

Plasma Osteopontin Increases After Bariatric Surgery and Correlates with Markers of Bone Turnover But Not with Insulin Resistance

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Context: Osteopontin (OPN) is a multifunctional protein involved in bone metabolism, cardiovascular disease, diabetes, and obesity. OPN levels are elevated in the plasma and adipose tissue of obese subjects, and are decreased with diet-induced weight loss.

Objective: We investigated the effect of bariatric surgery on plasma OPN concentrations in morbidly obese patients.

Setting: The study was performed at a university hospital.

Subjects: We investigated 40 obese patients aged 43.1 ± 1.8 yr, scheduled to undergo bariatric surgery. Roux-en-Y gastric bypass (RYGB) was performed in 30 subjects (27 females, three males), and laparoscopic adjustable gastric banding (LAGB) in 10 subjects (eight females, two males).

Study Design: All patients were studied before and 1 yr (10.3–14.8 months) after the intervention.

Main Outcome Measures: OPN, leptin, C-reactive protein, insulin, the homeostatic model assessment insulin resistance index, calcium, 25-hydroxyvitamin D, C telopeptide, and osteocalcin were determined.

Results: Both bariatric procedures significantly reduced body weight, body mass index, insulin, leptin, and C-reactive protein 1 yr after surgery. Plasma OPN increased from 31.4 ± 3.8 to 52.8 ± 3.7 ng/ml after RYGB ($P < 0.001$) and from 29.8 ± 6.9 to 46.4 ± 10.6 ng/ml after LAGB ($P = 0.042$). Preoperative OPN correlated with age, insulin, the homeostatic model assessment insulin resistance index, and postoperative OPN. Postoperative OPN correlated with C telopeptide and osteocalcin.

Conclusions: One year after RYGB and LAGB, plasma OPN levels significantly increased and correlated with biomarkers of bone turnover. Unlike other proinflammatory cytokines, OPN does not normalize but increases further after bariatric surgery. (*J Clin Endocrinol Metab* 93: 2307–2312, 2008)

Osteopontin (OPN) is a conserved multifunctional glycoprotein that is secreted by many cell types (1–3). Its structure contains several signaling motifs that allow binding to calcium and adhesion to different membrane receptors, including

integrins and CD44 variants (2, 4). OPN controls bone remodeling, and functions as a proinflammatory cytokine in regulating immune processes, chronic inflammation, and tumorigenesis (1, 5–8).

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Abbreviations: BMI, Body mass index; CRP, C-reactive protein; HOMA, homeostatic model assessment; LAGB, laparoscopic adjustable gastric banding; LDL, low-density lipoprotein; OC, osteocalcin; OPN, osteopontin; RYGB, Roux-en-Y gastric bypass.

Recently, several studies have highlighted the involvement of OPN in certain components of the metabolic syndrome: plasma OPN is elevated in cardiovascular disease (9), diabetes (10), and in obese subjects when compared with an age-matched normal control group (11, 12). OPN expression is increased in the presence of elevated proinflammatory cytokine concentrations (1) and hyperglycemia (13) but decreases after treatment with peroxisome proliferator-activated receptor- α agonists (14). In addition, *OPN*^{-/-} mice on a high-fat diet displayed reduced adipose tissue inflammation and improved insulin resistance when compared with the wild-type controls (15).

Diet-induced weight loss in obese subjects is associated with normalization, namely reduction, of plasma OPN concentrations (11). To our knowledge there is no evidence on the effect of bariatric surgery on this complexly regulated molecule. Bariatric procedures are increasingly used as the treatment of choice for morbid obesity because they achieve significant weight loss and reduce mortality rates (16). The reduction in mortality rate is attributed to a considerable reduction in comorbidities such as cardiovascular disease, diabetes, and cancer (17). Loss of adipose tissue mass is accompanied by a decrease in insulin resistance, and in plasma concentrations of adipokines, inflammatory markers and cytokines (18). Nevertheless, bariatric surgery procedures are complicated by gastrointestinal complaints, bone resorption, and bone loss (18, 19). Accumulating evidence has revealed that weight regain happens in the long term (20).

The present study aimed to investigate the changes in plasma OPN 1 yr after two commonly used bariatric procedures: laparoscopic adjustable gastric banding (LAGB), a strictly restrictive procedure; and laparoscopic Roux-en-Y gastric bypass (RYGB), a mixed restrictive malabsorptive procedure. Prompted by the association of OPN with metabolic diseases, inflammation, and bone metabolism, we explored the changes in metabolic, inflammatory, and bone turnover parameters.

Subjects and Methods

Study subjects

A total of 40 Caucasians with morbid obesity was recruited from the cohort scheduled for bariatric surgery. Exclusion criteria were: age less than 18 yr, previous bariatric surgery or recent (greater than 5%) weight change, diabetes mellitus (21), uncontrolled hypertension, myocardial infarction during the last year, chronic kidney or liver disease, and thyroid disease and malignancy. The study was approved by the institutional review board, and informed consent was obtained from all participants before enrollment.

RYGB was performed in 30 patients (27 females and three males, aged 42.9 \pm 2 yr) and LAGB in 10 patients (eight females and two males, aged 43.9 \pm 4.5 yr). Both procedures were performed at a university hospital by the same team of surgeons. Among the 35 women studied, 23 were premenopausal (18 in the RYGB group and five in the LAGB group) and 12 postmenopausal (nine in the RYGB group and three in the LAGB group). Only two women were taking oral contraceptives (RYGB group). Patients were prescribed supplementations of vitamin D and calcium (one tablet containing 600 mg calcium and 400 IU vitamin D₃, twice daily) throughout the post-surgery study period. Data were collected at two time points: before bariatric surgery and 1 yr after bariatric surgery. The time elapsed between the two time points varied from 10.3–14.8 months. At each visit, subjects underwent a thorough clinical examination, and blood samples were collected in the fasting state. Fasting glucose, triglycerides, total cholesterol, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein-cholesterol, albumin, calcium, 25-hydroxyvitamin D, creatinine, liver enzymes, and C-reactive protein (CRP) were quantified using routine certified tests.

Measurement of hormones and bone markers

PTH, osteocalcin (OC), and C telopeptide (β -crosslaps, C-terminal telopeptide of type I collagen, CTX) were routinely determined by electrochemiluminescence immunoassays (Elecys; Roche Diagnostics, Mannheim, Germany). For the other parameters, blood samples were immediately cooled, centrifuged within 30 min, and then frozen at -80°C . Samples taken on both study days from an individual subject were analyzed in one assay and in duplicates. Plasma OPN was measured using a commercially available sandwich immunoassay (quantikine ELISA kit; R&D Systems, Inc., Minneapolis, MN), with an intraassay and interassay coefficient of variation of 2.9 and 5.4%, respectively. Leptin was measured using the Fluorokine human leptin kit and the obesity Multi-Analyte Profiling Base Kit (R&D Systems). Insulin and C peptide were

TABLE 1. Clinical and biochemical parameters of obese subjects before and 1 yr after bariatric surgery

	RYGB group (n = 30)			LAGB group (n = 10)			
	Baseline	% Change	P ^a	Baseline	% Change	P ^a	P ^b
Weight (kg)	127.9 \pm 2.9	-26.4 \pm 1.8	<0.001	130 \pm 8.1	-18.2 \pm 2.1	<0.001	0.008
BMI (kg/m ²)	47.7 \pm 1.1	-26.4 \pm 1.8	<0.001	47.6 \pm 2.1	-18.2 \pm 2.1	<0.001	0.008
Fasting insulin ($\mu\text{U/liter}$)	28.2 \pm 3.5	-33.1 \pm 16	0.002	23.8 \pm 3.2	-24.5 \pm 8.6	0.021	0.031
HOMA insulin resistance index	6.1 \pm 1	-47.1 \pm 5.4	<0.001	4.97 \pm 0.9	-14.1 \pm 13	0.195	0.020
Fasting triglycerides (mg/dl)	167.9 \pm 17.9	-16.7 \pm 7.6	0.038	185.8 \pm 33	-8.5 \pm 9.4	0.108	0.315
Total cholesterol (mg/dl)	197.1 \pm 5.3	-18.6 \pm 2.7	<0.001	231.3 \pm 14.9	-6.47 \pm 4.3	0.127	0.028
LDL cholesterol (mg/dl)	119.1 \pm 4.9	-24.9 \pm 3.9	<0.001	140.4 \pm 13.4	-2.2 \pm 4.4	0.287	0.002
HDL cholesterol (mg/dl)	50.1 \pm 1.6	2.2 \pm 3.5	0.776	47.1 \pm 2.7	20.2 \pm 13.7	0.133	0.208
CRP (mg/dl)	1.23 \pm 0.15	-66.2 \pm 4.4	<0.001	1.07 \pm 0.23	-61.8 \pm 1.8	0.018	0.886
Creatinine (mg/dl)	0.8 \pm 0.01	-0.2 \pm 1.8	0.892	0.9 \pm 0.06	1 \pm 11.6	0.636	1.000
Albumin (g/liter)	42.2 \pm 0.4	-3.66 \pm 1	0.001	43.1 \pm 0.6	-0.98 \pm 1.7	0.560	0.293
Total calcium (mmol/liter)	2.3 \pm 0.01	0.91 \pm 0.56	0.129	2.3 \pm 0.03	2.01 \pm 1.9	0.354	0.871
25-hydroxyvitamin D	44 \pm 4.3	3.9 \pm 11.7	0.760	42.3 \pm 2.3	7.2 \pm 13.4	0.531	0.978

Data are presented as mean \pm SE. HDL, High-density lipoprotein.

^a P values for comparison between baseline and postoperative values (paired t test).

^b P values for comparison between surgery-induced changes in the RYGB and LAGB groups (Mann-Whitney U test).

TABLE 2. Hormone and peptide plasma levels of obese subjects before and 1 yr after bariatric surgery

	RYGB group (n = 30)			LAGB group (n = 10)			<i>P</i> ^b
	Baseline	After surgery	<i>P</i> ^a	Baseline	After surgery	<i>P</i> ^a	
OPN (ng/ml)	31.4 ± 3.8	52.8 ± 3.7	<0.001	29.8 ± 6.9	46.4 ± 10.6	0.042	0.414
Leptin (ng/ml)	110.2 ± 7.3	36.4 ± 4.9	<0.001	87.2 ± 21.2	42.4 ± 8.9	0.024	0.073
C telopeptide (ng/ml)	0.3 ± 0.03	0.87 ± 0.06	<0.001	0.25 ± 0.02	0.57 ± 0.1	0.032	0.278
OC (ng/ml)	14.5 ± 1.3	41.1 ± 3.5	<0.001	16.4 ± 1.4	27.5 ± 4.9	0.041	0.053
PTH (pg/ml)	48.8 ± 3.2	54.2 ± 3	0.081	44.9 ± 5.3	49.5 ± 7.3	0.393	0.978
TSH (μU/ml)	2.2 ± 0.2	1.82 ± 0.2	0.032	2.5 ± 0.6	1.4 ± 0.3	0.198	0.262

Normal ranges: PTH, 15–65; and TSH, 0.44–3.77.

^a *P* values for comparison between baseline and postoperative values (paired *t* test).

^b *P* values for comparison between surgery induced changes in the RYGB and LAGB groups (Mann-Whitney *U* test).

determined using commercially available RIAs (LINCO Research, Inc., St. Charles, MO). The homeostatic model assessment (HOMA) insulin resistance index was calculated as the product of fasting glucose (expressed as mg/dl) and insulin (expressed as μU/ml) divided by the constant 405.

Statistical analysis

All data are expressed as mean ± SEM. Baseline and postoperative values were compared using the paired Student's *t* test. Surgery induced changes between the two groups (LAGB vs. RYGB) were tested with the Mann-Whitney *U* test. Linear regression analysis was performed to evaluate the relationships between OPN and other parameters. The statistical software package SPSS release 12.0.1 (SPSS, Inc., Chicago, IL) was used. *P* values less than 0.05 were considered statistically significant.

Results

Preoperative values and RYGB- and LAGB-induced changes of clinical and biochemical parameters are presented in Table 1. Baseline characteristics were similar between the two groups. Both surgical procedures significantly decreased weight, body mass index (BMI), plasma insulin, CRP, and leptin (Tables 1 and 2). The reduction in the HOMA insulin resistance index, triglycerides, total cholesterol, LDL-cholesterol, and albumin reached significant levels only in the RYGB group.

Plasma OPN increased from 31.4 ± 3.8 to 52.8 ± 3.7 ng/ml after RYGB (*P* < 0.001) and from 29.8 ± 6.9 to 46.4 ± 10.6 ng/ml after LAGB (*P* = 0.042) (Fig. 1, A and B, and Table 2). C telopeptide (marker of bone resorption) and OC (marker of bone formation) also increased in both groups after surgery (Table 2), whereas total calcium and PTH did not change significantly (Tables 1 and 2). TSH showed a mild but significant decrease in the RYGB group (Table 2).

There was a weak but significant negative correlation of baseline OPN levels with age (Fig. 2A), but no relation to menopausal status in women. Preoperative plasma OPN concentrations correlated positively and highly significantly to the respective postoperative values.

At baseline, plasma OPN levels correlated positively to insulin ($R^2 = 0.205$; *P* = 0.003) and the HOMA insulin resistance index ($R^2 = 0.154$; *P* = 0.024). However, both of these associations disappeared 1 yr after bariatric surgery (Fig. 2, C and D). In addition, preoperative values of OPN were not associated with markers of bone turnover, whereas postoperative OPN cor-

related with both OC (Fig. 2, E and F) and C telopeptide (Fig. 2, G and H).

The difference between OPN levels at both study time points was negatively associated with the difference in plasma albumin ($R^2 = 0.106$; *P* = 0.046). There were no significant correlations between OPN and the remaining parameters that were studied.

Discussion

We show here that plasma OPN levels increase significantly and correlate to biomarkers of bone turnover 1 yr after RYGB and

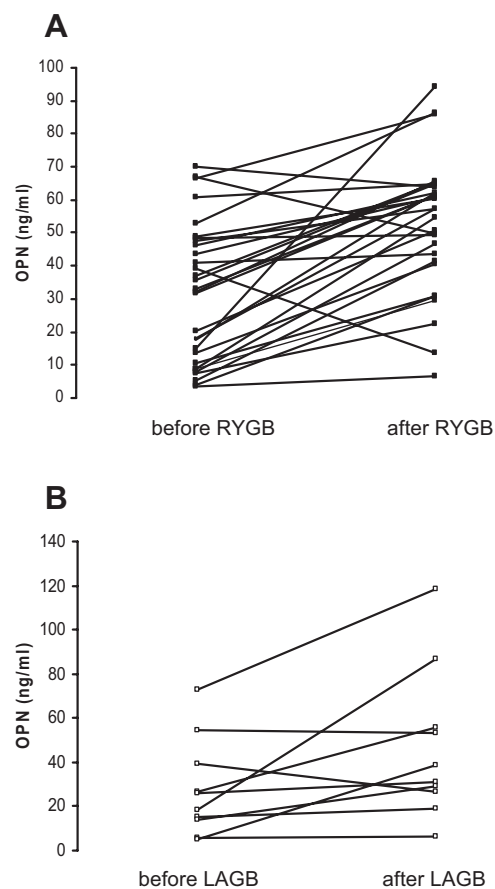


FIG. 1. Bariatric surgery induced changes in plasma OPN. Differences between preoperative and postoperative OPN plasma concentrations after RYGB (A) and LAGB (B).

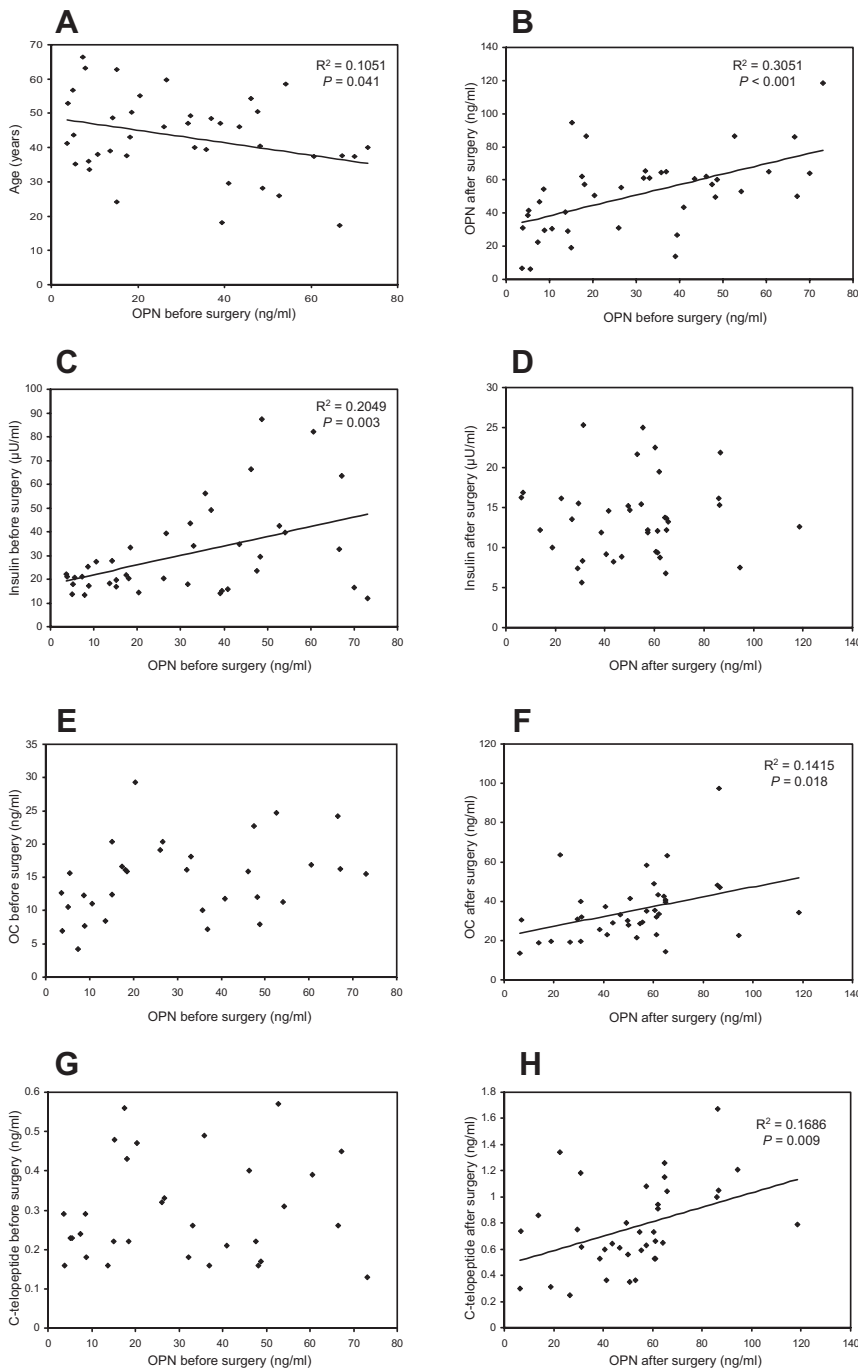


FIG. 2. Linear regression analysis of correlations between plasma OPN and other variables. A, Baseline OPN vs. age. B, Preoperative OPN vs. postoperative OPN. C, Preoperative plasma insulin vs. preoperative OPN. D, Postoperative plasma insulin vs. postoperative OPN. E, Preoperative plasma OC vs. preoperative OPN. F, Postoperative plasma OC vs. postoperative OPN. G, Preoperative plasma C telopeptide vs. preoperative OPN. H, Postoperative plasma C telopeptide vs. postoperative OPN.

LAGB. Bariatric surgery achieved a significant reduction in body weight, BMI, leptin, insulin, and CRP, however, OPN changes or postoperative values were not correlated to these variables. RYGB ($n = 30$) was more effective than LAGB ($n = 10$) in reducing weight, BMI, insulin, the HOMA insulin resistance index, total cholesterol, and LDL cholesterol. The effects on OPN and markers of bone turnover were not significantly different between the two surgical procedures. Nevertheless, the differ-

ence in patient numbers between the two groups should be kept in mind when interpreting these results.

An interesting finding is the weak but significant negative correlation of OPN with age, which to our knowledge has not been previously reported (Fig. 2A). A possible link with age-related changes in bone mineral density is hypothetical and remains to be investigated. Of the patients studied here, 87% were female, and baseline OPN levels were not influenced by the menopausal status.

Chronic inflammation is one of the hallmarks of obesity and metabolic syndrome (22). Increased levels of cytokines, activation of the hypothalamic-pituitary-adrenal axis, and elevated markers of inflammations (such as CRP) accompany morbid obesity. OPN is a complex protein with proinflammatory cytokine functions, which promotes macrophage adhesion and migration *in vitro* (6). Recently, Gómez-Ambrosi *et al.* (11) showed that obese patients have higher plasma OPN concentrations than matched lean subjects. These levels decline after diet-induced weight loss. Within the adipose tissue, OPN expression is restricted to adipose tissue macrophages (12). OPN mutant mice display reduced lean body mass, adipose tissue inflammation, and insulin resistance when compared with their wild-type counterparts (15). It is hypothesized that OPN might be an important player in the pathophysiology of adipose tissue inflammation and cytokine-induced insulin resistance (12, 15).

Gastric bypass surgery decreases systemic inflammation and cytokine levels (23). In addition, it reduces the amount of adipose tissue macrophages as well as the expression of genes involved in macrophage attraction such as monocyte chemoattractant protein, plasminogen activator urokinase receptor, and colony stimulating factor. Taking these data together, we expected a postoperative decrease in plasma OPN. The opposite finding presented in this study does not seem to have a direct association with

the accompanied weight loss and reduction in insulin resistance.

When discussing the origin of elevated circulating OPN after bariatric surgery, we should keep in mind that known substantial sources are osteoblasts, endothelial and epithelial cells, macrophages, and cancer cells (2). Bariatric surgery procedures are complicated by bone loss and increased markers of bone turnover (18, 20). We found a significant correlation between postoperative OPN values and markers of bone turnover (Fig. 2,

E–H), and, therefore, hypothesize that bone might be the source of high plasma OPN concentrations 1 yr after bariatric surgery. Indeed, many studies have already established a strong association between obesity surgery and bone loss (18, 19). OPN represents a component of the noncollagenous bone matrix secreted by osteoblasts and osteoclasts that is critical for the remodeling of mature bone (1). Bone remodeling is the outcome of two sequential events: resorption of preexisting bone by osteoclasts and *de novo* bone formation by osteoblasts (24). OPN is secreted from both osteoclasts and osteoblasts (1). OPN deficient mice have impaired bone resorption and hypermineralized fragile bones (25, 26). Therefore, increased OPN levels after gastric surgery might be under the control of the same mechanisms that mediate bone resorption.

Body weight is an important determinant of bone mass (27). Several studies have found an association of high body weight with higher bone mass and with lower bone loss (28). This relationship seems to be also dependent on age and sex (29). Fat tissue is an endocrine organ that releases adipokines, such as leptin, which influence not only the peripheral insulin sensitivity, but also the function of many organs (30). Leptin regulates osteoblast proliferation and bone formation by acting at the hypothalamus, and through a combined regulation of two antagonistic pathways (31, 32). Serum leptin level is a significant and independent predictor of bone mineral density in postmenopausal women (33). Moreover, epidemiological data from 800 elderly men and women from the population-based Framingham Osteoporosis Study reveal that weight loss is an independent risk factor for osteoporosis, whereas serum 25-hydroxyvitamin D, or calcium intake, is not significantly related to changes in bone mineral density (34). We show here that markers of bone turnover increase also in the presence of normal calcium and 25-hydroxyvitamin D plasma levels, thereby supporting the hypothesis that increased bone turnover might, at least in part, be due to increased weight loss (20).

RYGB patients show a reduced absorption of true fractional calcium and increased markers of bone resorption, despite adequate substitution with calcium and vitamin D (35). Our data confirm that increased bone resorption exists also in the presence of unchanged plasma calcium levels. Therefore, calcium release from bone might compensate for the decreased intestinal absorption. Calcium metabolism is controlled by several hormones and peptides influenced by weight loss (19, 32, 35). We hypothesize that postoperative OPN changes are induced by the same mechanisms that mediate increased bone turnover, the latter being several and not yet completely understood.

Bone is not only a target of hormones. A recent study by Lee *et al.* (36) investigated the existence of a feedback control of bone on energy metabolism. OC deficient mice presented decreased β -cell mass, glucose intolerance, and insulin resistance (36), whereas OPN deficient mice showed improved insulin sensitivity (15).

The mechanisms underlying long-term weight regain after bariatric surgery are not fully understood (20). A role is attributed to appetite-regulating hormones and changes in energy intake and expenditure over time (20, 37, 38). A possible regula-

tion of energy metabolism by the skeleton merits further investigation in these patients.

In conclusion, we report here that plasma OPN, a proinflammatory cytokine linked to the development of insulin resistance, increases 1 yr after RYGB and LAGB. It is suggested that bone might be the source of enhanced OPN concentrations. Further prospective studies are needed to elucidate whether postoperative circulating OPN concentrations, and/or the degree of bone loss, relate to future changes in body weight and insulin resistance.

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