Effects of Weight Loss after Bariatric Surgery for Morbid Obesity on Vascular Endothelial Growth Factor-A, Adipocytokines, and Insulin

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Background: Adipocytes regulate blood vessel formation, and in turn endothelial cells promote preadipocyte differentiation through the expression of proangiogenic factors, such as vascular endothelial growth factor (VEGF)-A. Some adipocytokines and hormones also have an effect on vascular development.

Objectives: Our objectives were to analyze the relationship between weight and circulating VEGF-A in morbidly obese subjects before and after bariatric surgery, and investigate the relationship between circulating VEGF-A and certain adipocytokines and hormones regulating adipocytes.

Methods: A total of 45 morbidly obese women and nine lean females were included in the study. Patients underwent bariatric surgery: vertical banded gastroplasty (n = 17), gastric bypass (n = 17), and biliopancreatic diversion (n = 11). Serum samples for VEGF-A, adiponectin, leptin, ghrelin, and insulin were obtained preoperatively and 9–12 months after surgery.

Results: Obese patients showed significantly higher VEGF-A levels than controls (306.3 ± 170.3 *vs.* 187.6 ± 91.9 pg/ml; $P = 0.04$), decreasing to 246.1 ± 160.4 after surgery ($P < 0.001$), with no differences among surgical procedures. In controls there was an inverse correlation between VEGF-A and ghrelin ($r = -0.85$; $P < .01$), but not in obese patients. Leptin and insulin concentrations were increased in obese patients, with a significant decrease shown after weight loss with surgery. Conversely, adiponectin concentrations were lower in obese patients, with a significant increase shown after weight loss with surgery. Ghrelin was higher in controls than obese patients, decreasing after gastric bypass and biliopancreatic diversion, but not after vertical banded gastroplasty.

Conclusion: Serum VEGF-A levels are significantly higher in obese patients than in lean controls, decreasing after weight loss with bariatric surgery, behaving similarly to other hormones related to adipose mass like leptin and insulin. **(*J Clin Endocrinol Metab* 93: 4276–4281, 2008)**

Growth of any tissue requires the formation of a functional and mature vasculature. Adipose tissue, unlike other organs, grows and develops continuously throughout life. *In vitro* studies showed that differentiating adipocytes and adipose tissue explants trigger blood vessel formation (1, 2) and that in turn adipose tissue endothelial cells promote preadipocyte differentiation (3). Furthermore, adipose tissue growth in mice can be impaired with angiogenesis inhibitors or inactivation of proangiogenic factors (4–6).

Vascular endothelial growth factor (VEGF)-A plays a pivotal role in both physiological and pathological angiogenesis through the increase of proliferation and migration of endothelial cells (7)

0021-972X/08/$15.00/0

Printed in U.S.A.

doi: 10.1210/jc.2007-1370 Received June 21, 2007. Accepted August 13, 2008.

First Published Online August 19, 2008

Abbreviations: BMI, Body mass index; BPD, biliopancreatic diversion; HOMA, homeostasis model of assessment; VEGF, vascular endothelial growth factor.

and permeabilization of blood vessels by inducing fenestrations in the endothelium (8). VEGF-A is highly expressed in adipose tissue, and its expression increases significantly during adipocyte differentiation in several experimental models (9–12). This close relationship between adipogenesis and VEGF-A induced angiogenesis has been demonstrated in *in vitro* and in *in vivo* studies. The inhibition of angiogenesis by VEGF receptor-2 blocking antibodies not only reduced angiogenesis but also inhibited murine adipose cell differentiation (13).

Adipocyte-derived factors may play critical roles in regulation of tissue function by control of angiogenesis. Adiponectin is a circulating adipocyte-derived cytokine with antiatherogenic, antiinflammatory, and antidiabetic properties, which is decreased in obese individuals, whereas active angiogenesis occurs in the adipose tissue (14). However, the interaction between adiponectin and angiogenesis has not been clarified. Some studies showed that adiponectin can stimulate new blood vessel formation in ischemic tissue (15), and stimulate the differentiation of human endothelial cells into capillary like structures *in vitro* and blood vessel growth *in vivo* in a rabbit corneal assay (16). In contrast to these findings, it has been reported that adiponectin inhibits tumor neovascularization through its ability to induce endothelial apoptosis (17). Leptin has also been found to regulate angiogenesis. Leptin, another adipocyte-derived cytokine that plays a role in the control of satiety and energy expenditure, has promoted angiogenesis in several *in vitro* and *in vivo* studies (18, 19). Furthermore, leptin may potentiate VEGF-A-mediated angiogenesis because leptin increased endothelial cell VEGF-A expression and secretion in a dose-dependent manner (20, 21) and acted synergistically with VEGF-A-driven angiogenesis in cornea pockets assays (22).

Some other hormones could have a role in the relationship between adipogenesis and angiogenesis. Ghrelin, a hormone isolated from the stomach, releases GH and stimulates appetite. Ghrelin and its receptors are expressed in endothelial cells, but their function in blood vessels remains controversial. Some studies have demonstrated an antiangiogenic effect of ghrelin in human and rat *in vitro* assays and *in vivo* animal models (23, 24). In contrast, a very recent study found that ghrelin significantly increased proliferation and migration of human microvascular endothelial cells *in vitro* (25). Finally, insulin has activated hypoxia inducible factor-1, the most potent stimulus for VEGF-A synthesis and secretion (26).

Modulation of development of the vascular network in adipose tissue may constitute a strategy to affect obesity. Therefore, it is important to obtain information on expression and functional roles of pro- and antiangiogenic components. However, most of the data come from *in vitro* studies or animal models. Very little is known about the relationship between adipogenesis and angiogenesis in humans.

The aim of this study was to investigate the relationship between body mass index (BMI) and serum concentrations of VEGF-A in obese human subjects and its modification after weight loss with bariatric surgery. We also analyzed the association of circulating VEGF-A levels with other adipocytokines and hormones as adiponectin, leptin, ghrelin, and insulin in morbidly obese patients before and after weight loss with bariatric surgery. We selected patients who underwent different surgical techniques to search for differences in the analyzed hormone levels due to different anatomical alterations.

Patients and Methods

Patients

A total of 45 obese women (age range 20–65 yr, mean age 39.3 ± 12.3), all with BMI more than 40 kg/m^2 (mean BMI 49.9 ± 8.1 kg/m^2), was included in this study. Nine patients were affected by type 2 diabetes, eight patients had hypertension, and four had obstructive sleep apnea. All of the patients underwent open surgery for morbid obesity: vertical banded gastroplasty (n = 17), gastric bypass (n = 17), and biliopancreatic diversion (BPD) (n = 11). Serum samples were obtained preoperatively and 9–12 months after surgery. We also included nine apparently healthy lean adult controls, all of them females (age range 24–30 yr, mean age 26.1 ± 2.5). The mean BMI was 20.6 kg/m^2 ± 0.7. The protocol was approved by the local ethical committee, and written informed consent was obtained from all subjects.

Laboratory methods

Subjects were instructed to fast from 2200 h the evening before blood sampling. Blood samples were obtained between 0800 and 0900 h, and were transported within 1 h to the laboratory in an insulated container. Samples were centrifuged, and the sera were divided into separate aliquots and stored at −80 C. All determinations were performed in duplicate. Serum VEGF-A was measured with the Quantikine Human VEGF Immunoassay (R&D Systems, Oxon, UK), an ELISA, with intraassay variation of 5.3% and interassay variation of 8.8%. Estimation of adiponectin, leptin, and ghrelin was performed with a RIA (LINCO Research, Inc., St. Charles, MO). The intraassay variations were 3.6, 4.6, and 5.02%, and the interassay variations were 9.2, 6.9, and 12.8% for the adiponectin, leptin, and ghrelin assays, respectively. Insulin was measured with a RIA INSIK-5 kit (DiaSorin, Vercelli, Italy), with intraassay variation less than 5% and interassay variation of 6.2%. Insulin resistance was measured by homeostasis model of assessment (HOMA) (27).

Body composition was determined in 36 patients, previously to surgery, by using a tetrapolar whole body multifrequency bioelectrical impedance analyzer (Bioscan, Barcelona, Spain). Measurements were performed in the subjects, within 30 min of voiding, in a supine position on a flat, nonconductive couch, with their limbs abducted from the trunk. A tetrapolar arrangement of gel electrodes was placed at defined anatomical sites to one hand, wrist, ankle, and foot of each patient, following the instructions of the manufacturer.

Statistical analysis

The results are expressed as mean ± SD. Nonparametric tests were applied according to the distribution of the sample. When controls and patients were compared, the Mann-Whitney *U* test or comparison of the mean, according to the dispersion of the sample, was used. The Wilcoxon signed rank test for two variables was used to compare the differences between concentrations previous to surgery and 9–12 months after surgery in all the analyzed variables. The Spearman rank correlation test was applied to study the relation between VEGF-A levels and adiponectin, leptin, ghrelin, insulin, glucose, and HOMA in controls, and in patients before and after surgery. $P < 0.05$ was considered significant. The control group was significantly younger than the patient group. Despite the fact that the variables did not follow a normal distribution to correct for age, their residuals did. Therefore, we performed a lineal regression adjusting the independent effect of each hormone on VEGF-A, and adjusting for age. All statistical analyses were done with SPSS version 12.0 (SPSS, Inc., Chicago, IL).

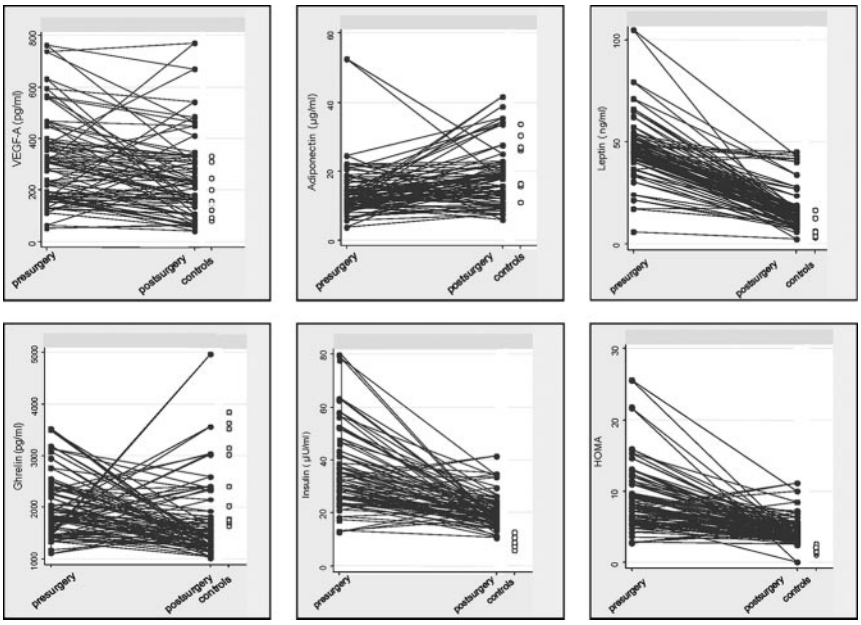

FIG. 1. VEGF-A, adiponectin, leptin, ghrelin, insulin levels, and HOMA in lean controls and morbidly obese patients before and after weight loss with bariatric surgery.

Results

The morbidly obese patients have a significantly higher concentration of circulating VEGF-A than nonobese controls (306.3 ± 170.3 *vs.* 187.6 ± 91.9 pg/ml; $P = 0.048$). In obese patients the circulating levels of VEGF-A significantly decreased with weight loss after surgery (246.1 ± 160.4 pg/ml; $P < 0.001$) (Fig. 1). The difference remained significant for every surgical technique (Table 1). Despite a significant decrease in VEGF-A levels and weight after bariatric surgery, serum VEGF-A did not show any significant correlation with weight, BMI, or fat mass measured by the bioelectrical impedance analyzer. The mean values of VEGF-A were higher, though not significantly, in obese patients after surgery than in controls.

Concentration of circulating adiponectin was significantly lower in the morbidly obese patients than in nonobese controls (13.5 ± 7.6 *vs.* 22.6 ± 7.5 µg/ml; $P = 0.028$). In obese patients the circulating levels of adiponectin significantly increased with weight loss after surgery (17.6 ± 8.4 µg/ml; $P < 0.001$) (Fig. 1). The difference remained significant for every surgical technique (Table 1). The median values of adiponectin were lower, though not significantly, in obese patients after surgery than in controls.

Serum leptin levels were significantly higher in morbidly obese patients than nonobese controls (46.4 ± 15.6 *vs.* 7.3 ± 4.2 ng/ml; $P < 0.001$) (Fig. 1). In obese patients the concentration of circulating leptin significantly decreased with weight loss after surgery (Table 1) but was still higher than in controls (16.8 ± 9.9 *vs.* 7.3 ± 4.2 ng/ml; $P = 0.033$) (Fig. 1).

Obese patients before surgery had a significantly lower concentration of circulating ghrelin than lean controls (2020.6 ± 621.8 *vs.* 2780.6 ± 837.5 pg/ml; $P = 0.012$) (Fig. 1). There was a significant decrease in ghrelin concentrations in the patients who underwent gastric bypass and BPD after surgery (1495.9 ± 333.8 and 1259.2 ± 226.0 pg/ml; $P < 0.001$, respectively), but not in those who had a vertical banded gastroplasty performed.

Presurgical insulin levels and HOMA were significantly increased in morbidly obese patients compared with lean controls (35.7 ± 15.6 *vs.* 8.5 ± 2.0 µIU/ml, $P < 0.001$; 8.7 ± 4.5 *vs.*1.6 ± 0.4 µIU/ml, $P < 0.01$, respectively) (Fig. 1), and significantly decreased with every surgical procedure (Table 1). The median values were still significantly higher in obese patients after surgery than in controls.

The percent variation of BMI did not correlate with the percent change of VEGF-A, ghrelin, leptin, adiponectin, glucose, or insulin concentrations, or with insulin resistance by HOMA.

Serum levels of VEGF-A did not correlate significantly with any of the parameters measured (ghrelin, leptin, adiponectin, glucose, insulin, or insulin resistance) before or after surgery in obese patients. The percent variation of VEGF-A levels did not correlate with the percent change in these parameters either. In healthy controls there was significant inverse correlation between VEGF-A and ghrelin levels ($r = -0.85$; $P < 0.01$).

The control group was significantly younger than the patient group. Despite the fact that the variables did not follow a normal distribution to correct for age, their residuals did. Therefore, we performed a lineal regression adjusting the independent effect of each hormone on VEGF-A, and adjusting for age. We found that the unadjusted effect of insulin and HOMA changed in relation to the adjusted effect for age, but not in the case of adiponectin, leptin, and ghrelin. We did not find and independent effect of age on VEGF-A concentrations.

Discussion

It has been reported that VEGF-A levels are significantly elevated in overweight and obese patients compared with lean subjects (28), but there are no data regarding variations in VEGF-A levels with weight loss. We showed a significant decrease in VEGF-A with weight reduction in morbidly obese subjects after bariatric surgery. In addition to the stimulation of the capillary net in the adipose tissue, VEGF-A could have some other important effects on obese subjects as the contribution to the previously documented increased risk of metastatic disease in obese subjects with prevalent cancers such as breast and prostate (29, 30). The pivotal role of VEGF-A in cancer progression is well known. In fact, new therapies for cancer include anti-VEGF-A monoclonal antibodies (bevacizumab) and VEGF-A receptor blockade (sorafenib and sunitinib). VEGF-A is also involved in endothelial IL-8 production (31), a cytokine with atherogenic properties, which is increased in obese subjects and related to fat mass (32). Thus, VEGF-A might indirectly increase the cardiovascular risk in obese patients.

TABLE 1. Levels of VEGF-A, adiponectin, leptin, ghrelin, insulin, HOMA, and glucose according to the surgical technique

	VBG 0 months (n = 17)	VBG 9–12 months (n = 17)	GBP 0 months (n = 17)	GBP 9–12 months (n = 17)	BPD 0 months (n = 11)	BPD 9–12 months (n = 11)	Total patients 0 months (n = 45)	Total patients 9–12 months (n = 45)	Controls (n = 9)
Weight (kg)	108.5 (105–127)	86 (76.7–97.5)[a]	135 (116–143.7)	89.7 (83–101.2)[a]	115 (111–130.7)	85 (80–102.7)[b]	116.5 (109.8–137.2)	87 (81–99.2)[a]	54 (52.5–56)[c]
VEGF-A (pg/ml)	305.6 (177–368.7)	224.8 (98.8–297.9)[a]	257.4 (171.3–383.9)	234.9 (159.6–332.1)[b]	327.2 (131.5–467.6)	219 (70.4–331.6)[b]	296 (171.3–385.2)	226.3 (127.1–331)[a]	157.3 (107.4–278.6)[d]
Adiponectin (μg/ml)	10.9 (9–17.6)	13.4 (9.3–19.6)[e]	12.9 (10.7–17)	17.8 (12.8–20.9)[e]	11.1 (9.4–14.1)	17.7 (14.2–33.2)[b]	11.7 (9.4–15.9)	15.7 (11.4–20.6)[a]	26.1 (15.5–28.5)[d]
Leptin (ng/ml)	42.9 (31.7–48.1)	9.5 (7.4–14.7)[a]	47.2 (41.8–51.8)	17.8 (12.3–18.6)[a]	49.5 (44–65)	15.3 (14.1–26.9)[b]	45.8 (40.7–51.2)	14.9 (10.5–18.3)[a]	5.6 (3.6–9.3)[c]
Ghrelin (pg/ml)	1718 (1455–2145.5)	1925 (1470–2805.5)	2005 (1454.7–2524)	1407 (1271.5–1610.5)[b]	1832 (1614–2431)	1215 (1103–1453)[b]	1867.5 (1469.7–2384.5)	1419 (1247.5–1868)[b]	3024 (1891.50–3578)[d]
Insulin (μIU/ml)	28.4 (24.6–39.9)	18 (14.7–20.6)[a]	34 (22.5–46.6)	20.2 (15.7–25.1)[b]	35.1 (25–52.4)	17.7 (13.4–23.3)[b]	30.9 (25–43.9)	18.2 (14.8–23.3)[a]	7.9 (7.3–9.9)[c]
HOMA (μIU/ml)	7.1 (5.4–9.2)	3.8 (3.3–4.9)[a]	7.8 (5–9.9)	4.6 (3.1–5.6)[b]	8.5 (5.9–12.9)	3.4 (2.4–4.9)[b]	7.6 (5.4–10.9)	3.8 (3.1–5.2)[a]	1.4 (1.3–1.8)[c]
Glucose (mg/dl)	92 (84–111)	90 (86–93.5)	92.5 (85.7–96.2)	88.5 (86–95)	95 (86–112)	86 (80–98)[e]	92.5 (85–106.7)	88.5 (86–94.25)[e]	79 (73.5–80)[c]

Results are expressed as median (interquartile range). GBP, Gastric bypass; VBG, vertical banded gastroplasty.

[a] $P < 0.001$, 9- to 12-month results compared with basal results in patients.

[b] $P < 0.01$, 9- to 12-month results compared with basal results in patients.

[c] $P < 0.01$, control results compared with basal results in patients.

[d] $P < 0.05$, control results compared with basal results in patients.

[e] $P < 0.05$, 9- to 12-month results compared with basal results in patients.

Adipose tissue mass is sensitive to changes in vascularization. In experimental studies adipose tissue growth is angiogenesis dependent, and several antiangiogenic molecules have prevented obesity in high-calorie diet-fed mice as well as in genetically leptin-deficient ob/ob mice (4, 5). Our data agree with previous studies supporting an antiangiogenic effect of ghrelin in human and rat *in vitro* assays and *in vivo* animal models (23, 24). These studies indicate that ghrelin exerts its effects through the inhibition of the tyrosine kinase and MAPK activities in the endothelial cells. An additional antiangiogenic effect through the inhibition of VEGF-A is possible. We showed that this reverse relationship between ghrelin and VEGF-A is present only in subjects with normal weight, but not in obese patients. Lean subjects have higher levels of circulating ghrelin that might inhibit VEGF-A production and endothelial cell proliferation. Ghrelin levels start to decrease with weight gain and, consequently, its antiangiogenic effects, favoring adipose tissue growth and perpetuating a vicious circle in obese patients. Therefore, it is possible to hypothesize that a failure in this balance between ghrelin and VEGF-A favors weight gain.

Changes in circulating ghrelin concentrations in patients undergoing bariatric surgery depend on the surgical procedure. According to our results, gastric bypass surgery has markedly suppressed ghrelin concentrations, whereas restrictive techniques even increased circulating ghrelin levels (33–35). Data on ghrelin concentrations after BPD are controversial. Some authors could not find a significant decrease in ghrelin levels after BPD (33). However, Lee *et al.* (34) demonstrated, in agreement with our results, that patients who had undergone a BPD exhibited diminished circulating ghrelin concentrations. Different studies have performed different follow-up measurements after surgery, which may explain some of the conflicting results. For example, Hanusch-Enserer *et al.* (35) found that plasma ghrelin was not different before and 6 months after gastric banding but increased significantly 12 months after surgery. Presumably, the reduction in serum/plasma ghrelin levels in derivative techniques is not determined by weight loss but, rather, depends on the surgically induced disruption of fundic-derived factors participating in food intake signaling. In contrast, variations in adiponectin, leptin, and insulin concentrations after bariatric surgery reflect the decrease in adipose tissue and are not related to the surgical procedure applied.

In our study healthy controls were significantly younger than patients. When we corrected for age, we found, in agreement with previous studies, that VEGF-A, ghrelin and leptin concentrations are not affected by age (36–38). It has been reported that circulating levels of adiponectin decline with age (37), but we did not find any effect of age on adiponectin concentrations. Nevertheless, we showed, as published previously, that insulin resistance is related to aging (39), and this could, somehow, mask the results.

Many questions remain regarding the mechanisms by which VEGF-A production is increased in obese subjects. An important source is inflammatory cytokines secreted by adipose tissue. VEGF-A can be produced by nonadipocyte stromal cells from human sc fat (40) as well as human adipose progenitor cells in response to adipocyte-derived cytokines, mainly TNF-α (41).

A limitation of this study is the fact that it has been done only in females, and results may not be valid in males in whom a separate study needs to be done.

With an increasing incidence of obesity worldwide, rational strategies are required to control adipogenesis. Preliminary evidence indicates that developing blood vessels in fat tissue may represent a potential target for regulating fat cell development. Further investigations are needed to clarify the relevance of blocking angiogenesis as a future treatment for obesity.

Acknowledgments

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This research project was supported by grants from Fundación Mutua Madrileña (2005), Universidad Complutense de Madrid (2006), and Vegenat SA.

Disclosure Statement: The authors have nothing to disclose.

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