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ORIGINAL ARTICLE

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Serum IGF-binding protein 2 (IGFBP-2) concentrations change early after gastric bypass bariatric surgery revealing a possible marker of leptin sensitivity in obese subjects

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Abstract

Purpose Expression of IGFBP-2 in mice is regulated by leptin. Over-expression of IGFBP-2 is associated with reduced caloric intake and resistance to weight gain. Hormonal variations contributing to weight loss occur very early after bariatric surgery but have not been fully elucidated. We evaluated IGFBP-2 serum changes after bariatric surgery and their relationship with leptin variations to test the hypothesis that an increase of leptin sensitivity may explain some of the effects of gastric bypass.

Methods This is a historical prospective study. Fifty-one obese patients (41 women e 10 men), 9 non-obese surgical controls and 41 lean matched controls were studied. Serum IGFBP-2 and leptin were measured after bariatric bypass surgery at various time points up to 18 months, after non-bariatric laparoscopic surgery in a control group, and in lean matched controls.

Results Compared to lean controls, serum IGFBP-2 levels were lower in obese patients. After gastric bypass, IGFBP-2 significantly increased at 3 days and became normal before the occurrence of relevant changes in body weight, remaining stable up to 18 months after surgery. IGFBP-2/leptin ratio increased early after surgery and became normal after one year.

Conclusions After gastric bypass, serum IGFBP-2 increases in a window of time when variations of hormones mediating the effects of bariatric surgery occur. Our results suggest that IGFBP-2, a leptin-regulated protein, may be an in-vivo marker of leptin action. If this is the case, an early improvement of leptin sensitivity might contribute to the anorectic effect of gastric bypass.

Keywords IGFBP-2 · Bariatric surgery · Gastric bypass · Obesity · Leptin sensitivity

Introduction

Insulin-like growth factors (IGFs) are pivotal regulators of growth, metabolism and lipogenesis. IGF-binding protein 2 (IGFBP-2), a 34 kDa plasma protein, is one of six homologous proteins that can bind IGFs [1]. IGFBP-2 is considered a regulator of IGFs action through a high-affinity binding that modulates the interaction with IGF receptors, but its role in human physiology may go beyond the function of a mere binding protein. It is proved that some regulating functions are mediated by IGFs while other effects are displayed independently from them. Indeed, increasing evidence indicates that IGFBP-2 acts *per se* as a signaling factor in cancer and metabolism [2, 3].

Liver is considered the principal anatomical site of IGFBP-2 production [1], but adipose tissue and muscle [4–6] may contribute to the serum concentrations of the protein. IGFBP-2 exhibits an inhibitory effect towards adipogenesis and visceral lipogenesis [7, 8]; reduced IGFBP-2 levels have been documented in serum of obese and diabetic patients and in subjects with metabolic

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syndrome [9, 10]. IGFBP-2 circulating concentrations are negatively associated with HOMA index, while are inversely related to BMI and insulin levels [11]. For the above mentioned reasons serum IGFBP-2 levels have been proposed as a marker of metabolic syndrome [12, 13] and of risk for acute myocardial infarction and mortality [14].

IGFBP-2 liver expression is up-regulated by leptin [6, 15, 16], suggesting a possible role of this binding protein in leptin-mediated metabolic regulation [9]. In *ob/ob* obese mice and lean wild type mice, IGFBP-2 over-expression is associated with reduced caloric intake, resistance to weight gain and to hepatic steatosis [9, 16]. It is unclear whether or not IGFBP-2 retains an insulin-sensitizing effect [9, 17].

Gastric bypass bariatric surgery is a very effective procedure capable of inducing a significant, stable weight loss [18], and resolution of many co-morbidities, especially metabolic [19], associated with severe obesity. The mechanisms mediating these actions have not been fully elucidated, but likely involve neural signals and gastro-intestinal hormones such as GLP-1, PYY, ghrelin [20–23] and others not yet identified. Hormonal variations contributing to these effects typically occur very early after surgery.

As of this writing, it is not clear if leptin, a hormone secreted by adipocytes and a pivotal regulator of body weight homeostasis [24], which affects neuroendocrine functions, food intake, and possibly locomotor activity [25], is involved in post-surgery weight loss [26]. Indeed, severe obesity is a condition characterized by "leptin resistance" and an increase of leptin sensitivity induced by bariatric surgery, might contribute to weight loss. However, leptin sensitivity cannot be measured since no reliable in vivo markers of leptin activity has been described so far.

It was then an intriguing hypothesis to test if, in obese patients submitted to gastric bypass, IGFPB-2 serum levels change during the early window of time when variations of hormones mediating the effects of bariatric surgery occur. The relationship between the variations of concentrations of IGFBP-2 and leptin, a hormone that regulates its production in vivo, was also matter of our investigation to test the hypothesis that an increase of leptin sensitivity may explain some of the effects of gastric bypass.

Patients and Methods

Study design and participants

GROUP A: Fifty-one unrelated consecutive patients (41 women e 10 men) were enrolled among those who were candidate to bariatric bypass surgery at the Obesity Center of the Endocrine Unit of the University Hospital of Pisa, Italy. Age of the patients was between 24 and 59 years (mean ± SD: 43 ± 8 years), and body weight between 92 and 184 kg (mean ± SD: 128 ± 24 kg) while body mass index (BMI) between 34,9 and 67,5 (mean ± SD: 48 ± 7 kg/m^2). Clinical, biochemical, instrumental examinations and indications for the type of bariatric surgery of each patient followed the Italian guidelines for obesity [27]. Each patient was treated according to appropriate protocols for her/his condition and was evaluated by a multidisciplinary team. All patients signed a written consent for the treatment of their clinical data for purpose of research. The study was approved by the Ethics Committee of the North West Vast Area (CEAVNO).

Exclusion criteria were: history of bariatric surgery and cardiac surgery, acute and chronic inflammatory disease, liver, cardiac or renal failure, untreated endocrine disorders, use of corticosteroids, self reported alcohol consumption >20 g/day, use of illicit drugs.

Anthropometric measures were performed in the morning after a night of fasting. Body weight was measured to the nearest kg while height, waist and hip circumference were determined to the nearest centimeter.

Patients were counseled periodically at 1, 3, 6, 12, 18 months after surgery in order to follow standardized dietary regimens, take nutritional supplements, increase their physical activity.

GROUP B: A control group submitted to abdominal surgery was selected for this study. We enrolled 9 (4 women and 5 men) non-obese consecutive patients candidate to laparoscopic surgery. Exclusion criteria were diabetes mellitus, advanced cancer, age >65 years, liver disease, cardiac disease, untreated endocrine disorders. Mean age was 55 ± 6 years while body mass 26 ± 3 kg/m^2 (mean ± SD). Indication to surgery were the presence of an adrenal mass in one case, cholecystectomy in one case, early stage non-metastatic colorectal cancer in six cases and explorative laparoscopy in one case.

GROUP C: A second control group was chosen as a normal reference. Forty-one healthy, controls (28 women and 13 men) who volunteered for blood sampling, were selected. Mean age of this group was 45 ± 12 years (mean ± SD) with a body mass 23 ± 2 kg/m^2 (mean ± SD). We excluded overweight subjects, subjects affected by any chronic inflammatory disease, hepatic diseases, self reported alcohol consumption >20 g/day, diabetes, autoimmune disorders or use of any type of pharmacological treatment possibly interfering with liver function.

Patients and controls gave their written informed consent for blood sampling and data collection.

Venous blood samples for measurement of IGFBP-2 and leptin were obtained in the early morning (7.30–9 a.m.) after an overnight fasting.

IGFBP-2 was measured before and 3 days (mean 2.4 ± 0.8 SD), 1 month (32.6 mean ± 8.9), 3 months (101.8 mean ± 14.3), 6 months (mean 202.9 ± 54.3), 12 months (371.6

mean ± 29.9) and 18 months (mean 551 ± 76.4) after surgery in obese patients **(GROUP A)**.

IGFBP-2 was measured before and 3 days after surgery (mean 2.8 ± 0.6) in the surgical lean control group **(GROUP B)**.

Leptin was measured before and 3 days (mean 2.4 ± 0.8 SD), 1 month (32.6 mean ± 8.9) and 12 months (371.6 mean ± 29.9) after surgery in obese patients **(GROUP A)**.

Leptin was measured before and 3 days after surgery (mean 2.8 ± 0.6) in the surgical lean control group **(GROUP B)**.

IGFBP-2 and leptin were also measured in the forty-one healthy controls **(GROUP C)**.

Surgical procedure

Gastric bypass was performed by a reference center for bariatric surgery; gastroplasty was conducted creating a 30 cm^3 gastric pouch by transecting vertically the native stomach with a 45 mm linear tristapler and longitudinally with an application of 60 mm linear tristaple under the control of a 36 French bougie. Right vagus nerve fibers were spared. Roux-en-Y bypass was obtained by the creation of an alimentary limb of 150 cm and a biliopancreatic limb of 120 cm.

Hormonal assays

Serum IGFBP-2 levels were measured by a specific ELISA (*Mediagnost, Reutlingen, Germany*) [28] with a sensitivity of 0.2 ng/ml. Sera were prepared after blood centrifugation at 3000 rpm for 15 min at 4 °C and kept stored at −20 °C until measurement. The measurement was conducted within 3–4 months from sample collection and preliminary trials have shown stability of the results over longer periods of storage.

Serum human leptin levels were measured by a specific RIA (*Mediagnost, Reutlingen, Germany*) with a sensitivity of 0.1 ng/ml and low intra (5%) and inter assay (5.3%) variability. Sera were prepared as indicated above.

Evaluation of body composition

In a subgroup of 12 obese patients, body composition was analyzed by Dual Energy X-rays Absorptiometry (Dexa) before surgery. Total and regional lean body mass and fat body mass were measured by Dexa (Hologic QDR4500A, Hologic Inc. Walthan, MA). Dexa scans were analyzed with the manufacturer's whole-body version (Hologic Inc.). Peripheral values of lean and fat mass were calculated by adding up values measured in superior and inferior limbs.

Statistical analysis

The Shapiro-Wilk test was used to assess normality of data. Changes (Δ) at each time point were analyzed by paired Student's t-test when assessing differences in mean values, or by Wilcoxon signed-rank test when assessing differences in variables with skewed distribution such as IGFBP-2/leptin ratio. Similarly, the unpaired Student's t-test or the Mann-Whitney U test were used to assess differences between groups at each visit. The Pearson (r) and Spearman (ρ) correlation coefficients were calculated to quantify associations between Gaussian and skewed variables, respectively.

A P-value less than or equal to 0.05 was considered statistically significant. Data are presented as mean ± SD or as median with interquartile range (IQR). Statistical analyses were performed using SPSS (version 25, IBM Corp, Armonk, NY, USA).

Results

The anthropometric characteristic of patients before surgery are shown in Table 1, together with those of lean controls. As expected, after bariatric surgery, obese patients lost progressively body weight and consequently their BMI significantly declined over time, this reduction (Fig. 1) was associated with a progressive reduction of serum leptin (Fig. 2). Leptin/BMI ratio was higher in obese subjects compared to lean controls and normalized at one month after surgery, remaining constant up to one year (Fig. 2).

Serum IGFBP-2 levels measured before bariatric surgery were on average 53% lower ($p < 0.001$) than those measured in lean controls (Table 1).

No relationships were observed between IGFBP-2 concentrations and BMI (data not shown). Pre-surgery results of Dexa analysis in a subgroup of 12 patients showed no

Table 1 Characteristics of the study groups

	Bariatric patients	Lean controls	Surgical controls
Age (years)	43.3 ± 8.5	45.3 ± 12.0	54.6 ± 6.4
Sex (F/M)	41/10	28/13	4/5
BMI (Kg/m^2)	48.1 ± 7.2*	22.6 ± 2.3	25.8 ± 3.9
IGFBP-2 (ng/mL)	187.2 ± 100.5*	398.8 ± 175.7	336.7 ± 224.6
	159.6 (122.0 to 244.5)	397.0 (259.8 to 510.7)	268.0 (188.5 to 420.2)
Leptin (ng/mL)	43.7 ± 22.0*	9.0 ± 7.6	5.6 ± 6.4
	37.1 (29.2 to 56.7)	5.8 (4.0 to 11.8)	3.4 (2.2 to 6.3)
IGFBP-2/leptin	4.6 ± 3.1*	86.7 ± 85.8	120.2 ± 143.3
	4.0 (2.7 to 3.4)	63.8 (21.1 to 107.5)	63.8 (48.0 to 140.4)

Data are reported as mean ± SD or median (interquartile range). *SD* standard deviation. *BMI* body mass index. *F* female, *M* male. Student's *t*-test for unpaired variates vs lean controls and surgical controls. *$p <$ 0.001

correlation between total amount of fat and IGFBP-2 concentrations (data not shown), while there was a positive correlation between leptin and total fat ($p < 0.05$). We have not observed any gender difference in serum IGFBP-2 concentrations either before or after bariatric surgery.

Strikingly, 3 days after gastric bypass, serum IGFBP-2 levels increased on average by 77 % ($\Delta = 151$ ng/mL, 95% CI: 96 to 206, $p < 0.001$) up to 348 ng/mL, not significantly different from values measured in lean controls (399 ng/mL, Fig. 3).

In the months that followed bariatric surgery, mean IGFBP-2 levels steadily increased in all patients and remained normal at later time points, when body weight was stabilized (Fig. 3). No relationships were observed between changes in IGFBP-2 and changes in BMI at each follow-up visit. Interestingly, when IGFBP-2 concentrations were adjusted for BMI, IGFBP-2/BMI ratio recapitulated very tightly IGFBP-2 trends (Fig. 3) confirming that IGFBP-2 variations are largely independent from adipose tissue reduction and body composition variations due to surgical intervention.

Since it was possible that the surgical procedure *per-se* played a role in the early variations of IGFBP-2, we did measure the protein in patients who underwent non-bariatric laparoscopic surgery (indicated as control surgery in the figures) and no significant IGFBP-2 increase was observed 3 days (Fig. 3) after surgery.

Median IGFBP-2/leptin ratio, which may serve as a peripheral indicator of leptin sensitivity, was 4.0. It increased significantly at 3 days (median increase + 6.6, 95% CI: 3.6 to 11.4, $p < 0.0001$) and at 1 month (median increase + 12.2, 95% CI: 9.6 to 17.6, $p < 0.0001$) after bariatric surgery, whereas it did not significantly change in the surgical control group (Table 1, Fig. 4).

This results means that early after bariatric surgery, IGFBP-2/leptin ratio increases on average by 182% (after 3 days) and 412 % (after 1 month), indicating that relevant leptin-sensitization may already occur during this window of time (Fig. 4). When measured after one year, the IGFBP-2/leptin ratio was comparable to that of the control group ($p = 0.79$, Fig. 4).

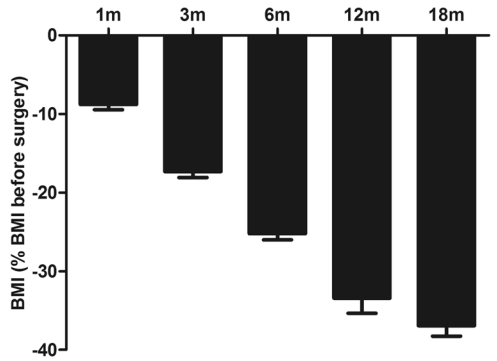
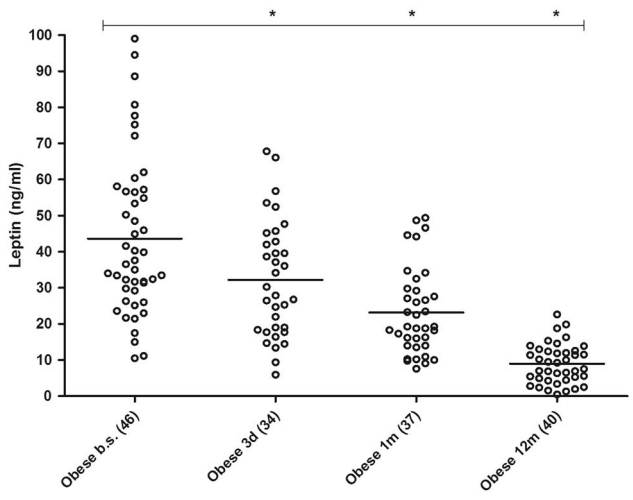


Fig. 1 BMI expressed as percentage (%) reduction after gastric bypass bariatric surgery compared to baseline values

Discussion

The number of bariatric procedures is steadily increasing worldwide because bariatric surgery is an effective therapeutic approach that guarantees significant and durable



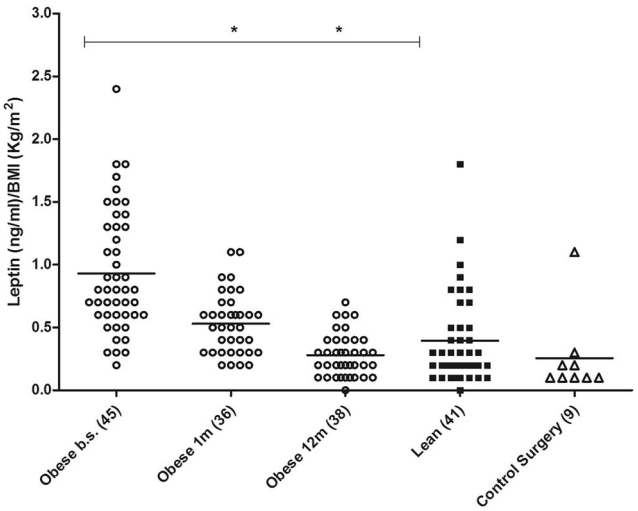


Fig. 2 Serum leptin before and after gastric bypass bariatric surgery (left panel). Concentrations were measured at different time points: before surgery, 3d = 3 days after surgery, 1m = 1 month after surgery, 12m = 1 year after surgery. Leptin concentrations are also displayed corrected by BMI (right panel) in obese (circles), lean controls (closed squares) and surgical controls (triangles). One month after gastric bypass surgery Leptin/BMI, in obese patients, significantly decreased remaining normal and overlapping with normal weight controls. Mean values are represented with a horizontal black bar. The sample size of each group is also reported in brackets. Student's paired t-test versus values before surgery, *$p < 0.0001$

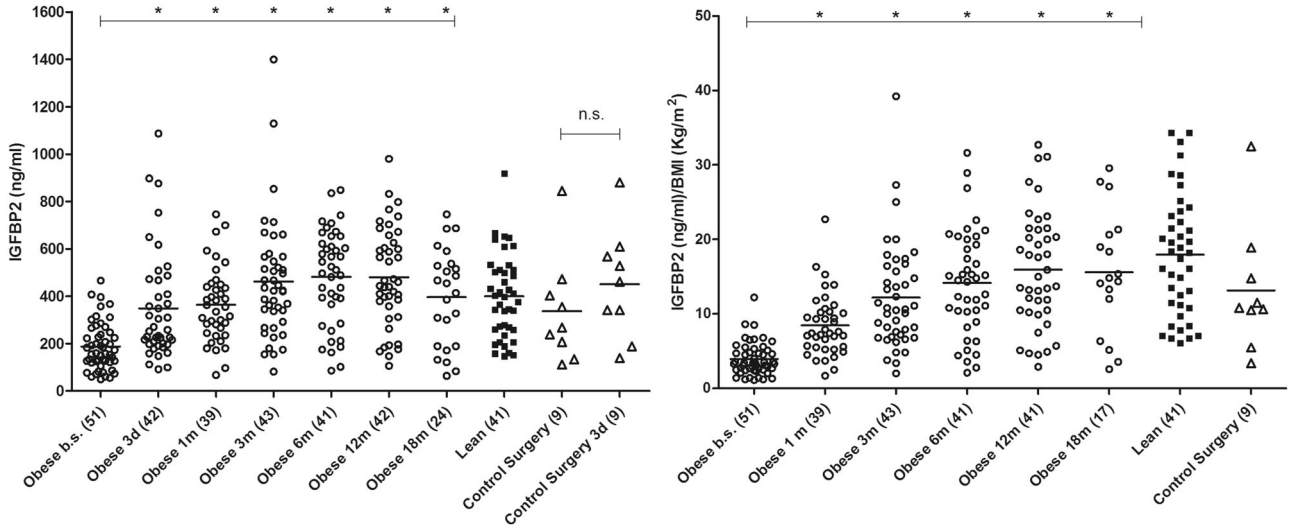


Fig. 3 Serum IGFBP-2 levels (left panel) in obese (circles), lean controls (closed squares) and surgical controls (triangles). IGFBP-2 values are also displayed corrected for BMI values (right panel). IGFBP-2 concentrations were measured at different time points: b.s. = before surgery, 3d = 3 days after surgery, 1m = 1 month after surgery, 3m = 3 months after surgery, 6m = 6 months after surgery, 12m = 1 year after surgery, 18m = 1 year and half after surgery. Mean values are represented with a horizontal black bar. The sample size of each group is also reported (n). Student's paired t-test versus values before surgery (*$p < 0.001$, n.s. = not significant)

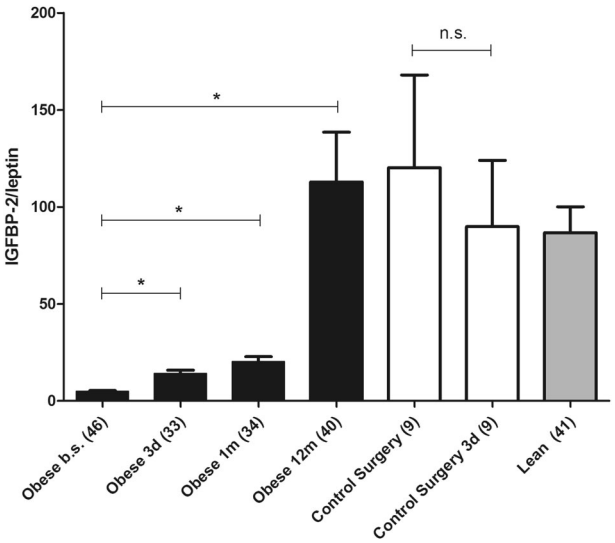


Fig. 4 IGFBP-2/leptin ratio measured in obese patients (black bars), in the surgical control group (white bars) and in lean controls (grey bar). When measured after one year, the IGFBP-2/leptin ratio was comparable to that of the control group. Values are represented as mean ± SD. Student's paired *t* test for paired variates vs. values before surgery (*$p < 0.0001$, n.s. = not significant). b.s. = before surgery, 3d = 3 days after surgery, 1m = 1 month after surgery, 12m = 1 year after surgery

weight loss, associated with improvements of co-morbidities and an increase in life expectancy [29, 30].

After gastric bypass, weight loss is achieved by the combined effects of gastric restriction, mild malabsorption of nutrients and, most importantly, by sustained changes of concentrations of hormones (and neural signals) involved in the regulation of energy balance [20, 22].

Increasing evidence has been gathered indicating a possible role of IGFBP-2 in directly regulating body weight homeostasis and adipogenesis [7, 9, 16]. In particular, IGFBP-2 inhibits adipogenesis especially at the visceral level [8] and it increases the production of cytokines with metabolic protective effect [7, 9]. Furthermore, in mouse models, IGFBP-2 over-expression favors weight loss, improves metabolic profile, and protects against high fat diet weight gain [9, 16].

In mice, IGFBP-2 mRNA and serum concentrations are increased by leptin treatment. This effect can occur even for minimal changes of leptin levels, and it is interpreted as the result of activation of efferent signals from the brain to the liver or other peripheral organs [6, 15, 16]. Interestingly, reduced IGFBP-2 levels have been previously documented in the serum of obese subjects, diabetics and in patients with metabolic syndrome [9, 10]: low IGFBP-2 levels in obese patients, despite increased levels of serum leptin, may be justified at least in part by the reduced effect of the hormone in this condition, as a consequence of leptin resistance.

Leptin resistance is defined as the reduced capacity of elevated endogenous leptin to suppress appetite and weight gain [31]. By this definition, most obese patients are leptin resistant. Leptin resistance develops as an acquired signaling down-regulation involving intracellular messengers or neural circuitries regulating energy homeostasis; these mechanisms have been dissected in animal models [32] but have been poorly proven in humans.

It was then interesting for us to study the possible variations of serum IGFBP-2 levels in obese individuals after weigh loss intervention. As expected, in our study, basal serum IGFBP2 concentrations were significantly reduced in

obese patients compared to lean matched controls. Surprisingly, they rose rapidly after gastric bypass despite a marked reduction of serum leptin. This increase was on average 76% already 3 days after intervention, a time interval too precocious to be dependent from variations of body composition and likely induced by the pleiotropic changes in hormonal signals occurring after surgery. Such an early increase of IGFBP-2 has been documented 5 days after biliopancreatic diversion surgery [33], a bariatric approach associated with severe malabsorption and malnutrition [34], therefore complicated by more confounding factors than our study. In our study, IGFBP-2 concentrations steadily increased up to 6 months when they stabilized at levels comparable to those measured in healthy, lean controls.

We suppose that the observed increase in serum protein levels originates preferentially from increased liver secretion [35], but we cannot rule out that other tissues [6] provide a contribution or that changes in IGFBP-2 concentrations are related to reduced catabolism. In this regard, a limitation of this study, is the lack of data of changes in body composition after surgery.

Variations of IGFBP-2 of such a magnitude are not observed after short-term experimental dietary restriction: a 50% caloric reduction did not modify IGFBP-2 levels [36] while a 600 Kcal/day hypocaloric diet in obese patients was associated with an increment of only 10% [37]. These observations suggest that serum changes of IGFBP-2 after gastric bypass do not reflect the amount of weight loss but are driven and potentiated by other signals.

Thinking of the possibility that the surgical procedure *per-se* may affect the protein concentrations, we did measure IGFBP-2 in a surgical control group consisting of patients who underwent laparoscopic non-bariatric surgery. In this case IGFBP-2 did not change significantly.

Based on this observations we suppose that after surgery, the acute serum increase of IGFBP-2, might be mediated by improved leptin sensitivity as can also be suspected by the precocious normalization of the leptin/BMI ratio. To test this hypothesis, we have employed the IGFBP-2/leptin ratio, an index of how much IGFBP-2 is produced per unit of circulating leptin. Pre-surgical IGFBP-2/leptin ratio was very low, compared to the tight values documented in lean controls, but increased progressively over time, becoming normal one year after gastric bypass. These findings show that after surgery, more IGFBP-2 is produced in presence of a reduced amount of serum leptin and that a smaller amount of leptin circulates per unit of BMI: a further indirect evidence of an improvement of leptin sensitivity. All these data, taken together, could back our hypothesis that gastric bypass improves leptin sensitivity and that IGFBP-2 concentrations reflect leptin action. In this scenario, the potentiation of leptin signaling would favour weight loss and weight maintenance.

According to our hypothesis, the absence of leptin, in mice, significantly attenuates the body weight reduction after gastric bypass and fails to improve glucose tolerance [38, 39].

Additionally, the administration of an amylin analog, capable of leptin-sensitizing effects and overcoming leptin resistance, is an effective weight loss treatment in obese patients [40, 41]. Although pharmacological administration of recombinant leptin after gastric bypass did not further improve body weight loss outcomes, these results do not exclude the possibility that surgery determine sensitization to the endogenous hormone [42].

An open question is if IGFBP-2 may act *per-se* as a factor defending against weight gain since this effect has been described in few occasions in animal studies together with an improvement of liver steatosis [9, 16]. If IGFBP-2 exerts a direct metabolic action, its effects may be mediated a) by direct translocation into the nucleus [43, 44] b) upon binding to integrins and other components of the extracellular matrix [7, 45] or c) by specific receptors, yet to be identified [46].

In conclusion, by this study we have shown that: 1) patients affected by morbid obesity have low serum levels of IGFBP-2. 2) After gastric bypass, IGFBP-2 serum levels increase very precociously and become normal before the occurrence of significant changes in body weight. 3) The normalization of IGFBP-2 serum concentrations is stable over time and persists up to 18 months from surgery not being correlated with the degree of weight loss. 4) IGFBP-2/leptin ratio, a potential indicator of leptin sensitivity, rapidly raises after bariatric surgery and becomes normal after one year, suggesting that IGFBP-2 might be an in-vivo marker of leptin action. If this is the case, an early improvement of leptin sensitivity might contribute to the anorectic effect of gastric bypass.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest related to this study.

Ethical approval All procedures performed in this study were in accordance with the ethical standards of the Local Ethical Committee and with the 1964 Helsinki declaration and its later amendments.

Informed consent Informed consent was obtained from all individuals included in this study.

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