### Statistical Analysis

We compared preoperative data using student's unpaired $t$ test or Fisher's exact test. All nonparametric data achieved parametric distribution after logarithm transformation and $p$ values reflect comparison of logarithm values. Postoperative changes

were parametric and analyzed using paired *t* tests. We analyzed associations between parameters with multiple linear regression and stepwise backward elimination. SAS 9.4 (SAS institute, Cary, NC, USA) was used for analysis.

## Results

### Baseline Associations

We present baseline data of patients with and without diabetic retinopathy in Table 1. Patients with diabetic retinopathy had 24 % higher plasma levels of bioactive IGF (95 % CI 15–33 %, $p = 0.03$). We adjusted changes in each biomarker for age, sex, duration of diabetes and change in BMI, $HbA_{1c}$, and diabetic retinopathy status in multiple linear regression analysis. Using this approach, baseline levels of bioactive IGF were 61 % higher in women than in men (95 % CI 43–82 %, $p < 0.001$) and IGF-II was 23 % higher in women than in men (95 % CI 14–34 %, $p < 0.001$). Adjustment only for sex strengthened the association between bioactive IGF and diabetic retinopathy ($p = 0.006$) as compared to the univariate analysis. Total IGF-I levels were associated with longer duration of diabetes in the multivariate analysis ($p = 0.001$).

### Postoperative Changes

As we identified a sex difference in bioactive IGF and total IGF-II levels, we plotted changes in bioactive IGF and total IGF-II for each sex separately. However, there was no indication of sex-specific differences in the response (Figs. 1 and 2), and consequently, changes in men and women were analyzed together.

Only three patients had worsening of diabetic retinopathy postoperatively. In multiple regression analysis we performed regression of the change in each outcome variable, including worsening of diabetic retinopathy. However, this analysis did not reveal any significant associations.

We have depicted the postoperative changes in each biomarker in Fig. 3a, b, c, d, e, f, g, h, i, k, l, including an indication of the changes for the patients who experienced worsening of diabetic retinopathy. Bioactive IGF increased 0.13 μg/l (95 % CI 0.00–0.26, $p = 0.05$) at 3 months, but returned to preoperative levels 12 months postoperatively. IGFBP-3 decreased by a mean of 401 ng/ml (95 % CI 209–595, $p < 0.001$) 3 months

**Table 1** Preoperative characteristics stratified by diabetic retinopathy status, mean ± SE, median (IQR), or n (%)

| | No diabetic retinopathy | Diabetic retinopathy | *p* |
|---|---|---|---|
| N | 18 | 18 | |
| Age (years) | 48.0 ± 1.8 | 47.7 ± 2.1 | 0.90 |
| Male sex | 9 (50) | 8 (44) | 1.00 |
| BMI ($kg/m^2$) | 40.6 ± 1.3 | 38.0 ± 1.9 | 0.13 |
| Duration of diabetes (years) | 4 (2–9) | 7 (2–13) | 0.55 |
| Systolic blood pressure (mmHg) | 125 ± 3 | 127 ± 3 | 0.64 |
| Diastolic blood pressure (mmHg) | 85 ± 2 | 89 ± 2 | 0.20 |
| Current smoker | 8 (47) | 5 (29) | 0.48 |
| Glucose metabolism | | | |
| HbA1c (%) | 6.3 ± 0.2 | 6.4 ± 0.2 | 0.82 |
| Glucose (mmol/l) | 6.4 ± 0.2 | 6.8 ± 0.5 | 0.49 |
| HOMA-IR | 1.7 (1.2–2.3) | 2.1 (1.4–2.7) | 0.26 |
| Lipids | | | |
| HDL (mmol/l) | 1.0 ± 0.1 | 1.0 ± 0.0 | 0.37 |
| LDL (mmol/l) | 2.1 ± 0.2 | 2.5 ± 0.3 | 0.23 |
| Triglycerides (mmol/l) | 1.6 ± 0.1 | 1.7 ± 0.3 | 0.79 |
| IGF system | | | |
| Bioactive IGF (ng/ml) | 0.84 (0.68–0.96) | 1.27 (0.87–1.40) | 0.03 |
| Total IGF-I (ng/ml) | 131 (110–140) | 137 (114–179) | 0.24 |
| Total IGF-II (ng/ml) | 531 (434–645) | 566 (513–656) | 0.87 |
| IGFBP-1 (ng/ml) | 8.9 (5.4–17.6) | 6.4 (3.4–10.2) | 0.17 |
| IGFBP-3 (ng/ml) | 3643 (3153–4912) | 4308 (3189–4785) | 0.86 |
| Total IGF-I/IGFBP-3 molar ratio | 0.14 (0.11–0.16) | 0.15 (0.11–0.20) | 0.30 |

*P* values are either unpaired student's *t* test or Fisher's exact test. For the *t* test, parametric distributions for data presented as median (IQR) were achieved using logarithm transformation

*IGF-I* insulin-like growth factor I, *IGFBP* IGF binding protein, *HOMA-IR* homeostasis model assessment of insulin resistance

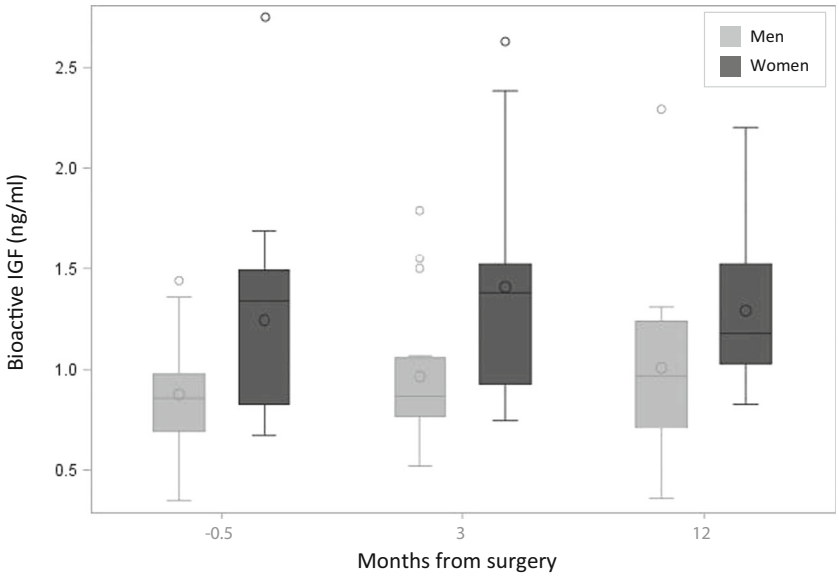


**Fig. 1** Postoperative development in bioactive IGF in men and women

postoperatively and by 613 ng/ml (95 % CI 414–811, $p < 0.001$) 12 months postoperatively. There were no significant changes in total IGF-I, but total IGF-II decreased 32 ng/ml (95 % CI 14–50, $p = 0.001$) at 3 months and 49 ng/ml (95 % CI 25–72, $p < 0.001$) at 12 months. Using a multivariate analysis, adjusting for the same parameters as in the baseline analysis, bioactive IGF was no longer significantly elevated at 3 months ($p = 0.11$). In contrast, IGFBP-3 remained decreased at 3 ($p = 0.05$) and 12 months ($p < 0.001$), and because total IGF-I remained unchanged, the ratio between IGF-I and IGFBP-3 tended to increase after 3 months ($p = 0.10$) and increased at 12 months ($p = 0.01$).

Glucose and lipid metabolism improved significantly for all parameters, though for LDL and glucose the changes were not significant before 12 months.

## Discussion

The aim of this study was to evaluate baseline imbalances in the circulating IGF-system in obese patients with T2D with and without diabetic retinopathy and to establish associations between the IGF-system and diabetic retinopathy after gastric bypass surgery. Only three out of 36 patients had worsening of diabetic retinopathy levels. While there was a significant increase in IGFBP-1 and a significant decrease in IGFBP-3 that continued up to 12 months, the ability of serum IGF to activate the IGF-IR in vitro was only slightly increased at 3 months and unchanged at 12 months.

Prior to surgery, bioactive IGF was significantly elevated in patients with diabetic retinopathy and the difference was

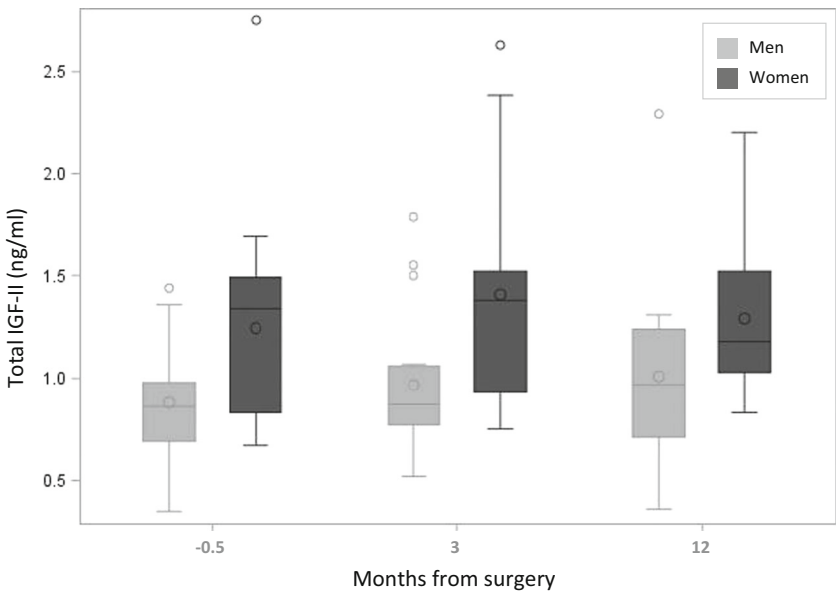


**Fig. 2** Postoperative development in IGF-II in men and women

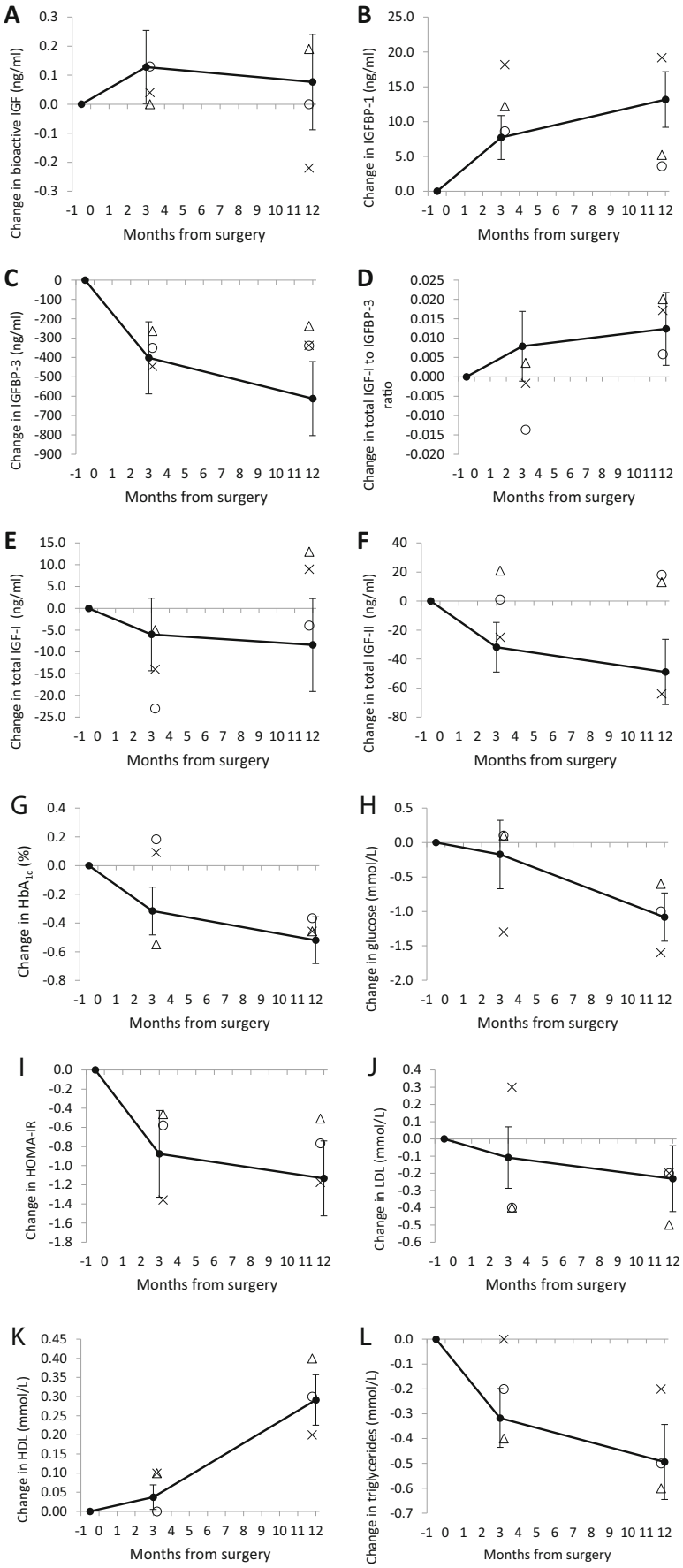


**Fig. 3** Postoperative changes in systemic biomarkers after gastric bypass surgery. *Error bars* = 95 % confidence limits for the mean change. XO△ represent the values for the three patients who experienced worsening of diabetic retinopathy postoperatively

strengthened following a multivariate analysis. Previous cross-sectional studies of circulating IGF-I in relation to diabetic retinopathy have been contradictory, with studies reporting elevated [20, 21], lowered [22] and similar [23–25] levels of circulating IGF-I when comparing patients with and without diabetic retinopathy. Some of this discrepancy could be related to the measurement of IGF-I as well as the regulatory action of IGFBPs. The KIRA assay employed in the present study determines the ability of serum to activate the IGF-IR in vitro and integrates the whole IGF system into one signal: IGF-IR activation [6]. To acknowledge that both IGF-I and IGF-II are able to activate the IGF-IR we have nominated the output of the assay bioactive IGF. The higher preoperative levels of IGF bioactivity are in accordance with the hypothesis that IGF-IR activation, as measured by the KIRA method, is associated with the pathogenesis of diabetic retinopathy. However, this finding should be confirmed in future studies.

A higher circulating IGF bioactivity in women than in men has previously been described [26] and may be attributed to the modulating effects of estrogen [27]. Our results are in agreement with this; however, another study did not find this association [28]. Total IGF-II levels were also higher in women and may have contributed to the difference, as IGF-II is able to activate the IGF-IR. Despite these sex differences, women and men appeared to have similar postoperative changes in both bioactive IGF and total IGF-II.

Several studies suggest that circulating GH and IGF-I participate in worsening of diabetic retinopathy [5]. Still, a causative role has never been unanimously established and intervention trials aiming to inhibit GH secretion, GH receptor activation or IGF-I have shown variable results [29–31]. We did not find a correlation between worsening of diabetic retinopathy and changes in any of the biomarkers tested, but we had only three patients with worsening. This clearly limits the power of our study.

The increase in bioactive IGF 3 months postoperatively was just significant at the 5 % level ($p = 0.05$), and it failed to reach significance in the multivariate analysis ($p = 0.11$). Therefore, the biological importance of this finding is likely to be weak. This contrasts to IGFBP-1 and IGFBP-3 that increased and decreased, respectively, with high statistical significance at both 3 and 12 months.

IGFBP-1 appears to be negatively associated with impaired glucose tolerance [32, 33], diabetes [34], and obesity [35, 36], though not unanimously [37]. Conversely, IGFBP-3 is positively associated with decreased hepatic insulin sensitivity and diabetes development [33, 37]. On this basis, the response in IGFBP-1 and IGFBP-3 after gastric bypass surgery demonstrated in the present study follows the well-known postoperative improvements in the metabolic syndrome. Pellitero et al. reported a decrease in IGFBP-3 and an increase in the total IGF-I to IGFBP-3 molar ratio in a study of both gastric bypass and gastric sleeve surgery in patients without diabetes [38]. In a cohort of women without diabetes, Giusti et al. reported a decrease in IGFBP-3 6 months after gastric bypass surgery that leveled off at 12–24 months, whereas total IGF-I remained stable [39]. These results for total IGF-I and IGFBP-3 in patients without diabetes are in agreement with our results for patients with T2D.

Interestingly, both IGFBP-1 and IGFBP-3 are known to have several IGF-IR independent actions [40, 41]. The dissociation between changes in bioactive IGF and IGFBP-1 and -3 suggests an IGF-IR independent association. However, our results may have been confounded by changes in BMI and $HbA_{1c}$. Unfortunately, our study design did not allow us to resolve this further, but we hypothesize that the IGFBP-1 to IGFBP-3 ratio is a sensitive marker for longitudinal changes in the metabolic profile that should be evaluated in future studies.

Studies of total IGF-I after gastric bypass surgery have reported a decrease after 3 weeks to 1 month [42, 43] and an increase at 1 year [43–45], whereas one study failed to observe any changes in IGF-I levels postoperatively [46]. In our study there were no significant changes in total IGF-I at any of the postoperative visits. In contrast, $HbA_{1c}$, fasting glucose and HOMA-IR, LDL and triglycerides all declined and HDL improved postoperatively as expected after the surgery.

### Limitations

Several important limitations of this study should be noted. Firstly, we lacked a proper control group, and our results are therefore limited to associations. Secondly, although we have measured many members of the IGF-system, we have not taken all binding proteins, the acid labile subunit or IGFBP-proteases into consideration in the present study. The application of the KIRA assay for the whole IGF system addresses some of this discrepancy, but for IGF-IR independent mechanisms we do not present a full overview of the changes in the IGF-system. Thirdly, from a statistical standpoint our study was modestly sized, which increases the risk of spurious correlations and type I errors. Fifthly, our patients had achieved a weight loss of 8 % preoperatively. We hypothesize that this led to smaller postoperative changes in systemic markers than in a setting without a preoperative weight loss. Finally, we limited our analysis to 3 and 12 months postoperatively and may have missed signals at other points in time. All 3-month signals were attenuated at 12 months with the exception of bioactive IGF, and a peak outside of 3 months could therefore be hypothesized for this particular marker.

## Conclusions

In obese patients with T2D, bioactive IGF is a potential biomarker for diabetic retinopathy and levels increased slightly

3 months after bariatric surgery. IGFBP-1 increased and IGFBP-3 decreased postoperatively. Only three patients had postoperative worsening of diabetic retinopathy, unrelated to changes in markers of the IGF-system. All markers of the metabolic syndrome improved.

**Compliance with Ethical Standard**

**Conflicts of Interest** Troels Brynskov was supported by unrestricted grants from the following nonprofit organizations: Fight for Sight Denmark, the Bagenkop Nielsen Myopia Foundation, and the Research Foundation of Region Zealand. The other co-authors declare no conflicts of interest.

**Ethical Approval** All procedures were in accordance with the regional ethical research committee (approval #SJ-205) and with the 1964 Helsinki declaration and its later amendments.

**Statement of Informed Consent** Informed consent was obtained from all individual participants included in the study.

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