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Involvement of serum vascular endothelial growth factor family members in the development of obesity in mice and humans☆

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Abstract

Adipose tissue is highly vascularized implying that angiogenesis takes place in its expansion. The aim of this study was to compare the concentrations of members of the vascular endothelial growth factor (VEGF) family in obesity. Serum concentrations of VEGFs were analyzed in 15 lean (BMI 20.3±2.5 kg/m^2) and 24 obese (BMI 47.6±5.9 kg/m^2) volunteers. Obese patients showed significantly increased circulating VEGF-A (150±104 *vs.* 296±160 pg/ml; $P<.05$), VEGF-B (2788±1038 *vs.* 4609±2202 arbitrary units; $P<.05$) and VEGF-C (13 453±5750 *vs.* 17 635±5117 pg/ml; $P<.05$) concentrations. Interestingly, levels of VEGF-D were reduced in obese individuals (538±301 *vs.* 270±122 pg/ml; $P<.01$). In addition, VEGF-A significantly decreased after weight loss following Roux-en-Y gastric bypass (BMI from 46.0±8.0 to 28.9±4.2 kg/m^2 $P<.0001$ *vs.* initial) from 345±229 to 290±216 pg/ml ($P<.01$). Moreover, in order to corroborate the human findings VEGF-A levels were analyzed during the expansion of adipose tissue in two dynamic models of murine obesity. Serum VEGF-A was significantly increased after 12 weeks on a high-fat diet (43.3±9.0 *vs.* 29.7±9.1 pg/ml; $P<.01$) or in *ob*/*ob* mice (52.2±18.0 *vs.* 29.2±7.7 pg/ml; $P<.01$) and was normalized after leptin replacement in the latter (32.4±14.0 pg/ml; $P<.01$ *vs.* untreated *ob*/*ob*). Our data indicates the involvement of these factors in the expansion of adipose tissue that takes place in obesity in relation to the need for increased vascularization, suggesting that manipulation of the VEGF system may represent a potential target for the pharmacological treatment of obesity.

Keywords: Obesity; VEGF; Adipose tissue; High-fat diet; *ob*/*ob* mice; Leptin

1. Introduction

Obesity is defined medically as a state of increased adipose tissue of sufficient magnitude to produce adverse health consequences [1,2]. However, the molecular mechanisms that lead to and take place in the development of obesity and the expansion of adipose tissue are not well understood [3]. Microarray technology has been applied to the analysis of gene expression profiles of adipose tissue in obese individuals in order to identify differential expression patterns and key genes involved in obesity [4]. Interestingly, this approach has drawn attention to the changes taking place in the expression of genes involved in diverse relevant physiological processes such as angiogenesis. Our group has previously shown the altered expression of genes involved in angiogenesis, namely, the up-regulation of vascular endothelial growth factor (VEGF)-B and the down-regulation of c-fos-induced growth factor (FIGF, also known as VEGF-D) in omental adipose tissue of obese patients [4].

Adipose tissue is highly vascularized implying that remodeling of the vascular network takes place in its expansion [5–7]. In this sense,

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CRP, C-reactive protein; DBP, diastolic blood pressure; EWAT, epididymal white adipose tissue; γ-GT, γ-glutamyltransferase; HDL, high-density lipoprotein; HOMA, homeostatic model assessment; LDL, low-density lipoprotein; PRWAT, perirenal white adipose tissue; QUICKI, quantitative insulin sensitivity check index; RYGBP, Roux-en-Y gastric bypass; SBP, systolic blood pressure; SCWAT, subcutaneous white adipose tissue; VEGF, vascular endothelial growth factor; WL, weight loss.

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blockade of neovascularization with angiogenesis inhibitors has been shown to effectively impair adipose tissue growth and even induce weight reduction and adipose tissue loss [5]. The crosstalk between adipocytes and endothelial cells takes place through several angiogenic regulators, which cooperatively control vessel growth [6]. VEGF is a key physiological and pathophysiological regulator of angiogenesis, and it is generally accepted that the VEGF/VEGFR system accounts for most of the angiogenic activity in adipose tissue [7]. Administration of anti-VEGF antibodies inhibits angiogenesis and the formation of adipogenic/angiogenic cell clusters, indicating that the coupling of adipogenesis and angiogenesis is essential for differentiation of adipocytes in obesity and that VEGF is a key mediator of that process [8]. The VEGF family in humans currently comprises at least five members, namely, VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor [9–12]. Several studies have evidenced alterations in members of the VEGF family in relation to obesity in mice [13–15] and humans [16,17]. The aim of the present study was to compare the concentrations of members of the VEGF family in lean and obese volunteers and to assess whether VEGF-A is changed within periods of negative energy balance. Furthermore, we aimed to analyze serum concentrations of VEGF-A in different models of obesity in mice, during active adipose tissue expansion.

2. Materials and methods

2.1. Subjects

In order to analyze the effect of obesity on VEGF-A, -B, -C and -D concentrations, 15 lean (seven male/eight female) and 24 obese (12 male/12 female) Caucasian subjects were recruited from healthy volunteers and patients attending the Department of Endocrinology at the University Clinic of Navarra. Subjects were classified according to body mass index (BMI) (lean, <25 kg/m^2; obese, ≥30 kg/m^2). Patients underwent a clinical assessment including medical history, physical examination, body composition analysis, co-morbidity evaluation as well as nutritional interviews performed by a multidisciplinary consultation team. All subjects were nonsmokers. Patients with signs of infection were excluded. Obese patients were not receiving statins, antidiabetic or antihypertensive medication. In addition, a group of 19 obese patients (seven male/12 female) were selected to prospectively assess the effect of weight loss achieved by Roux-en-Y gastric bypass (RYGBP) on the concentrations of VEGF-A. The interventions were carried out via a laparoscopic approach. The experimental design was approved, from an ethical and scientific standpoint, by the hospital's ethics committee responsible for research, and volunteers gave their informed consent to participate in all the studies.

2.2. Anthropometric measurements

Body weight was measured with a digital scale to the nearest 0.1 kg, and height was measured to the nearest 0.1 cm with a Holtain stadiometer (Holtain, Crymych, UK). BMI was calculated as weight in kilograms divided by the square of height in meters. Body fat was estimated by air-displacement plethysmography (Bod-Pod, Life Measurements, Concord, CA, USA). Data for estimation of body fat by this plethysmographic method has been reported to agree closely with the traditional gold standard, hydrodensitometry (underwater weighing). Furthermore, the Bod-Pod has been shown to predict fat mass and fat-free mass more accurately than dual-energy X-ray absorptiometry and bioelectrical impedance [18–20]. Blood pressure was measured after a 5-min rest in the semi-sitting position with a sphygmomanometer. Blood pressure was determined at least three times at the right upper arm and the mean was used in the analyses.

2.3. Analytical procedures

Blood samples were collected after an overnight fast in the morning in order to avoid potential confounding influences due to hormonal rhythmicity. Plasma glucose was analyzed by an automated analyzer (Roche/Hitachi Modular P800, Basel, Switzerland) as previously described [21,22]. Insulin was measured by means of an enzyme-amplified chemiluminescence assay (IMMULITE, Diagnostic Products Corp., Los Angeles, CA, USA). Insulin resistance was determined by means of the homeostatic model assessment (HOMA) index expressed as: glucose (mmol/L)×insulin (μU/ml)/22.5 [23]. Insulin sensitivity was calculated by using the quantitative insulin sensitivity check index (QUICKI) [24]. This index represents a simple accurate method for assessing insulin sensitivity in humans and is defined as $1/(\log[\text{insulin}_0]+\log[\text{glucose}_0])$. Total cholesterol and triglyceride concentrations were determined by enzymatic spectrophotometric methods (Roche). High-density lipoprotein (HDL) cholesterol was quantified by a colorimetric method in a Beckman Synchron CX analyzer (Beckman Instruments, Ltd., Bucks, UK). Low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald formula.

Uric acid, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, γ-glutamyltransferase (γ-GT) and creatinine were measured by enzymatic tests (Roche) in an automated analyzer (Roche/Hitachi Modular P800). Fibrinogen concentrations were determined according to the method of Clauss using a commercially available kit (Hemoliance, Instrumentation Laboratory, Barcelona, Spain). Homocysteine was determined by applying a fluorescence polarization immunoassay (Axis Biochemicals, Oslo, Norway), using an IMx analyzer (Abbott Laboratories, Abbott Par, IL, USA). Measurement of von Willebrand factor antigen was performed by a micro-latex immunoassay (Diagnostica Stago, Parsippany, NJ, USA). A standard curve was prepared with a universal reference (NISBC 91/666), and the results were expressed as percentage of the standard. Intra- and interassay coefficients of variation (CVs) were 4.0% and 8.0%, respectively. High-sensitivity C-reactive protein (CRP) was measured using the Tina-quant CRP (Latex) ultrasensitive assay (Roche). Leptin was quantified by a double-antibody RIA method (Linco Research, St. Charles, MO, USA); intra- and interassay CVs were 5.0% and 4.5%, respectively. Adiponectin was measured by ELISA (BioVendor, Brno, Czech Republic); intra- and interassay CVs were 6.7% and 7.8%, respectively. Resistin was quantified by ELISA (BioVendor). Intra- and interassay CVs were 4.3% and 6.8%, respectively. VEGF-A was determined by ELISA (R&D Systems, Minneapolis, MN, USA) with intra- and interassay CV of 5.4% and 7.3%, respectively. VEGF-C was quantified by an enzyme immunoassay (Immuno-Biological Laboratories, Hamburg, Germany); intra- and interassay CVs were 6.1% and 7.4%, respectively. VEGF-D was measured by ELISA (R&D Systems); intra- and interassay CVs were 4.3% and 7.5%, respectively.

As an immunoassay for VEGF-B was not commercially available, the serum concentrations of this protein were measured by Western blot. Briefly, 3 μl of 50-fold diluted serum was run in 12% SDS-PAGE, subsequently transferred to nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA, USA) and blocked in Tris-buffered saline with Tween 20 (TBS-T) containing 1% w/v I-Block (TROPIX, Bedford, MA, USA) for 1 h at room temperature. Blots were then incubated overnight at 4°C with an anti-human VEGF-B goat polyclonal antibody (sc-1876, Santa Cruz Biotechnology, Santa Cruz, CA, USA). The antigen–antibody complexes were visualized using peroxidase-conjugated anti-goat IgG antibody (1:5000) and the enhanced chemiluminescence ECL detection system (Amersham Biosciences, Buckinghamshire, UK). Two bands migrated at 33 and 31 kDa, respectively. Densitometric quantification of both bands corresponding to the isoforms VEGF-B$_{186}$ (33 kDa) and VEGF-B$_{167}$ (31 kDa) was performed together because it was impossible to do it separately. The intensity of the bands was determined by densitometric analysis with the Gel Doc EQ gel documentation system and Quantity One 4.5.0 software for quantitation of images (Bio-Rad).

2.4. Study in animals

Mice (all purchased from Charles River, L'Abresle, France) were maintained at an ambient temperature of 22±2°C on a 12:12 h light–dark cycle (lights on at 0800) under pathogen-free conditions. In the first experiment, 12-week-old male C57BL/6 mice were maintained during 12 weeks on a high-fat diet (n=8, rodent diet with 45 kcal% fat; D12451, Research Diets, New Brunswick, NJ, USA) or on a normal diet (n=10, rodent diet with 10 kcal% fat; D12450B, Research Diets). In a second experiment, 12-week-old male mice were treated with saline (nine wild-type and eight *ob*/*ob* mice) or leptin (PeproTech EC, London, UK) at a dose of 0.1 mg leptin/kg body weight per day intraperitoneally twice daily (seven *ob*/*ob* mice) for 18 days. In addition, a third group of *ob*/*ob* mice (n=7) was treated with saline and pair fed to the amount eaten by the leptin-treated group in order to dissociate the well-known appetite-reducing effect of leptin from other effects. Body weight and food intake were recorded. Blood samples were collected by cardiac puncture. All experimental procedures conformed to the European Guidelines for the Care and Use of Laboratory Animals, and the study was approved by the Ethical Committee for Animal Experimentation of the University of Navarra (041-2002 and 044-2003).

Serum glucose was analyzed by using a glucometer (Ascensia Elite, Bayer, Leverkusen, Germany). Serum insulin was measured by means of the Ultra Sensitive Mouse Insulin ELISA kit (Crystal Chem, Chicago, IL, USA). Intra- and interassay CVs were 5.5% and 4.8%, respectively. Circulating concentrations of leptin were measured by ELISA (Crystal Chem). Intra- and inter-assay CVs were 5.4% and 6.9%, respectively. Serum VEGF-A was determined by a Mouse VEGF Immunoassay (R&D Systems). Intra- and inter-assay CVs were 5.7% and 6.8%, respectively.

2.5. Statistical analysis

Data are presented as mean±S.D. Differences between groups of subjects were analyzed by two-tailed unpaired t tests. The effect of weight loss after RYGBP was analyzed by two-tailed paired t tests. In the mice experiments, differences were analyzed by two-tailed unpaired t test (high-fat experiment) and ANOVA followed by LSD test (*ob*/*ob* mice study). Correlations between two variables were assessed by Pearson's (r) or Spearman rank (ρ) correlation coefficients as appropriate. The calculations were performed using the SPSS statistical package for Windows version 15.0.1 (SPSS, Chicago, IL, USA). A P value less than .05 was considered statistically significant.

3. Results

3.1. VEGF Family members in obese patients

Clinical characteristics of the patients are summarized in Table 1. No statistically significant differences for age were found between the groups. Increased concentrations of glucose (P=.001) and insulin (P<.001) were observed in the obese subjects, who also showed higher HOMA index (P<.001) and lower insulin sensitivity than lean individuals as evidenced by the lower QUICKI (P<.0001). Circulating concentrations of total- (P<.001) and LDL cholesterol (P<.0001) were significantly increased in the obese individuals, while HDL cholesterol was reduced (P<.0001). The inflammatory markers fibrinogen and CRP were significantly increased (P<.0001) in obese patients. Obese individuals exhibited increased levels of ALT (P=.001) and AST (P<.05). As expected, obese patients showed hyperleptinemia (P<.0001) and hypoadiponectinemia (P<.01).

Obese subjects showed significantly increased circulating VEGF-A (P<.01), VEGF-B (P<.01) and VEGF-C (P<.05) concentrations (Table 2). Interestingly, concentrations of VEGF-D were significantly reduced in obese individuals (P<.01). No sexual dimorphism was observed for any of the VEGFs analyzed. A significant correlation was observed between BMI and VEGF-A (r=0.50, P<.001), VEGF-B (r=0.40, P<.05) and VEGF-D (r=−0.47, P<.001), while only a marginal association was detected with VEGF-C (r=0.31, P=.056). Body fat percentage was significantly correlated with VEGF-A (r=0.45, P<.01), VEGF-B (r=0.43, P<.01), VEGF-C (r=0.37, P<.05) and VEGF-D (r=−0.47, P<.001). Regarding endothelial function and inflammation, VEGF-A, VEGF-B, and VEGF-C concentrations were significantly correlated with CRP levels (r=0.35, P<.05; r=0.34, P<.05; and r=0.42, P<.05, respectively), while VEGF-D concentrations were associated with fibrinogen (r=−0.34, P<.05), von Willebrand factor (r=−0.27, P<.05) and CRP levels (r=−0.46, P<.001). All members of the VEGF family analyzed were significantly correlated with leptin: VEGF-A (r=0.44, P<.01), VEGF-B (r=0.43, P<.01), VEGF-C (r=0.37, P<.05) and VEGF-D (r=−0.47, P<.001). However, these associations were lost after adjusting for body fat percentage, suggesting that they are merely reflecting the correlation between the cardiovascular risk factors studied and adiposity. A detailed correlation analysis is provided in Table 3.

Table 1
Subject characteristics

	Lean (n=15)	Obese (n=24)	P
Sex, male/female	7/8	12/12	–
Age, y	33.5±11.2	34.4±8.4	.773
BMI, kg/m^2	20.3±2.5	47.6±5.9	<.0001
Body fat, %	18.3±4.5	50.8±5.9	<.0001
SBP, mmHg	110±11	125±19	<.05
DBP, mmHg	72±8	80±14	.096
Glucose, mmol/L	4.44±0.44	5.44±1.22	.001
Insulin, pmol/L	42.2±18.3	131.3±98.3	<.001
HOMA	1.16±0.52	4.48±3.61	<.001
QUICKI	0.383±0.034	0.319±0.028	<.0001
Triglycerides, mmol/L	0.65±0.22	1.51±0.95	.002
Cholesterol, mmol/L	4.00±0.83	5.07±0.83	<.001
LDL-Cholesterol, mmol/L	2.23±0.62	3.34±0.80	<.0001
HDL-Cholesterol, mmol/L	1.52±0.31	1.06±0.18	<.0001
Uric acid, mg/dl	4.1±0.8	6.5±1.6	<.0001
Fibrinogen, mg/dl	171±34	307±106	<.0001
Homocysteine, μmol/L	5.9±1.6	9.2±6.9	.080
CRP, mg/L	0.8±0.3	10.3±7.8	<.0001
Von Willebrand factor, %	84±34	115±48	<.05
ALT, UI/L	10.3±7.2	29.1±21.3	.001
AST, UI/L	10.8±7.8	18.3±12.8	<.05
Phosphatase alkaline, UI/L	68±21	111±34	<.0001
γ-GT, UI/L	12±4	28±28	0.008
Leptin, ng/ml	6.9±2.9	44.1±17.6	<.0001
Adiponectin, μg/ml	10.4±4.5	6.2±3.3	<.01
Resistin, ng/ml	7.4±2.0	9.0±2.9	.076

Data presented as mean±S.D. Differences between groups were analyzed by two-tailed unpaired t tests. P, Lean *vs.* obese individuals.

Table 2
Serum concentrations of VEGF-A, VEGF-B, VEGF-C and VEGF-D in lean and obese subjects

	Lean (n=15)	Obese (n=24)	P
VEGF-A, pg/ml	150±104	296±160	<.01
VEGF-B, au	2788±1038	4609±2202	<.01
VEGF-C, pg/ml	13 453±5750	17 635±5117	<.05
VEGF-D, pg/ml	538±301	270±122	<.01

au=Arbitrary unit. Data presented as mean±S.D. Differences between groups of subjects were analyzed by two-tailed unpaired t tests. P, Lean *vs.* obese individuals.

Table 3
Unadjusted correlation analysis between VEGFs and other variables

	VEGF-A	VEGF-B	VEGF-C	VEGF-D
Age	0.21	−0.12	0.04	−0.08
	0.203	0.488	0.823	0.507
BMI	0.50	0.40	0.31	−0.47
	0.001	0.012	0.056	<0.001
Body fat	0.45	0.43	0.37	−0.47
	0.005	0.007	0.022	<0.001
SBP	0.33	−0.14	0.08	−0.01
	0.089	0.471	0.692	0.989
DBP	0.16	−0.08	0.12	−0.14
	0.432	0.671	0.527	0.289
Glucose	0.30	0.08	0.42	−0.12
	0.065	0.619	0.008	0.332
Insulin	0.11	0.26	0.17	−0.26
	0.504	0.112	0.290	0.035
HOMA	0.14	0.25	0.26	−0.22
	0.388	0.137	0.115	0.070
QUICKI	−0.26	−0.29	−0.32	0.44
	0.118	0.082	0.050	<0.001
Triglycerides	0.06	0.35	0.14	−0.14
	0.728	0.045	0.435	0.276
Cholesterol	0.10	0.14	0.14	−0.11
	0.587	0.452	0.433	0.382
LDL-Cholesterol	0.24	0.19	0.19	−0.26
	0.180	0.295	0.280	0.049
HDL-Cholesterol	−0.36	−0.45	−0.29	0.41
	0.037	0.008	0.095	<0.001
Uric acid	0.32	0.32	0.18	0.26
	0.060	0.058	0.276	0.039
Fibrinogen	0.03	0.30	0.28	−0.34
	0.857	0.073	0.094	0.011
Von Willebrand factor	0.06	0.24	0.10	−0.27
	0.724	0.167	0.575	0.048
Homocysteine	−0.09	0.13	0.23	−0.20
	0.605	0.462	0.167	0.153
CRP	0.35	0.34	0.42	−0.46
	0.041	0.045	0.012	<0.001
ALT	0.37	0.08	0.19	−0.23
	0.026	0.640	0.247	0.063
AST	0.25	−0.07	0.15	−0.13
	0.141	0.675	0.361	0.300
Phosphatase alkaline	0.19	0.33	0.17	−0.28
	0.262	0.040	0.298	0.025
γ-GT	0.34	0.32	0.20	−0.13
	0.040	0.053	0.229	0.320
Creatinine	−0.21	−0.01	0.08	−0.09
	0.212	0.996	0.626	0.494
Leptin	0.44	0.43	0.37	−0.48
	0.007	0.009	0.026	<0.001
Adiponectin	−0.27	−0.31	−0.22	0.09
	0.108	0.057	0.190	0.647
Resistin	0.07	0.10	0.18	−0.01
	0.682	0.567	0.281	0.960

Data are Pearson's correlation coefficients (upper value) and associated P (lower) values.

Table 4
Effect of surgically induced weight loss in obese patients

	Before WL	After WL	P
Sex, M/F	7/12	–	–
Age, years	36.2±13.5	37.5±13.3	–
Body weight, kg	133±33	84±19	<.0001
BMI, kg/m^2	46.0±8.0	28.9±4.2	<.0001
Body fat, %	52.0±5.0	31.2±8.9	<.0001
Waist circumference, cm	127±17	92±12	<.0001
Waist-to-hip ratio	0.92±0.07	0.87±0.06	<.01
Glucose, mmol/L	5.22±0.67	4.50±0.50	<.001
Insulin, pmol/L	145.7±90.4	50.2±27.3	<.0001
HOMA	4.9±2.9	1.5±0.8	<.0001
QUICKI	0.311±0.023	0.373±0.040	<.0001
Triglycerides, mmol/L	1.24±0.44	0.87±0.27	<.01
Cholesterol, mmol/L	5.25±1.03	4.01±1.03	<.0001
LDL Cholesterol, mmol/L	3.59±0.88	2.35±0.67	<.0001
HDL Cholesterol, mmol/L	1.06±0.23	1.24±0.41	<.01
Uric acid, mg/dl	6.1±1.7	4.5±1.1	<.001
Fibrinogen, mg/dl	342±104	334±87	.758
Von Willebrand factor, %	116±45	99±39	.098
Homocysteine, μmol/L	7.6±2.6	6.9±2.0	.374
CRP, mg/L	9.6±7.8	2.3±3.5	<.01
ALT, UI/L	30±20	21±8	.085
AST, UI/L	17±7	16±5	.842
Phosphatase alkaline, UI/L	121±37	126±43	.654
γ-GT, UI/L	23±11	12±7	<.0001
Creatinine, mg/dl	0.87±0.15	0.79±0.11	<.05
Leptin, ng/ml	53.2±25.8	20.5±14.3	<.0001

Data presented as mean±S.D. Differences between groups of subjects were analyzed by two-tailed paired *t* tests. *P*, Before *vs.* after WL.

3.2. Effect of weight loss on VEGF-A concentrations in obese patients

After an average of 15 months following bariatric surgery, patients who had undergone RYGBP experienced a significant (P<.0001) decrease in body weight, BMI and body fat (Table 4) as well as a significant improvement in the initial glycemia (P<.001), insulinemia (P<.0001), and HOMA and QUICKI indices (P<.0001). Triglyceride concentration (P<.01), as well as total- (P<.0001) and LDL cholesterol (P<.0001) concentrations, was significantly reduced, while HDL-cholesterol levels were significantly increased (P<.01). Leptinemia (P<.0001) and CRP concentrations (P<.01) were also significantly improved. Loss of excess weight induced a statistically significant reduction in circulating VEGF-A concentrations (Fig. 1), which decreased from 345±229 to 290±216 pg/ml (P<.01).

3.3. Serum VEGF-A concentrations in mice on a high-fat diet

To corroborate the human findings regarding VEGF-A and to further explore the potential role of VEGF-A in developing obesity, a series of experiments were performed in two different murine models of obesity. After 12 weeks on a high-fat diet, mice exhibited a greater increase in body weight than those on a chow diet (34±11 *vs.* 22±8%; P<.05). Epididymal (P<.05), subcutaneous (P<.01) and perirenal (P<.01) fat depot weights, as well as leptin concentrations (P<.05), were increased in the mice on high-fat diet (Table 5). As observed in Fig. 2, weight gain in mice on the high-fat diet was accompanied by increased serum concentrations of VEGF-A (43.3±9.0 *vs.* 29.7±9.1 pg/ml; P<.01). Serum levels of VEGF-A were significantly correlated with body weight gain (r=0.49, P<.05) and subcutaneous depot weight (r=0.49, P<.05). No correlation was found between VEGF-A and leptin concentrations (r=0.38, P=.135).

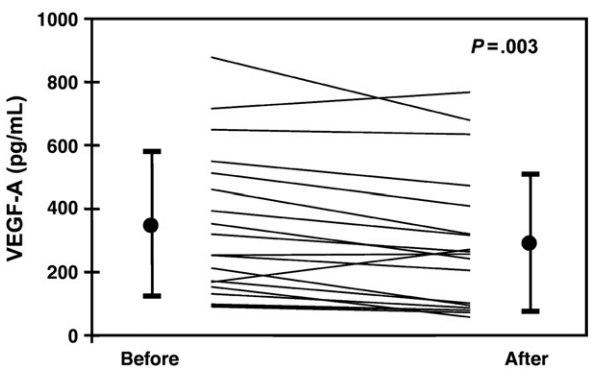

Fig. 1. Comparison of serum concentrations of VEGF-A determined in 19 obese patients before and after weight loss following RYGBP. Values are means±S.D. Statistical differences were assessed by two-tailed paired Student's *t* test.

Table 5
Body and adipose tissue weights together with circulating leptin concentrations in C57BL/6 mice after 12 weeks on a high-fat diet

	Chow diet (n=10)	High-fat diet (n=8)	P
Initial weight, g	22.4±1.4	21.9±1.5	.468
Final weight, g	27.3±2.2	29.4±3.6	.139
Weight gain, g	4.9±1.7	7.5±2.6	<.05
EWAT weight, g	0.44±0.14	0.84±0.45	<.05
SCWAT weight, g	0.24±0.06	0.40±0.16	<.01
PRWAT weight, g	0.23±0.10	0.45±0.21	<.05
Leptin, ng/ml	2.59±1.41	6.93±5.74	<.05

Data presented as mean±S.D. Differences were analyzed by two-tailed unpaired *t* test. *P*, Chow *vs.* high fat.

3.4. Serum VEGF-A concentrations in ob/ob mice. Effect of leptin replacement

As expected, leptin-deficient obese *ob*/*ob* mice showed increased body weight, food intake and serum insulin levels as compared to wild-type mice (Table 6). As depicted in Fig. 3, serum concentrations of VEGF-A were significantly increased in *ob*/*ob* mice as compared to wild-type littermates (52.2±18.0 *vs.* 29.2±7.7 pg/ml; P<.01). Leptin treatment significantly reduced body weight, food intake and insulin concentrations after 18 days of treatment. Serum VEGF-A levels were normalized after leptin replacement (32.4±14.0 pg/ml; P<.01 *vs.* untreated *ob*/*ob*). Pair-fed *ob*/*ob* mice exhibited an improvement in VEGF-A concentrations, showing a half-way phenotype between leptin- and saline-treated *ob*/*ob* mice. Serum levels of VEGF-A were significantly correlated with body weight increase during the treatment period (ρ=0.52, P<.01) (Fig. 3) and with serum insulin concentrations (ρ=0.49, P<.01).

4. Discussion

The adipose organ is unique because of its extraordinary growth potential. Accumulating scientific evidence indicates that angiogenesis is a process involved in the expansion of adipose tissue that takes place in obesity [5,6,25]. The present study shows that obese subjects exhibit increased circulating concentrations of the members of the VEGF family — VEGF-A, VEGF-B and VEGF-C — and reduced levels of VEGF-D. In addition, negative energy balance and the subsequent weight loss following RYGPB produce a significant reduction of serum VEGF-A concentrations. Furthermore, body weight increase in two different mice models of obesity, following a high-fat diet or due to leptin deficiency, is accompanied by an important increase in serum levels of VEGF-A.

Several authors have reported increased levels of VEGF-A in human obesity [16,26,27], although others have failed to find the same results [28]. Another study has described higher levels of VEGF-C and VEGF-D in obese subjects, which was only detected in women after gender segregation [27]. To our knowledge, this is the first study reporting increased concentrations of VEGF-B in obese individuals.

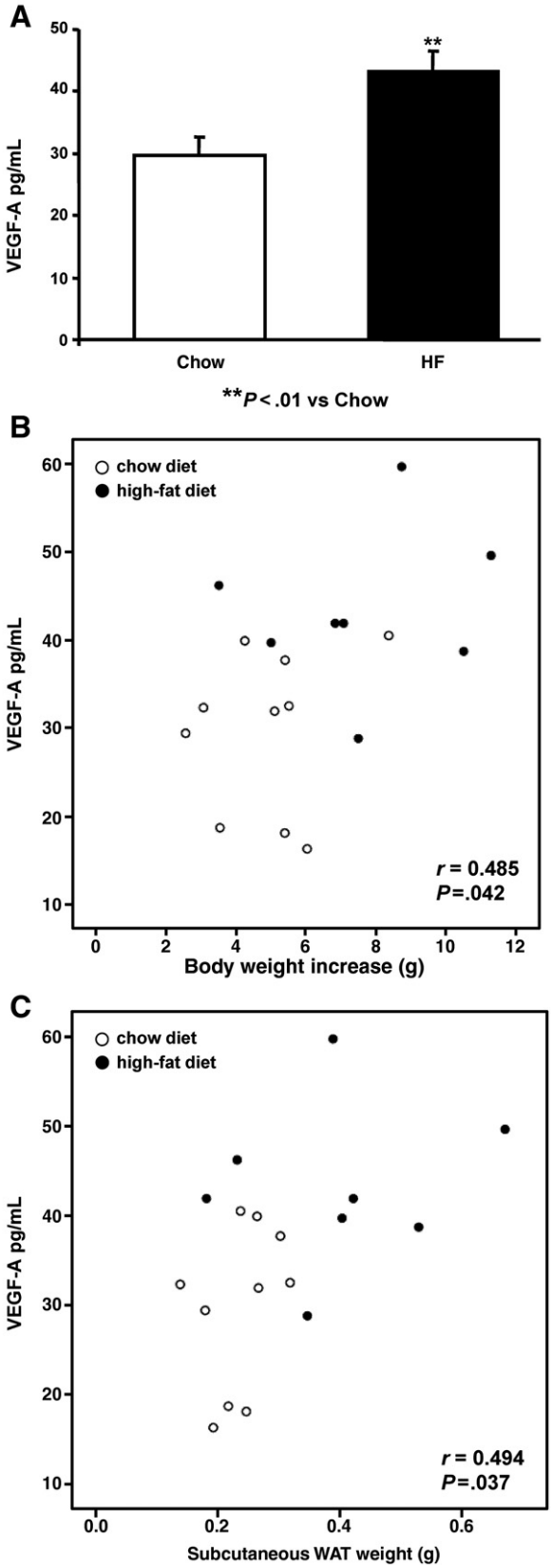

Fig. 2. Comparison of serum concentrations of VEGF-A of 12-week-old male C57BL/6 mice fed a chow (n=10, white bar) or a high-fat (n=8, black bar) diet during 12 weeks (A). Values are means±S.D. Statistical differences were assessed by two-tailed unpaired Student's t test. Correlations of body weight gain (B) and subcutaneous adipose tissue weight (C) with circulating concentrations of VEGF-A of high-fat diet (●) or chow diet (○) fed mice. Pearson's correlation coefficients (r) and P values are indicated.

Table 6
Body weight, food intake and serum insulin concentrations in *ob*/*ob* mice

	Wild type (n=9)	*ob*/*ob* (n=8)	*ob*/*ob*+Leptin (n=7)	*ob*/*ob* Pair fed (n=7)
Initial weight, g	22.9±0.9	41.3±2.0***	40.9±2.6***	42.4±1.9***
Final weight, g	23.5±1.1	48.3±1.8***	43.2±2.4***,†	44.4±2.0***,†
Food intake, g	3.2±0.2	5.5±0.5***	4.6±0.6***,†	4.7±0.6***,†
Insulin, ng/ml	0.2±0.1	14.2±3.1***	5.3±4.3**,†	16.4±4.9***,¶

Data presented as mean±S.D. Differences were analyzed by ANOVA followed by LSD test.
** P<.01 *vs.* wild type.
*** P<.001 *vs.* wild type.
† P<.001 *vs.* *ob*/*ob*.
¶ P<.001 *vs.* *ob*/*ob*+leptin.

VEGF-B is an angiogenic factor with mitogenic effect on endothelial cells, which promotes angiogenesis and vascular permeability and potentiates the effects of VEGF-A [12]. The increased VEGF-B and reduced VEGF-D circulating concentrations observed in obesity in the present study are in accordance with our previous work reporting mRNA expression of these genes in omental adipose tissue [4] that was further confirmed in the case of VEGF-D by another group [29].

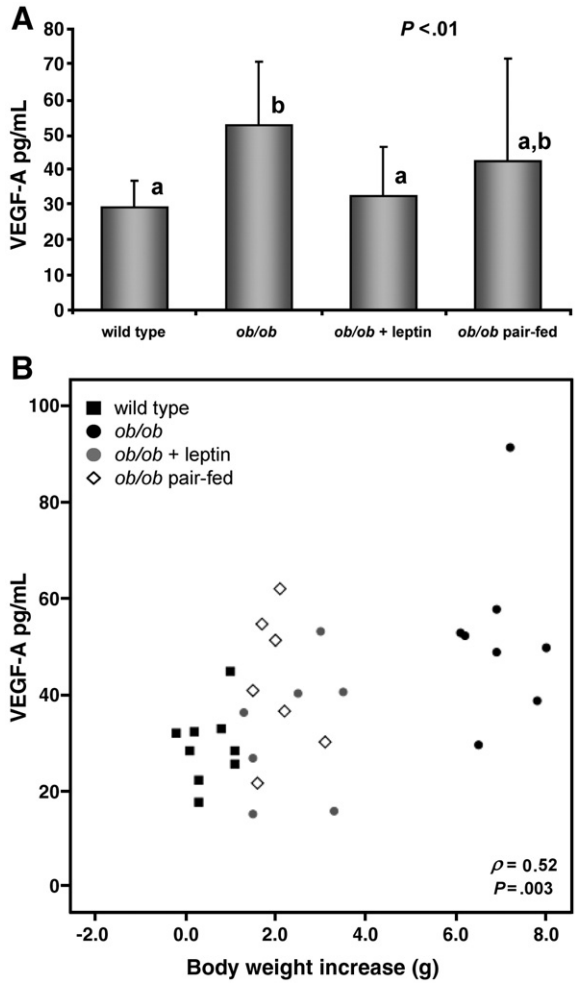

Fig. 3. Serum concentrations of VEGF-A in C57BL/6 wild-type and *ob*/*ob* mice receiving either vehicle or leptin. Serum concentrations of VEGF-A (A). Values are means±S.D. Columns not sharing a common superscript letter are significantly different (P<.01) by ANOVA followed by LSD tests. Correlations of body weight increase with circulating concentrations of VEGF-A (B). Spearman's rank correlation coefficient (ρ) and P value are indicated.

Increased serum VEGF-A, VEGF-B and VEGF-C levels in obesity could be the result of normal expression and secretion from an increased fat mass and/or increased expression and secretion from adipose or other tissues. The opposite has to be considered for VEGF-D. The discrepancies regarding VEGF-D between the study of Silha et al. [27] and ours are difficult to explain given the similarities in the sample population and the fact that the same commercial kit was used in both studies for measuring VEGF-D. This controversy needs further research in order to clarify the potential role of VEGF-D in obesity. As VEGF-D is mainly lymphangiogenic [12], the decrease in VEGF-D levels reported herein may be in relation to some cases of lymphatic vascular dysfunction related to human obesity [30]. However, it has been recently described that VEGF-D is unlikely to play an important role in adipose tissue development at least in mice [31]. The potential involvement of VEGF-D in adipose tissue pathophysiology together with getting more insight into its exact role in human obesity warrants further investigation.

A reciprocal regulation of adipogenesis and angiogenesis has been suggested, with the blockade of VEGF signaling inhibiting *in vivo* adipose tissue formation [32]. In this sense, it has been reported that inhibition of VEGFR tyrosine kinases impairs the development of adipose tissue in murine models of obesity [15]. Furthermore, an intense crosstalk between angiogenic vessels and preadipocytes has been proposed which will determine both processes, adipogenesis and angiogenesis [8,32–34].

VEGFs regulate both physiological and pathophysiological angiogenesis and are involved in vascular maintenance [12]. In this sense, VEGFs have received much attention regarding their potential involvement in endothelial dysfunction, atherogenesis and plaque instability, and myocardial and peripheral ischemia [12]. Members of VEGF family are involved in vascular inflammation and remodeling through increased proinflammatory and angiogenic mechanisms, and the elevated VEGF-A levels reported in obesity have been related to endothelial function rather than to adipose tissue angiogenesis [16]. In this sense, VEGF proteins have been related to an enhancement of sprouting as well as tube formation of vascular endothelial cells and to the pathological neovascularization observed in atherosclerosis [16]. We found several associations of the members of the VEGF family with markers of endothelial dysfunction or inflammation, which were lost after adjusting for body fat percentage. This observation indicates that these associations seem to be merely reflecting the correlation between the cardiovascular risk factors analyzed and adiposity. Furthermore, it also suggests that the changes observed in obese patients regarding VEGFs levels are more in relation to anthropometric variables and increased adipose tissue mass than to endothelial dysfunction or inflammation, although a relation to and/or an effect on obesity-related cardiovascular derangements cannot be discarded.

Because of its higher angiogenic potency, we analyzed the effect of negative energy balance following RYGBP on serum VEGF-A levels. The dramatic weight loss observed 15 months after the gastric bypass was accompanied by a significant decrease of VEGF-A concentrations. Similar findings have been observed after dietetic intervention [16] and other bariatric surgery procedures [35]. However, VEGF-A tended to increase without statistically significant differences after weight loss following a 6-month exercise program [26]. These discrepancies in the changes in VEGF-A after weight loss depending on the degree of the negative energy balance induced may be explained through an exercise-induced increase in the expression of VEGF-A by the skeletal muscle or by the relatively small BMI change (from 29.0 to 28.0 kg/m^2) [26]. Taken together, these observations indicate that adipose tissue reduction is accompanied by a concomitant decrease of circulating VEGF-A levels.

To corroborate the human findings and to further explore the potential role of VEGF-A in developing obesity, a series of experiments were performed in two different mice models of obesity. After 12 weeks on a high-fat diet, weight gain was accompanied by increased serum concentrations of VEGF-A. Our data are in accordance with previous findings in mice [36] and rats [37]. Serum VEGF-A levels were associated with body weight gain and with subcutaneous fat weight, but not with any other adipose tissue depot weight. Higher expression of VEGF-A in visceral adipose tissue has been reported in mice [14] and humans [17,38]. Furthermore, one group has observed that serum VEGF-A is associated with visceral adipose area in humans [16]. However, other authors have reported that VEGF-A correlates with subcutaneous adipose tissue area but not with the visceral depot in overweight men [26]. Although we have not assessed VEGF-A expression or secretion from adipose tissue, our data suggest that subcutaneous adipose tissue may contribute significantly to circulating VEGF-A levels or, reciprocally, its size may be regulated by VEGF-A levels.

Furthermore, in a different murine model of obesity, the leptin-deficient *ob*/*ob* mice, increased serum concentrations of VEGF-A were also observed. Paradoxically, a recent study reported normal expression of VEGF-A in epididymal adipose tissue and skeletal muscle of *ob*/*ob* mice [39]. Although VEGF-A is widely expressed and may regulate angiogenesis and vascular permeability in many tissues, its expression levels are particularly high in tissues such as the lungs, the liver and the kidneys [40,41]. However, increased plasma levels of VEGF-A and expression in adipose tissue have been reported in obese *db*/*db* mice, which are also obese due to the lack of functional leptin receptors [14]. Interestingly, leptin treatment, which significantly reduced body weight, concomitantly reduced serum levels of VEGF-A, independently of the reducing effects on appetite, as evidenced by the halfway phenotype observed in pair-fed *ob*/*ob* mice. To our knowledge, this is the first study analyzing the circulating concentrations of VEGF-A in *ob*/*ob* mice and the effect of leptin replacement. Although leptin has been associated with an *in vitro* stimulating effect on VEGF-A expression [42], our *in vivo* study suggests that leptin induces a systemic reduction in VEGF-A concentrations probably in relation to the reduction in body weight and adipose mass. On the other hand, the reduction of VEGF-A after leptin replacement may be in relation to the improvement observed in insulin levels. In this sense, we found a significant correlation between serum VEGF-A and insulin concentrations in agreement with a previous study in rats [37], while insulin has been shown to positively stimulate VEGF-A expression in adipose tissue [43,44].

In summary, the present study seems to indicate that the members of the VEGF family play a role in obesity. Data from obese patients and different murine obesity models suggests the involvement of these factors in the adipose tissue expansion that takes place in obesity. The exact role of the members of the VEGF family in obesity development still remains unclear as does the potential usefulness of anti-VEGF therapies in obesity treatment.

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