International Journal of Obesity (2021) 45:374–382
https://doi.org/10.1038/s41366-020-00687-0

ARTICLE

Bariatric Surgery

# Modifications of IGF2 and EGFR plasma protein concentrations in NAFLD patients after bariatric surgery

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Received: 21 May 2020 / Revised: 20 August 2020 / Accepted: 9 September 2020 / Published online: 17 September 2020


## Abstract

**Background** Nonalcoholic fatty liver disease (NAFLD) is strictly associated with the epidemic of obesity and is becoming the most prevalent liver disease worldwide. In severe obesity, bariatric surgery (BS) is the most effective treatment not only for obesity but also for the associated metabolic co-morbidities, NAFLD, among others. To date, noninvasive diagnostic/prognostic methods cannot evaluate hepatic improvements following surgery.

**Objectives** We aimed to measure plasma level of insulin-growth factor-2 protein (IGF2) and epithermal growth factor receptor (EGFR), and to assess their relationship with clinical and biochemical parameters during the 12 months follow-up.

**Methods** Demographic, clinical–biochemical data, and plasma IGF2 and EGFR were measured in 69 patients pre-operatively (T0) and 6 and 12 months (T6M and T12M, respectively) after BS. Liver biopsy was performed at T0. Relationships between IGF2, EGFR, and several biochemical parameters were performed using Pearson or Spearman correlation analysis.

**Results** IGF2 plasma level increases during follow-up, passing from 2.5 (1.8–15.5) at baseline to 13.3 (8.6–19.1) at T12M, $p < 0.001$. Conversely, EGFR showed a not significant reduction. At T12M, the plasma level of both markers was comparable to those of lean subjects. The clinical–biochemical parameters (BMI, glycated hemoglobin, HOMA-IR) also return to the normal range at T12M. Correlation analysis demonstrated that IGF2 was significantly associated with total bilirubin, direct bilirubin, and albumin at T0 while with blood glucose, ALT, GGT, and AST/ALT ratio at T6M and T12M.

**Conclusions** IGF2 plasma levels increase after bariatric surgery, and these changes are associated with the modification of hepatic biochemical parameters, even if other clinic or metabolic improvements cannot be excluded.

## Introduction

Nonalcoholic fatty liver disease (NAFLD) is becoming the most prevalent chronic liver disease worldwide [1]. The most recent reports estimate the world prevalence of NAFLD in children and adults in around 10% and 25%, respectively [2–4]. Nonalcoholic steatohepatitis (NASH) is a degenerative form of NAFLD that leads to damage and inflammation over time, with different stages of fibrosis. The fibrosis stage is the most significant predictor of mortality in NAFLD [5–7]. If left untreated or undiagnosed, it can lead to liver cirrhosis and eventually progress to liver failure. Unfortunately, despite the increase in awareness of this disease, there are still nonreliable noninvasive diagnostic tests [8].

Liver biopsy remains the gold standard for the diagnosis of NAFLD and the associated fibrosis stage [9]. However, it

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**Supplementary information** The online version of this article (https://doi.org/10.1038/s41366-020-00687-0) contains supplementary material, which is available to authorized users.

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is an invasive procedure that requires hospitalization, and although rare, complications may occur. Accordingly, the use of liver biopsy as a screening or diagnostic tool in a highly prevalent disease such as NAFLD is unfeasible.

Several reports evidence the global epidemic of obesity as the core factor behind the continually increasing of NAFLD prevalence [10, 11]. The World Health Organization estimated that over 1900 million adults over 18 years old are overweight, among which 650 million suffer from obesity [12]. In patients with obesity, the incidence of NAFLD was reported to be around 63–99% [13, 14]. Indeed, the major consistent information on NAFLD prevalence has been collected from studies on morbidly obese (MO) patients, where liver biopsy is performed during bariatric surgery (BS) [14]. BS is the most effective treatment not only for obesity but also for the associated metabolic co-morbidities [15]. Moreover, some studies have compared the long-term benefits of different abdominal surgery types (Roux-en-Y gastric bypass, adjustable gastric banding and vertical sleeve gastrectomy (Sleeve)) on liver function in patients with NAFLD. Contrasting evidence was obtained regarding the most effective type of intervention, and further randomized studies are necessary to achieve consistent results [16–18].

Insulin-like growth factors (IGFs) are pivotal regulators of growth, metabolism, and lipogenesis. Insulin-growth factor-2 (IGF2) is the predominant circulating IGF, known predominantly as a fetal and placental growth factor and more recently shown to be a key factor in the development of steatosis that accompanies NAFLD/NASH [19, 20]. The epithermal growth factor receptor (EGFR) signaling axis has received much attention because of the high density of EGFR protein in the hepatocyte membrane. Several studies proposed a key role of EGFR in liver regeneration, initiation of hepatocyte injury via lipotoxicity mechanisms, cirrhosis, and HCC, highlighting its role in the development of liver damage [21, 22]. Less known is the role of circulating EGFRs in liver disease, and only one study has described its diagnostic use in HCC [23].

As mentioned previously, there are not noninvasive diagnostic/prognostic tools to evaluate accurately the stage of NAFLD as well as the benefits of the current therapeutic approaches. Previously, we have presented data supporting that plasma levels of IGF2 protein, alone, or in combination with EGFR plasma levels are useful for noninvasive diagnosis of liver fibrosis in MO patients [24]. In the present retrospective study, we evaluated the plasma changes of those candidates at 6 and 12 months after BS, and with the overall metabolic improvements. Moreover, we analyze the eventual effects of the different bariatric surgeries on clinical biochemistry and candidates' plasma levels.

## Materials and methods

### Patient cohort and data analysis

Sixty-nine MO patients included in a BS program were consecutively enrolled (between March 2014 and October 2016) by a multidisciplinary team (bariatric surgeons, dieticians, hepatologists, psychologists, and psychiatrists). International guidelines (well-informed and motivated patients with acceptable operative risks, failure of non-surgical treatments, declared compliance to follow lifelong medical surveillance, BMI of 40 kg/m$^2$ or higher or between 35 and 40 kg/m$^2$ with obesity-related co-morbidities) were used to determine surgical eligibility. Liver biopsy was performed at all patients at the time of the surgical procedure (T0—Baseline). The exclusion criteria were documented liver disease, patients consuming alcohol more than 25 g/day, active duodenal/gastric ulcer disease, large hiatal hernia, previous major gastrointestinal surgery, patients infected with the HBV, HCV, and HIV. MO subjects gave their written informed consent before participating in this study, which was approved under protocol N. 22979 by the local ethical committee (Ente per la gestione accentrata dei servizi condivisi, Comitato Etico Regionale Unico, Regione Autonoma Friuli-Venezia-Giulia, Servizio Sanitario Nazionale). The same surgeons performed all surgical procedures.

Clinical and biochemical features were assessed first preoperatively (T0—baseline) and then at 6 and 12 months after surgery. Anthropometric parameters: height, body weight, sex, BMI (kg/m$^2$), alcohol intake, and smoking were recorded at T0 and follow-up visits (T6 and T12). Weight loss was expressed as the percentage of initial weight or initial excess weight, by considering a BMI of 25 kg/m$^2$ as a reference. Blood samples were collected after overnight fasting. Laboratory evaluation included routine liver metabolic markers (aspartate aminotransferase [AST], alanine aminotransferase [ALT], γ-glutamyltransferase [GGT], total bilirubin, albumin and the lipid profile (cholesterol, TAG, HDL, LDL)). Plasma glucose, glycated hemoglobin, and serum insulin were determined using enzymatic, immune- and immunoradiometric assays, respectively. The homeostatic model assessment for insulin resistance (HOMA-IR) was calculated as [(fasting insulin, mU/L) × (fasting glucose, mM)]/22.5 [25]. Diabetes was diagnosed according to the ESC-EASD guidelines [26].

Plasma levels of IGF2 and EGFR were determined by ELISA commercial kits and the ratio EGFR/IGF2 calculated as described previously [24]. Plasma samples from informed consenting healthy lean subjects were included in the study, as controls.

### Surgical technique and liver biopsy

Intraoperative wedge biopsies (5 × 5 mm) were taken from the free margin of the left lobe of the liver before starting with the bariatric operations. The procedures were conducted laparoscopically in all subjects and the surgical operations performed were gastric bypass and sleeve gastrectomy. Briefly, after positioning and ports were placed, gastric bypass tecnique involved the creation of a small gastric pouch completely separated from the bypassed stomach and the connection of the newly pouch directly to the bowel (biliary limb was measured 100 cm distal to the ligament of Treitz and the alimentary limb was measured up to 120 cm). In the sleeve gastrectomy, a vertical resection of the stomach was performed, beginning at 2 cm proximal to the pylorus and extending to the Angle of His, close to a 34 French orogastric tube inserted by the anesthesiologist. It is a restrictive procedure involving resection of two-thirds of the stomach. The success of BS was set to excess weight loss >50% and good glycemic control of T2DM with HbA1c lower than 6% [27].

### Liver histopathology

Liver biopsies were analyzed by a pathologist blinded to clinical data. Steatosis was graded according to the amount of fat present in the hepatocytes in a biopsy specimen on hematoxylin and eosin stain. Grading 0: normal (1–5%); 1: mild (6–33%); 2: moderate (34–66%); and 3: severe (67–100%). Biopsies showing no or minimal (<5%) steatosis and absent injury or fibrosis were considered as normal. The samples that showed more than 5% steatosis were labeled as NAFLD. The histological diagnosis of NASH was made following the NAFLD Activity Score [28, 29]. Ballooning degeneration was graded as none, few, and many and scored as 0, 1, and 2, respectively. Lobular inflammation was graded subjectively as 0: none; 1: mild; and 2: moderate. Fibrosis was assessed by Masson's trichrome staining and staged according to Kleiner criteria, from 0 to 4, with stage 0 = no fibrosis, 1 = perisinusoidal or periportal fibrosis, 2 = perisinusoidal and portal/periportal fibrosis, 3 = bridging fibrosis, and 4 = cirrhosis [28].

### Statistical analysis

Categorical variables are presented as counts or percentages, and continuous variables are expressed as means ± standard deviations. All parameters were analyzed in a paired manner. Comparison between groups of categorical variables was performed with the Chi-squared test. Differences for continuous variables were analyzed using *t*-test (when normally distributed according to D'Agostino and Pearson omnibus normality test) and by Mann–Whitney *U* test (when non-normally distributed). Multivariate repeated measurement ANOVA was used to assess changes in continuous variables over time. The relationships between IGF2, EGFR/IGF2, and other continuous variables were studied by correlation analysis using Pearson or Spearman's correlation coefficients. Statistical analysis was performed using GraphPad Prism 5.01.

## Results

### Clinical characteristics and liver histology

We studied a sample of 69 subjects enrolled between 2014 and 2016 in a bariatric program. As reported in Table 1 at baseline (T0) several clinic–biochemical parameters of the MO cohort were within the normal range except BMI (44.6 ± 6.4 kg/m$^2$), glycated hemoglobin (6.2 ± 0.9%), and HOMA-IR (5.4 ± 4.1); 16% of the subjects had type 2 diabetes. Histologically the prevalence of NASH was 67%, but only 25% presented moderately abnormal liver enzymes at baseline.

### Improvement in clinical/biochemical and metabolic parameters

At baseline, the BMI was 44.6 ± 6.4 kg/m$^2$ in the overall cohort. All clinical and metabolic parameters including BMI, body fat composition, diabetes, fasting blood glucose, HOMA-IR, HbA1c, HDL cholesterol, triglycerides, and cholesterol significantly improved at each time point of the follow-up (Table 1). For example, the mean percentage of total weight loss after 6 and 12 months was 25.8 ± 6.6% and 31.9 ± 9.4%, respectively. Liver enzymes, conjugated bilirubin, and transferrin also significantly improved 6 and 12 months after surgery (Table 1, hepatic biological feature, time effect). From a histological point of view, 90% of the patients presented NAFLD, and 62% of them were diagnostic with NASH. Regarding fibrosis, 25% of patients shown moderate/advance (F2–F3) and 65% minimal (F1) fibrosis stage.

### Plasma levels of IGF2, EGFR, and their ratio during follow-up

The circulating levels of IGF2 significantly increased during the follow-up (Fig. 1a). The median IGF2 level passed from 2.5 (interquartile range 1.8–15.5) to 7.8 (0.9–11.5) and 13.3 (8.6–19.1) at T6M and T12M, respectively ($p < 0.001$). Interestingly, IGF2 levels 6 months after surgery reached a concentration comparable to lean subjects (8.8 (6.8–12.5), $p < 0.05$). Conversely, as in Fig. 1b, the EGFR plasma level showed a slight but not significant reduction moving from

**Table 1** Demographic, clinical, and biochemical parameters before surgery (T0—baseline) and at the follow-up visits (T6M—6 months and T12M —12 months) after bariatric surgery.

| | Baseline (T0M) | | T6M | T12M | *p* | | |
|---|---|---|---|---|---|---|---|
| Clinical assessment | $n = 69$ | | $n = 69$ | $n = 69$ | T0M vs. T6M | T0M vs. T12M | T6M vs. T12M |
| Age, year | 44.1 ± 10.6 | | 44.1 ± 10.6 | 44.1 ± 10.6 | | | |
| Female, *n* (%) | 49 (74%) | | 49 (74%) | 49 (74%) | | | |
| BMI, kg/m$^2$ | 44.6 ± 6.4 | | 33.0 ± 5.6 | 30.0 ± 5.1 | * | * | ** |
| Weight loss, % | NA | | 25.8 ± 6.6 | 31.9 ± 9.4 | NA | NA | * |
| Excess weight loss, % | NA | | 62.3 ± 19.1 | 75.3 ± 24.2 | NA | NA | * |
| Body fat index, % | 67.3 ± 11.8 | | 46.5 ± 12.7 | 40.6 ± 13.1 | * | * | * |
| Metabolic assessment | | | | | | | |
| T2DM, *n* (%) | 11 (16%) | | 0 | 0 | NA | NA | NA |
| Fasting blood glucose, mg/L | 110.3 ± 24.9 | | 88.5 ± 10.4 | 86.4 ± 10.5 | * | * | ns |
| HOMA-IR | 5.4 ± 4.1 | | 1.9 ± 1.1 | 1.7 ± 1.3 | * | * | ns |
| HbA1c (%) | 6.2 ± 0.9 | | 5.6 ± 0.5 | 5.5 ± 0.4 | * | * | ns |
| HDL cholesterol, mg/dL | 48.1 ± 11 | | 53.3 ± 19.9 | 57.9 ± 14.7 | ns | * | ** |
| Triglycerides, mg/dL | 140.6 ± 85.4 | | 99.3 ± 40.7 | 86.5 ± 30.4 | * | * | ns |
| Cholesterol total, mg/dL | 203.5 ± 39.7 | | 185.3 ± 41.9 | 185.9 ± 47.7 | *** | ns | ns |
| Hepatic biological feature | | | | | | | |
| ALT, IU/L | 32.7 ± 28.8 | | 19.93 ± 10.7 | 20.52 ± 12.6 | * | * | ns |
| AST, IU/L | 24.2 ± 14.2 | | 18.8 ± 6.6 | 20.4 ± 8.9 | ** | ns | ns |
| GGT, IU/L | 35.3 ± 26 | | 21.6 ± 19.4 | 20.7 ± 20 | * | * | ns |
| Bilirubin total, mg/dL | 0.65 ± 0.33 | | 0.73 ± 0.34 | 0.73 ± 0.32 | ns | ns | ns |
| Direct bilirubin, mg/dL | 0.13 ± 0.11 | | 0.22 ± 0.10 | 0.20 ± 0.09 | * | * | ns |
| Albumin g/dL | 4.16 ± 0.31 | | 4.25 ± 0.29 | 4.26 ± 0.35 | ns | ns | ns |
| Transferrin mg/dL | 287 ± 48.5 | | 257 ± 45.0 | 270 ± 50.6 | * | ns | ns |
| Ferritin mg/dL | 83.2 ± 30.6 | | 79.4 ± 25.4 | 89.2. ± 30.3 | ns | ns | ns |
| Fe++ ug/dL | 69.0 ± 80.0 | | 80.8 ± 76.2 | 68.0 ± 69.4 | ns | ns | ns |
| Histological study | (*n*) | (%) | | | | | |
| Steatosis 0/1/2/3 | 7/26/19/17 | 10, 38, 27, 25 | | | | | |
| Lobular Inflammation 0/1/2 | 14/43/12 | 21, 62, 17 | | | | | |
| Balloning 0/1/2 | 13/22/34 | 19, 32, 42 | | | | | |
| Fibrosis 0/1/2/3/4 | 7/45/15/1/1 | 10, 65, 22, 2, 1 | | | | | |
| NO NAFL/NAFL/NASH | 5, 18, 46 | 7/26/67 | | | | | |

*$p < 0.001$; **$p < 0.01$; ***$p < 0.05$.

58.0 (48.5–67.2) at the baseline to 54.2 (45.1–65.0) at T12M. The EGFR/IGF2 ratio decreased from 14.9 (4.0–30.2) at baseline to 4.4 (3.4–6.3) at T12M ($p < 0.001$), reaching levels like those of lean controls (4.2 (2.7–6.0)).

## Associations between IGF2 and EGFR plasma level with clinical, metabolic, and hepatic parameters at T0, T6, and T12

The plasma level of IGF2 and its ratio with EGFR, correlation analysis at baseline, T6M, and T12M were correlated with the metabolic and hepatic function (Table 2). At baseline IGF2 was associated with total bilirubin ($\rho = -0.27$, $p = 0.012$), conjugated bilirubin ($\rho = -0.30$, $p = 0.004$), and albumin ($\rho = 0.25$, $p = 0.017$) and, as previously reported ($\rho = -0.26$, $p = 0.006$) with hepatic fibrosis and lobular inflammation [24, 30, 31]. Of notice, when the cohort was stratified according to fibrosis, the difference on IGF2 levels observed at baseline was lost at T6M and T12M after surgery, suggesting improvement of the histology (Fig. 2). At T6M, an association was observed between IGF2 and blood glucose levels ($\rho = -0.27$, $p = 0.019$) and at T12 with ALT ($\rho = -0.44$, $p = 0.002$), GGT ($\rho = -0.35$, $p = 0.05$), and with AST/ALT ratio ($\rho = 0.35$, $p = 0.004$). A comparable relationship was observed for EGFR/IGF2 ratio (see Table 2).

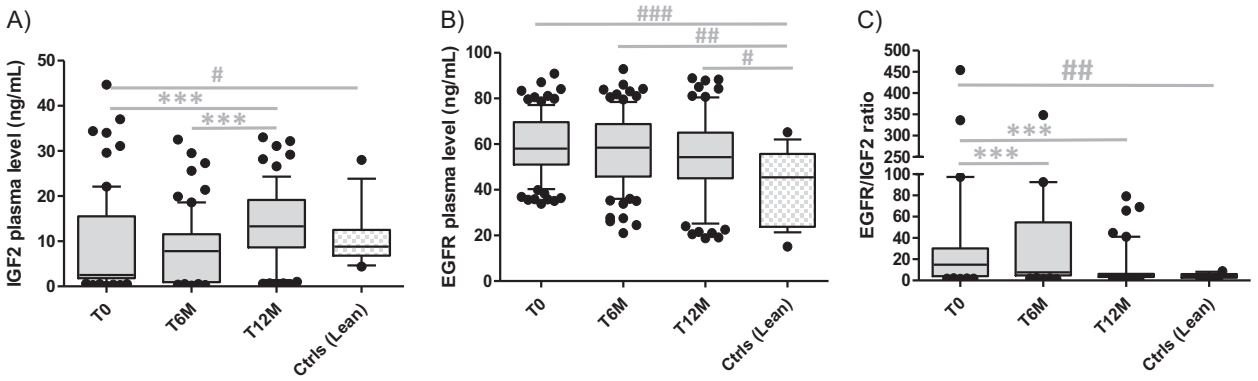


**Fig. 1 Plasma levels before surgery and during the follow-up.** Plasma concentrations were determined by ELISA commercial kits at baseline (T0) and during follow-up at 6 and 12 months after bariatric surgery. Plasma samples from lean subjects were used as controls (Ctrls, $n = X$). **a** IGF2, **b** EGFR, and **c** EGFR/IGF2 ratio. $^{***}p <$ 0.0001 between time-points in the follow-up. $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ vs. Ctrls.

**Table 2** Correlations between IGF2 plasma levels and the EGFR/IGF2 ratio with different clinic–biochemical parameters of interest at the baseline and during follow-up.

| | *IGF2* | | | | | | EGFR/IGF2 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| XY pairs ($n = 69$) | T0 | | T6M | | T12M | | T0 | | T6M | | T12M | |
| Variable | Rho | *p* | Rho | *p* | Rho | *p* | Rho | *p* | Rho | *p* | Rho | *p* |
| BMI | −0.11 | 0.25 | 0.015 | 0.9 | −0.086 | 0.45 | −0.05 | 0.64 | 0.066 | 0.56 | 0.02 | 0.87 |
| Fasting blood glucose | −0.19 | 0.07 | −0.27 | 0.019* | 0.11 | 0.34 | 0.16 | 0.16 | 0.2 | 0.09 | −0.11 | 0.4 |
| Triglycerides | 0.06 | 0.6 | −0.03 | 0.67 | −0.037 | 0.76 | 0.015 | 0.9 | −0.038 | 0.75 | −0.11 | 0.4 |
| HDL | 0.12 | 0.31 | 0.22 | 0.07 | 0.09 | 0.47 | −0.18 | 0.1 | −0.19 | 0.13 | 0.09 | 0.47 |
| AST | −0.14 | 0.25 | −0.1 | 0.36 | −0.21 | 0.1 | 0.07 | 0.53 | 0.085 | 0.47 | 0.21 | 0.09 |
| ALT | −0.15 | 0.21 | −0.036 | 0.76 | −0.44 | 0.002** | 0.027 | 0.81 | 0.046 | 0.7 | 0.38 | 0.002** |
| GGT | −0.14 | 0.25 | 0.17 | 0.15 | −0.35 | 0.05* | −0.048 | 0.67 | −0.14 | 0.24 | 0.35 | 0.005** |
| Total bilirubin | −0.27 | 0.012* | 0.054 | 0.64 | −0.13 | 0.25 | 0.38 | 0.005** | −0.06 | 0.62 | 0.2 | 0.12 |
| Direct bilirubin | −0.3 | 0.004** | 0.23 | 0.064 | 0.04 | 0.76 | 0.5 | 0.001** | −0.2 | 0.11 | 0.08 | 0.55 |
| Albumin | 0.25 | 0.017* | 0.12 | 0.33 | 0.07 | 0.6 | −0.36 | 0.001** | −0.07 | 0.56 | −0.04 | 0.76 |
| Transferrin | −0.04 | 0.75 | 0.09 | 0.44 | 0.19 | 0.13 | 0.034 | 0.77 | −0.07 | 0.56 | −0.11 | 0.4 |
| Fibrosis Kleiner | −0.26 | 0.006** | NA | NA | NA | NA | 0.21 | 0.05* | NA | NA | NA | NA |
| Lobular inflammation | −0.28 | 0.011* | NA | NA | NA | NA | 0.31 | 0.004** | NA | NA | NA | NA |
| AST/ALT | 0.014 | 0.9 | −0.052 | 0.66 | 0.35 | 0.004** | −0.028 | 0.79 | −0.27 | 0.04 | −0.25 | 0.046 |

*p* value corresponds to Ho: rho = 0 (the two variables do not vary together at all).
$^{*}p < 0.05$; $^{**}p < 0.01$.

## Effects of BS on plasma IGF2 and EGFR level

As shown in Fig. 3, plasma levels of IGF2 and EGFR were not affected by the type of intervention. For example, IGF2 presented a trend of increase from baseline to T12M, with values of 13.2 (3.8–18.4) and 13.8 (9.5–20.2) for RGYB and SG, respectively. Additional data regarding the comparison between RGYB and SG procedures demonstrate only significant differences for cholesterol (at T6M and T12M, $p < 0.001$) and transferrin levels (at T12M, $p < 0.05$), respectively (Table 1S, Supplementary Materials).

## Discussion

NAFLD is becoming the most common chronic liver disease in the developed world [1]. In 2015, the global obesity pandemic was estimated to affect a total of 107.7 million children and 603.7 million adults. Data from 195 countries established that the prevalence of obesity has been doubled from 1980 to 2015 in more than 70 countries [32], and obesity has shown to be the main contributing factor in the global NAFLD burden [11, 33]. The mathematical modeling of these epidemics estimates

about 1.12 billion and 100.9 million people affected by obesity [34] and NAFLD [35], respectively. Consequently, it is expected that NAFLD would represent the first cause of liver transplant by 2025 [36].

NAFLD is a progressive wide spectrum disease that involves several factors and requires interaction with different organs. Despite the intrinsic complexity of NAFLD pathogenesis, some promising therapeutic alternatives have been emerged recently to halt NASH progression to cirrhosis, and therefore preventing liver-related severe outcomes. However, to date, there are still no approved therapies to NAFLD, and the most effective medical approach is to persuade obese patients to adopt a healthful diet (low-fat, low-calorie) and to increase physical activity. BS represents an additional opportunity, been an effective long-term intervention in the treatment of obesity and related co-morbidities [37].

This retrospective study was designed to monitor the variation in plasma of two proteins (IGF2 and EGFR) during the outcome (at 6 and 12 months after bariatric intervention). These two markers were selected based on our previous data showing how their plasma concentration correlates with hepatic inflammation and fibrosis [24].

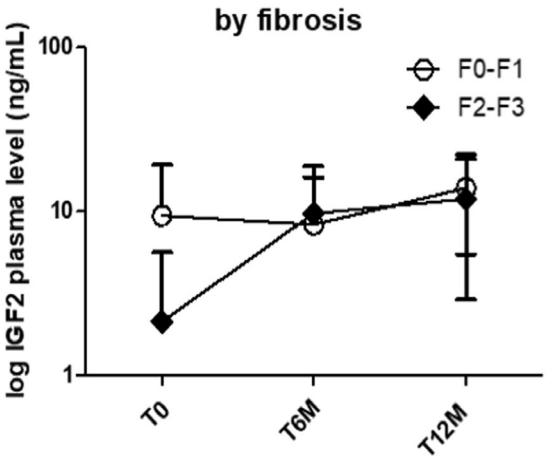


**Fig. 2 Plasma level of IGF2 at baseline and during follow-up in NAFLD patients stratified according to liver fibrosis.** (Stage of fibrosis was established by histological analysis of the wedge liver biopsy performed at baseline).

The most relevant finding of our study was that IGF2 plasma levels increased after BS reaching levels similar to those observed in lean people after 12 months. These changes were paralleled by the improvement of the hepatic such as ALT and GGT, but not with parameters evaluating morphometric or metabolic improvements. Of notice the type of surgery did not influence the effect of the biomarkers. The main strength of our study was the use of a well-characterized cohort of patients with histological evaluation at baseline. On the contrary, the main limitation of the study is the lack of liver histology data during the follow-up, together with the use of lean subjects in the control group. However, since a reduction in IGF2 plasma levels was associated with histological stages of NAFLD in obese children [30] and adults with liver fibrosis [31, 24] and cirrhosis [38]. It is tempting to speculate that the increase of IGF2 we observed may be related to an improvement in both steatosis and fibrosis.

On the other hand, the role of IGF2 in the development of the chronic liver disease is difficult to discern given the lack of data regarding the IGF2 action in the liver. Its local activity seem to be related to hepatocyte proliferation and tissue damage repair [39]. Moreover, even though the expression of IGF2 is highest in placenta and liver, the IGFs are ubiquitous; we are not able to exclude on one side the contribution of different tissues (adipose, endocrine, and gastrointestinal) affected by BS, as factors influencing our.

To date, several studies on animal models demonstrated a potential role of the IGF system components (IGF1, IGF2, IGFBPs, IGF receptors, and IGFBP-specific proteases) in the pathogenesis of NAFLD/NASH. Nishizawa et al. proved that administration of IGF1 ameliorates steatosis, inflammation, and fibrosis in a NASH and cirrhosis mouse model. Less well-known is the role of IGF2, but it could be a key factor for steatosis initiation in NAFLD [25]. Previous studies suggested a relationship between serum IGF1, IGF2 levels, and advanced fibrosis in NAFLD patients [30, 31, 40–43,], and IGF1/IGFBP3 ratio was recognized as the major predictor of liver inflammation in a cohort study on children with NAFLD [30]. Even though recent evidence associate modifications at serum level of IGFs with different histological abnormalities of the NAFLD

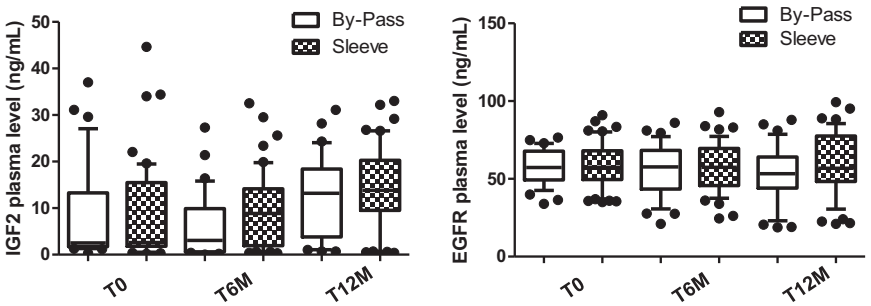


**Fig. 3 Plasma level of IGF2 and EGFR preoperatively and during follow-up when the cohort was stratified by surgical procedure.** IGF2 and EGFR plasma levels were not affected by the type of intervention (Roux-en-Y gastric bypass (By-Pass) or vertical sleeve gastrectomy (Sleeve)).

spectrum [19, 43–45,]; further epidemiological data assessing simultaneously IGFs will be essential to understand their role in chronic liver disease fully.

The effect of weight loss (by BS) on the IGFs serum levels was reported by Brynskov who mentioned that levels of IGF1 remain stable, IGFBP1 increased, whereas IGFBP3 and IGF2 levels decreased 3 and 12 months after gastric bypass in obese T2D patients [46]. Conversely, several studies showed that low IGF2 levels are predictors of weight gain and obesity in children and adults with and without T2D [47–49]. Our data are in line with these observations, at least regarding IGF2 levels and independently of the type of intervention (ByPass or SG).

To date, it is well evidenced that patients with NAFLD experiment histological and biochemical improvements after BS as well described in the meta-analysis performed by Bower et. al. which included a total of 29 bariatric studies and showed a significant reduction in the weighted incidence of the histological features observed in NAFLD, in liver enzyme levels (AST, ALT, ALP, and gamma-GT) and improvements in glucose metabolism and lipid profile [5, 14, 37, 50]. Moreover, Baldwin et al. have recently published the first meta-analysis exclusively comparing RGYB and SG in the amelioration of NAFLD, using four separate criteria (ALT, AST, NAFLD activity score, and NAFLD fibrosis score). Their findings corroborate the improvements in biochemical and histological parameters in NAFLD patients after surgery previously reported and support, that both surgeries may be equally effective [51]. Our follow-up data are in line with this conclusion.

Furthermore, our study does not exclude other factors implicated in our observations. Bariatric surgeries are no longer seen only restrictive and malabsorptive procedures; rather, they are considered metabolic procedures. They involve complex physiological changes where gut adaptation influences the signaling pathways in several other organs, including the liver and the brain, regulating hunger, satiety, body weight, glucose metabolism, and immune functions [52]. It is worth mentioning that both surgeries cause significant elevations of the same gastrointestinal peptides (GLP-1, CCK, PPY) [53]. Also, changes in serum level of hormones (ghrelin, leptin, GLP-1, GLP-2, FGF19, FGF15) [52], growth factors (IGF1, FGF, EGF) [52], transcription factors (FXR) [54], and cytokines [54] have been reported, particularly after RGYB. Interestingly, it was recently demonstrated that an increase in leptin after BS is associated with the rise in IGFBP2, one of the IGFs binders and activity regulator [55]. Our data are in line with these observations, and IGFBP2 could at least in part, contrasting the effects of IGF2 evidenced in our settings.

Moreover, BS modify also the microbiota profile, which in turn leads to the alteration of bile acid metabolism, an increase of glucose uptake and insulin sensitivity, among others [52]. In this regards, one crucial host pathway modulated by microbiota is that of the insulin-like growth factor 1, but others IGFs such as IGF2 could also be strongly modulated [56].

In conclusion, we showed that IGF2 plasma levels increased after BS. These changes, at least in our cohort, were directly/indirectly associated with modifications related to hepatic biological improvements (ALT, GGT, AST/ALT), but other metabolic parameters are not excluded. Further studies to understand the source IGF2 (tissue and/or microbiota) are needed and to understand their implications not only at the hepatic level but also to other tissues affected by NAFLD and obesity.

**Acknowledgements** Finanziamento Ricerca di Ateneo—U05SPFRA14—FRA 2014 (CdA dd. 19.12.2014) and Fondazione Italiana Fegato. PJG by Fondazione Umberto Veronesi (Grants 2015-2017). NR is funded by Fondazione Cassa di Risparmio di Trieste (CRTrieste) and by Fondazione Italiana Fegato ONLUS.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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