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Endothelial Progenitor Cells in Morbid Obesity

– Pathogenetic Implications –

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Background: The aim of this study was to assess the relationship among anthropometric indexes of adiposity (body mass index [BMI], waist circumference [WC]), endothelial progenitor cells (EPC) and carotid intima-media thickness (IMT) in patients with morbid obesity, and the effect of diabetes and weight loss.

Methods and Results: BMI, WC, IMT and circulating EPC (defined as CD34+/KDR+/CD45- cells) were assessed in 100 patients (37 with diabetes). Fifty patients underwent bariatric surgery, and in 48 of them a complete re-assessment after an average follow-up of 252±108 days was carried out. In 29 of them subcutaneous and visceral adipose tissue samples were obtained at the time of intervention and analyzed for the presence and number of EPC. EPC were directly correlated with weight, BMI, WC and insulin level, and inversely with mean IMT. All correlations were confined to non-diabetic patients. EPC were found in both subcutaneous and visceral adipose tissue specimens. Circulating EPC significantly decreased after weight loss (P=0.002).

Conclusions: EPC are positively related to markers of adiposity in severe obesity, when not complicated by diabetes. Weight loss is associated with decrease in EPC level. EPC are inversely correlated with IMT, confirming their protective role also in severe obesity. Diabetes has a negative modulating action. (*Circ J* 2014; **78:** 977–985)

Key Words: Diabetes; Endothelial progenitor cell; Obesity; Weight loss

Obesity is a recognized risk factor for coronary artery disease (CAD), but the strength of the relationship between the degree of obesity and CAD is controversial.[1] Indeed, although morbid obesity is a strong predictor of premature death,[2] the prevalence of cardiovascular disease in severely obese subjects (body mass index [BMI] >40 kg/m^2) is still largely unknown.[3]

Interestingly, post-mortem studies including large numbers of morbidly obese subjects consistently identified an unexpectedly low prevalence of severe coronary atherosclerosis in this patient type.[4–8] Accordingly, we previously found that insulin-sensitive morbidly obese subjects have preserved vascular function associated with an increased level of circulating endothelial progenitor cells (EPC).[9] These findings could help explain the protection against atherosclerosis observed in this patient type,[10] given that EPC provide an endogenous repair mechanism, counteracting ongoing risk factor-induced endothelial injury. Level, function and migratory capacity of EPC are reduced not only in patients with established CAD but also in those with cardiovascular risk factors, including obesity and, to a greater extent, diabetes mellitus (DM).[11,12] Information on the role of EPC in the setting of morbid obesity, however, is still very limited.

The aim of the present study was to assess the relationship between fat tissue amount and circulating EPC in morbid obesity, whether this relationship influences sub-clinical atherosclerosis and the eventual impact of DM. Finally, in order to further clarify the link between fat tissue amount and EPC, we investigated the presence of CD34+/KDR+/CD45- cells in adipose tissue and the impact of weight loss, achieved by bariatric surgery, on their circulating level.

Methods

Subjects and Design

The participants were enrolled from among those referred for evaluation for bariatric surgery at the Obesity Clinic of the Agostino Gemelli Hospital, at the Catholic University of the Sacred Heart in Rome, from May 2009 to December 2010. We

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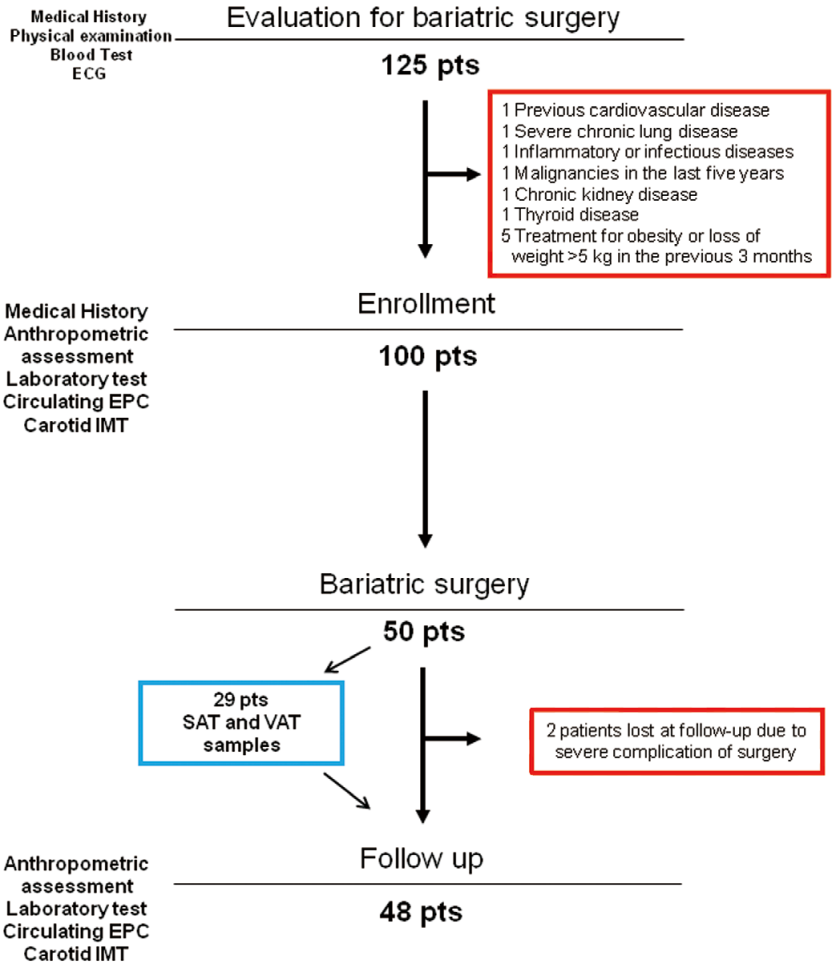

Figure 1. Subject selection. One-hundred and twenty-five obese people undergoing evaluation for bariatric surgery were screened on medical history, physical examination, blood tests and electrocardiography (ECG). Initial exclusion criteria were those reported in the first dashed panel. Cardiovascular disease was ruled out on the basis of medical history, history of coronary revascularization procedures, or the presence of left bundle branch block, Q or QS waves on ECG. Chronic kidney disease was defined as creatinine clearance $<60\,ml \cdot min^{-1} \cdot 1.73\,m^{-2}$. We finally enrolled 100 obese patients. Anthropometric characteristics, laboratory tests, circulating endothelial progenitor cell (EPC) level and carotid intima-media thickness (IMT) were assessed. Fifty participants underwent bariatric surgery. In 29 of them paired subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) samples were obtained at the moment of surgical intervention and were analyzed for the presence and number of CD34+/KDR+/CD45- cells. In 48/50 patients who underwent bariatric surgery a complete re-assessment after an average follow-up of 252±108 days was obtained. Two patients were lost at follow-up, due to severe complications of surgery, including 1 death. Dotted lines represent subjects initially excluded from the study or lost at follow-up.

screened 125 consecutive obese patients. Participants had to be in good general health, with a normal medical history and physical examination. Twenty-five obese patients were excluded because they fulfilled the pre-specified exclusion criteria (Figure 1).

Thus, we enrolled 100 patients (43 male; BMI, 47.4±7.3 kg/m^2; age, 41.8±9.3 years; 37 with DM [DM+] and 63 without [DM–]).

Medical history including cardiovascular risk factors (age, gender, DM according to American Diabetes Association criteria, dyslipidemia according to National Cholesterol Education Program screening criteria, hypertension according to Joint National Committee 7 criteria, cigarette smoking [>1 cigarette/day] and family history of early CAD), full anthropometric assessment and routine laboratory tests were obtained for all participants. In addition, circulating EPC level and carotid intima-media thickness (IMT) were measured in all patients.

A total of 50 of the 100 patients underwent bariatric surgery. In 29 of them (10 DM+ and 19 DM–), paired subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) specimens were obtained at the time of surgery. Biopsies were analyzed for the presence and number of CD34+/KDR+/CD45- cells, as described herein.

In 48 of the 50 patients who underwent bariatric surgery, a complete re-assessment, including EPC and IMT measurements, was obtained after an average follow-up of 252±108 days. Two patients were lost at follow-up, due to severe complications of surgery, including 1 death.

This study complies with the Declaration of Helsinki; the lo-

Table 1. Baseline Subject Characteristics

	Overall (n=100)	DM(–) (n=63)	DM(+) (n=37)	P-value
Age (years)	41.8±9.3	41.7±10.1	42.1±7.7	0.850
Sex (F/M)	57F/43M	38F/26M	19F/17M	0.536
Weight (kg)	135.9±27.2	133.9±27.6	139.6±26.6	0.314
BMI (kg/m^2)	47.4±7.3	46.5±7.3	49.0±7.2	0.096
Fat mass (kg)	63.6±14.9	61.9±12.9	66.6±17.8	0.181
Fat-free mass (kg)	71.3±18.4	70.1±17.6	73.6±19.9	0.424
WC (cm)	136.3±13.3	134.5±13.5	138.7±12.8	0.228
Smokers	28 (28)	16 (25.3)	12 (32.4)	0.494
Family history of CAD	30 (30)	19 (30.1)	11 (29.7)	0.999
Hypertension	51 (51)	25 (39.7)	26 (70.2)	0.004
OSAS	46 (46)	26 (41.3)	20 (54.1)	0.299
Total cholesterol (mg/dl)	185.2±37.1	186.9±38.5	182.2±35.5	0.545
LDL-C (mg/dl)	110.0±35.9	115.4±34.5	100.7±31.3	0.041
HDL-C (mg/dl)	43.9±14.7	44.9±13.5	42.1±16.9	0.371
Triglycerides (mg/dl)	166.0±89.3	145.1±81.0	201.6±92.8	0.002
Fasting glucose (mg/dl)	113.1±40.8	92.8±12.4	147.8±48.5	<0.001
Fasting insulin (μU/ml)	17.5±11.6	17.8±11.9	16.9±12.2	0.754
Glycated hemoglobin (%)	6.6±1.7	5.7±1.3	7.9±1.6	<0.001
HOMA-IR	4.6±3.6	4.0±2.6	5.9±5.0	0.026
Metabolic syndrome	69 (69)	36 (57.1)	33 (89.2)	<0.001
Maximum IMT (mm)	0.820±0.253	0.771±0.238	0.901±0.259	0.012
Mean IMT (mm)	0.693±0.209	0.641±0.195	0.781±0.204	0.001
Circulating EPC (%)	0.0060±0.0096	0.0067±0.0109	0.0047±0.0070	0.317
EPC in SAT (%)	0.3745±0.8835	0.4132±0.066	0.3008±0.3974	0.751
EPC in VAT (%)	0.7092±2.2001	0.9471±0.1817	0.2571±0.3602	0.432
Statins	6 (6)	3 (4.7)	3 (8.1)	0.667
Insulin	7 (7)	0 (0)	7 (18.9)	<0.001
Oral hypoglycemic drugs	25 (25)	2 (3.1)	23 (62.1)	<0.001

Data given as mean±SD or n (%).
BMI, body mass index; CAD, coronary artery disease; DM, diabetes mellitus; EPC, endothelial progenitor cells; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; IMT, intima-media thickness; LDL-C, low-density lipoprotein cholesterol; OSAS, Obstructive sleep apnea syndrome; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WC, waist circumference.

cally appointed ethics committee approved the research protocol, and informed consent was obtained from all subjects.

Anthropometric Measurements

Anthropometric measurements were taken according to standardized procedures. Height was measured in centimeters using a stadiometer. Weight, percent body fat and fat-free mass were measured with Tanita bioimpedance balance (Tanita International Division, West Dryton, UK). Waist circumference (WC) was measured just above the uppermost lateral border of the right ileum using the National Health and Nutrition Examination Survey protocol.

Laboratory Analyses

Peripheral blood samples were taken in the morning, after an overnight fast, from an antecubital vein, after minimal venostasis. Plasma glucose was measured using the glucose oxidase method (Beckman, Fullerton, CA, USA). Insulin was assessed on radioimmunoassay (Abbott Diagnostic Milan, Italy). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the formula: fasting plasma glucose×fasting plasma insulin/22.5. Serum total cholesterol, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol were measured on automated enzymatic assay, while serum triglycerides were measured using the enzymatic colorimetric method.

Measurement of Carotid IMT

IMT was measured using an echographic high-resolution transducer (Aplio; Toshiba SSA-770 A, Japan). A single experienced operator (F.G.) who was blinded to all clinical and laboratory data performed all ultrasound scans. All examinations were digitized and analyzed offline by an independent reader (R.D.B.). IMT measurement was performed at the carotid level with subjects in the supine position. A minimum of 3 frames (taken at the tip of the R-wave on the electrocardiogram) of the far wall of the right and left common carotid arteries (longitudinal section), 1 cm proximal to the carotid bifurcation, were digitized and measured. Measurements were carried out by tracing the leading edge of the lumen-intima and the media-adventitia interfaces. Three to 6 measurements at both common carotid arteries were taken, yielding mean IMT (the average thickness across the 1-cm segment of each of the carotid arteries) and maximum IMT (the single highest measurement).[13] All the measurements were conducted in plaque-free areas; a plaque was defined as IMT>1.5 mm.[14]

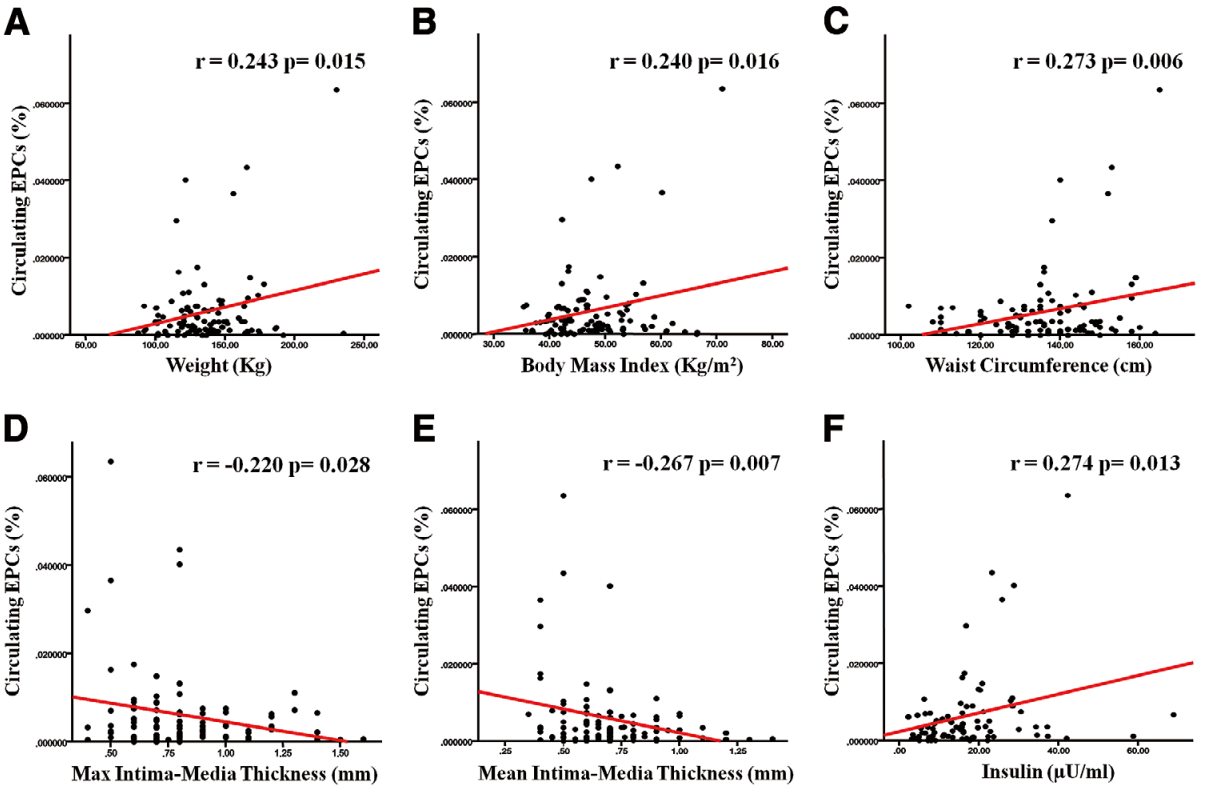

Figure 2. Correlations between endothelial progenitor cells (EPC) and (**A**) weight, (**B**) body mass index, (**C**) waist circumference, (**D**) maximum intima-media thickness, (**E**) mean intima-media thickness, and (**F**) fasting insulin.

Table 2. EPC Correlations vs. DM Status

	Circulating EPC	
	DM(–)	DM(+)
Weight (kg)	0.402***	–0.135
BMI (kg/m²)	0.409***	–0.120
WC (cm)	0.389**	–0.043
Mean IMT (mm)	–0.275*	–0.203
Maximum IMT (mm)	–0.225*	–0.164
Fasting insulin (µU/ml)	0.284*	0.250

*P≤0.05; **P≤0.01; ***P≤0.001.
Abbreviations as in Table 1.

Measurement of Circulating EPC

Blood samples were kept at room temperature and analyzed within 2 h of collection. A total of 100 µl of EDTA-anticoagulated peripheral blood was incubated for 15 min in the dark with 5 µl of the following monoclonal antibodies (mAbs): CD34 FITC (Beckman Coulter, Miami, FL, USA), CD45-PC5 (Beckman Coulter) and VEGFR2-PE (KDR; R&D Systems, Minneapolis, MN, USA). Appropriate fluorochrome-conjugated isotype-matched mAb purchased from the different manufacturers were used as controls for background staining. After incubation, cells were processed with immuno-Prep reagent system (Beckman Coulter) using Coulter Q-prep (Beckman Coulter) and then 200,000 events were collected and acquired on flow cytometry EPICSXL (Beckman Coulter) and analyzed using Expo32 (Beckman Coulter). EPC were defined as CD34+/KDR+/CD45- cells[11] and are expressed as absolute percentage of cells per total number of cytometric events after electronic gating on viable cells. A minimum of 200,000 events was collected.

Assessment of CD34+/KDR+/CD45- Cells From Adipose Tissue

Stromal vascular fraction (SVF) was obtained from each sample using the following technique: SAT and VAT samples were immediately transported to the laboratory and processed on receipt. Fat tissue was thoroughly minced with scissors, digested for 30 min in phosphate-buffered saline (PBS; Lonza, Verviers, Belgium) containing 2% bovine serum albumin (BSA; Sigma, St. Louis, MO, USA) and 2 mg/ml collagenase A (Roche, Mannheim, Germany), under constant shaking (130 g, 37°C). After successive filtrations through a 40-µm cell strainer (BD Falcon, Franklin Lakes, NJ, USA), the floating mature adipocytes were eliminated on centrifugation (400 g, room temperature, 10 min), and the pellet containing the SVF was resuspended in erythrocyte lysis buffer (155 mmol/L NH4Cl; 5.7 mmol/L K2HPO4; 0.1 mmol/L EDTA, pH 7.3) for 10 min; after successive filtrations through 100-, 70-, and 40-µm sieves, the cells were re-suspended in PBS 2%. Then FACS analysis on CD34+/KDR+/CD45- cells was performed as already described for circulating EPC. CD34+/KDR+/CD45- cells in the SVF from SAT and VAT specimens are expressed as absolute percentage of cells per total number of cytometric events after electronic gating on viable cells. A minimum of 200,000 events was collected.

Statistical Analysis

Normal distribution was tested with the D'Agostino-Pearson's test. Continuous variables were compared using the t-test, Mann-Whitney, ANOVA or Kruskal-Wallis tests, as appropriate. Correlations were evaluated on Spearman's or Pearson's r tests. Categorical variables were compared with the use of Fisher's exact test. Moreover, we performed multiple regression analysis for the baseline characteristics to identify the independent variables affecting the circulating and the intra-adipose tissue CD34+/KDR+/CD45- level. Continuous data are presented as mean±SD. Differences of P<0.05 were considered significant. Statistical analysis was done using STATISTICA version 7.0 (StatSoft, Tulsa, OK, USA).

Results

Circulating EPC, Adiposity and Carotid IMT

The baseline subject characteristics are listed in Table 1. In the whole group EPC significantly correlated with several indexes of adiposity. Specifically, a positive correlation was found with weight (r=0.243, P=0.015; Figure 2A), BMI (r=0.240, P=0.016; Figure 2B) and WC (r=0.273, P=0.006; Figure 2C). Moreover, circulating EPC level significantly and negatively correlated with maximum carotid IMT (r=–0.220, P=0.028; Figure 2D) and mean carotid IMT (r=–0.267, P=0.007; Figure 2E). Interestingly, EPC were directly correlated with fasting insulin level (r=0.274, P=0.013; Figure 2F).

Finally, we did not find any significant difference in EPC level according to Obstructive sleep apnea syndrome (OSAS) status (OSAS+, 0.00587; OSAS–, 0.00625; P=0.845).

DM Status

Mean and maximum IMT, as well as the proportion of carotid plaque, were significantly higher in the DM(+) than the DM(–) patients (P=0.001). No difference was found in circulating EPC between DM(+) vs. DM(–) patients (Table 1).

The aforementioned correlations had remarkably different trends when considered according to DM status (Table 2). Specifically, all correlations observed in the whole group were confirmed or even strengthen in DM(–) subjects (EPC–weight: r=0.402, P=0.001; EPC–BMI: r=0.409, P=0.001; EPC– WC: r=0.389, P=0.002), although they were lost in DM(+) patients (EPC–weight: r=–0.135, P=0.425; EPC–BMI: r=–0.120, P=0.480; EPC– WC: r=–0.043, P=0.801).

The same held true for the correlations between EPC and carotid IMT. Indeed, although the inverse correlation between EPC and IMT found in the whole group was confirmed in DM(–) subjects (EPC–mean IMT: r=–0.275, P=0.029; EPC–maximum IMT: r=–0.225, P=0.076), it was lost in DM(+) patients (EPC–mean IMT: r=–0.203, P=0.228; EPC–maximum IMT r=–0.164, P=0.333). Finally, EPC remained significantly correlated with fasting insulin level in DM(–) patients (r=0.284, P=0.036), but not in DM(+) patients (r=0.250, P=0.219; Table 2).

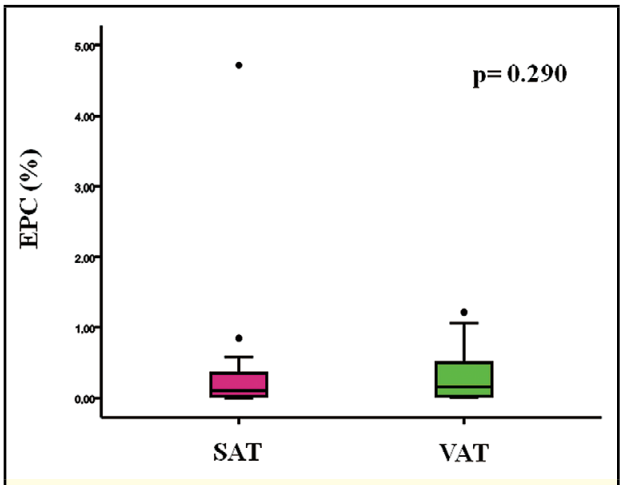

Figure 3. CD34+/KDR+/CD45- endothelial progenitor cells (EPC) from adipose tissue. No significant difference was found in CD34+/KDR+/CD45- cells between subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT).

CD34+/KDR+/CD45- Cells From Adipose Tissue

CD34+/KDR+/CD45- cells were found in all VAT and in 25/29 SAT specimens. CD34+/KDR+/CD45- cell frequencies were similar in SAT and VAT in the whole group (P=0.290; Figure 3) as well as in DM(+) and DM(–) patients. Furthermore, CD34+/KDR+/CD45- cell frequencies in SAT and in VAT were similar in DM(+) and DM(–) patients (P=0.30 and P=0.42, respectively). No significant correlation was found between intra-adipose tissue CD34+/KDR+/CD45- cells and circulating EPC (SAT: r=0.007, P=0.973; VAT: r=–0.061, P=0.753).

On multiple regression analysis, we found that IMT was the only predictor of circulating EPC and SAT EPC (Table 3).

Effects of Weight Loss

Characteristics of the group at follow-up and main differences with baseline are listed in Table 4.

Change in weight ranged between 18.1 kg and 75 kg weight loss (mean±SD, 37±12.7 kg, P<0.001). Change in BMI ranged between 6.7 kg/m^2 and 21.4 kg/m^2 (mean±SD, 12.4±3.7 kg/m^2, P<0.001). Seven out of 17 DM(+) patients (41.2%) were normoglycemic at follow-up. Similarly, 11 out of 20 hypertensive patients (55%) had normal blood pressure at follow-up. Furthermore, total and LDL-C, triglycerides as well as fasting glucose and insulin were significantly reduced at follow-up. Finally, IMT was reduced, but this did not achieve statistical significance (Table 4).

Circulating EPC level significantly decreased after bariat-

Table 3. Predictors of Circulating and Adipose Tissue EPC

Variable	Weight	BMI	WC	Baseline insulin	Mean IMT
Circulating EPC	β=–0.091 P=0.718	β=0.174 P=0.4	β=0.230 P=0.28	β=0.235 P=0.058	β=–0.286 P=0.02
SAT EPC	β=–0.845 P=0.106	β=0.506 P=0.226	β=0.411 P=0.282	β=–0.1 P=0.626	β=–0.513 P=0.032
VAT EPC	β=–0.673 P=0.207	β=0.306 P=0.474	β=0.403 P=0.308	β=–0.123 P=0.563	β=–0.445 P=0.069

Abbreviations as in Table 1.

Table 4. Effect of Weight Loss

	Baseline (n=48)	Follow-up (n=48)	P-value
Age (years)	39.8±8.0	40.9±7.9	<0.001
Weight (kg)	141.8±28.7	104.8±21.1	<0.001
BMI (kg/m^2)	47.8±7.3	35.4±5.7	<0.001
Fat mass (kg)	65.5±14.8	35.9±11.8	<0.001
Fat-free mass (kg)	76.9±19.0	68.8±15.5	<0.001
WC (cm)	137.6±12.5	113.4±12.9	<0.001
Hypertension	22 (45.8)	9 (18.7)	<0.001
DM	17 (35.4)	10 (20.8)	<0.001
Total cholesterol (mg/dl)	185.6±40.1	135.8±39.2	<0.001
LDL-C (mg/dl)	110.3±36.3	71.3±31.6	<0.001
HDL-C (mg/dl)	42.9±14.4	39.3±14.1	0.195
Triglycerides (mg/dl)	175.5±89.8	132.5±64.9	<0.001
Fasting glucose (mg/dl)	106.9±28.6	68.9±13.4	<0.001
Fasting insulin (μU/ml)	19.3±13.1	9.8±4.4	0.001
Glycated hemoglobin (%)	6.6±1.9	5.6±0.6	<0.001
HOMA-IR (mmol/L×μU/ml)	4.9±3.3	1.6±0.8	<0.001
Metabolic syndrome	35 (72.9)	17 (35.4)	<0.001
Maximum IMT (mm)	0.828±0.235	0.778±0.217	0.246
Mean IMT (mm)	0.697±0.192	0.641±0.160	0.069
Circulating EPC (%)	0.0073±0.0122	0.0018±0.0037	0.002

Data given as mean±SD or n (%).
Abbreviations as in Table 1.

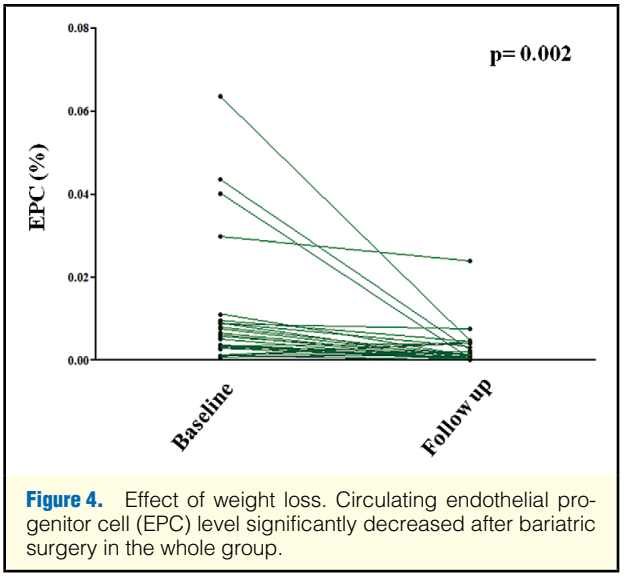

Figure 4. Effect of weight loss. Circulating endothelial progenitor cell (EPC) level significantly decreased after bariatric surgery in the whole group.

ric surgery in the whole group (P=0.002; Figure 4) as well as in DM(−) patients (0.0079±0.0136% vs. 0.0021±0.0045%; P=0.0013), and DM(+) patients (0.0063±0.0095% vs. 0.0013±0.0017%; P=0.046).

Of note, the correlations between EPC and anthropometric variables, IMT and insulin found at baseline, were no longer observed after weight loss.

Moreover, no significant correlation was found between EPC from SAT and VAT and circulating EPC after weight loss (EPC–SAT r=0.179, P=0.353; EPC–VAT r=0.178, P=0.356).

To assess whether the decrease in EPC level was associated with weight loss, we assessed the correlation between the changes in anthropometric variables and changes of circulating EPC. We found a direct correlation between reduction of EPC and changes in markers of adiposity, which reached statistical significance for weight (r=0.287, P=0.048) and borderline significance for BMI (r=0.276, P=0.058) and WC (r=0.271, P=0.063). We found no significant difference in EPC level when subdividing the group according to bariatric surgery under similar morbidity of DM (not operated patients: DM(+), n=20 vs. DM(−), n=30; P=0.415).

Discussion

The present study has shown that circulating EPC are significantly correlated with several indexes of adiposity in a large group of morbidly obese individuals. We also found CD34+/KDR+/CD45- cells in both subcutaneous and visceral human adipose tissue, suggesting that fat tissue could be a source of EPC. Taken together, these findings suggest a key role of abnormally expanded adipose tissue in determining circulating EPC level. We also found that circulating EPC are inversely correlated with carotid IMT, confirming their putative protective role against atherosclerosis also in the setting of severe obesity. Notably, these correlations are lost in DM(+) patients, thus confirming that DM negatively modulates potential beneficial effect of fatness per se, documented in our previous study.[9] Finally, we found that significant weight loss, achieved by bariatric surgery, is associated with reduction of circulating EPC level.

EPC, Obesity and DM and the Effects of Weight Loss

EPC are bone marrow-derived circulating cells able to differentiate into mature endothelial cells,[11] thus contributing to the regeneration of damaged endothelium. They have been found to be reduced in number and function in patients with CAD[15] as well as in those with cardiovascular risk factors.[16] In particu-

lar, DM has largely been investigated for its detrimental effects on progenitor cells. Several studies reported not only decreased but also functional impairment of EPC from DM(+) patients, and depletion of EPC has been found to negatively correlate with disease severity score.[17,18] Thus EPC dysfunction is emerging as a novel notion in the pathogenesis of vascular complications of DM.[19,20]

With regard to obesity, data on the relationship between adiposity and EPC are controversial. Indeed, while some studies suggest that obesity is associated with decreased number and function of progenitor cells, with weight loss being associated with reversal of this condition,[21,22] other studies show that obesity could promote the mobilization of several types of progenitor cells, prompting the hypothesis of a beneficial effect of overgrown adipose tissue.[23,24]

Of note, all these studies were conducted in small subject groups without including patients with severe obesity. More importantly, they used different definitions of EPC and none of them included what we now consider as true EPC, defined as CD34+/KDR+/CD45- cells.[11] Moreover, given that obesity is often associated with major cardiovascular risk factors, including dyslipidemia, DM and hypertension, which could affect EPC per se, several studies have actually been focused on metabolic syndrome[25,26] rather than on obesity. In our previous study we assessed non-DM, insulin-sensitive subjects, in order to dissect out the pure effect of increased adiposity, when not complicated by metabolic derangement.[9] In this setting, we found that morbidly obese patients had better flow-mediated dilation and lower IMT than obese patients, with values similar to those found in lean subjects. In the same study, morbidly obese patients had a higher level of circulating EPC than obese patients,[9] thus suggesting that fatness per se, when not associated with insulin resistance, might somehow confer protection towards atherosclerosis, possibly through EPC production and/or mobilization.

In the present study we expanded these previous observations by assessing the relationship between EPC and adiposity in morbidly obese patients who were candidates for bariatric surgery, including both DM(+) and DM(−) patients.

We found a significant and positive correlation between EPC and markers of adiposity, suggesting a direct role of adipose tissue in mediating either their production or their release. This finding is in sharp contrast to those of Müller-Ehmsen et al, who reported an inverse correlation between BMI or WC and EPC.[21] They also found weight loss-dependent increase of these cells. The 2 studies, however, are considerably different. First of all, the subject groups studied are diverse. Indeed, most of their patients were obese (mean BMI, 31 kg/m^2), while we focused on morbidly obese subjects (mean BMI, 47 kg/m^2). This is of particular importance, considering that profoundly different mechanisms probably take place in morbid obesity as compared to obesity, which led us to specifically focus on severe obesity. Moreover, the Müller-Ehmsen et al patients went through a diet program combined with increased physical activity, the latter contributing itself to EPC increase,[27] while the present patients underwent bariatric surgery, achieving an impressive weight loss (mean, 37 kg vs. 6 kg). Last, Müller-Ehmsen et al focused on other cell subtypes: CD34+ progenitor cells as well as those characterized by the double positivity CD34+/KDR+, CD34+/CD133+ and CD34+/CD117+, thus a broader cell type was considered, mostly represented by hematopoietic cells, while we focused on a very well-defined cell population, characterized by the absence of the pan-leukocyte antigen CD45. Notably, the increase in circulating progenitor cells after weight loss in their study was not observed for all subpopulations studied: specifically, the CD34+/KDR+ cell level did not change at follow up.

Another interesting finding of the present study is the significant correlation between EPC and fasting insulin level in the whole group as well as in DM(−) patients, suggesting that insulin could be the mediator of EPC mobilization when severe obesity is not complicated by DM. Insulin therapy has been reported to increase the number of circulating progenitor cells in poorly controlled DM, and human insulin has been shown to act as a factor enhancing the clonogenic potential of EPC via activation of the insulin-like growth factor-1 (IGF-1) receptor.[28,29] Thus, insulin has growth promoting and protective vascular effects in vitro and in vivo,[30,31] partly possibly through IGF-1-mediated modulation of circulating progenitor cells.

Considering the whole subject group, EPC negatively correlated with IMT, thus confirming their protective role against atherosclerosis also in the setting of morbid obesity, an issue never investigated before. The only predictor of EPC level on multivariate analysis was IMT. This result, together with the inverse correlation between EPC level and IMT, could confirm the mechanistic link between the number of EPC and vascular health, with EPC level being the mirror of vascular healing. Moreover, the fact that none of the variables included in multivariate analysis was particularly strong in predicting EPC level, suggests that either some other factors, not considered in the present study, or some factors with low power due to the small sample size (especially for adipose tissue EPC), might affect, to a greater extent, EPC number. It is worth noting that all correlations of circulating EPC with indexes of obesity and IMT were confined to DM(−) patients, confirming the independent detrimental role played by DM, which is able not only to abolish the relation between fatness and EPC, but also to offset their protective role against early atherosclerosis as assessed using IMT measurement.

Our group has recently demonstrated the powerful effect of bariatric surgery in inducing DM remission as well as in ameliorating lipid and blood pressure profile.[32] These results are confirmed in the present study, in which we also showed that all patients treated with bariatric surgery achieved significant weight loss and had a consistent parallel reduction of circulating EPC. Moreover, after weight loss, all correlations between circulating EPC and indexes of adiposity, IMT or insulin were no longer observed. This is probably due to the fact that lower degrees of obesity are probably characterized by different pathophysiological mechanisms, consistent with our previous results.[9]

Adipose Tissue as a Source of PC

Our previous observation of higher EPC level in the most obese people,[9] led us to hypothesize that adipose tissue could play a direct role in their production or release; in this study we addressed this issue by looking for these cells in adipose tissue specimens obtained during bariatric surgery. Several studies have proposed that adipogenesis and neovascularization are reciprocally regulated and tightly linked,[33,34] and described the presence of cells with progenitor properties in adipose tissue. In particular, the presence of a cell population expressing the stem cell marker CD34 has been shown in the SVF of human adipose tissue.[35] These cells could differentiate into endothelial cells and participate in vessel formation.[36]

We have further expanded these previous observations by showing for the first time that subcutaneous and VAT contain CD34+/KDR+/CD45- cells, similar to the true circulating EPC.

Of note, in the present study the CD34+/KDR+/CD45- cell level did not differ between SAT and VAT, either when considering the whole subject group, or when considering DM(+) vs. DM(−) patients. This suggests that the differences associ-

ated with the regional distribution of fat depots, with VAT being more closely associated with an adverse metabolic risk profile than SAT, are no longer apparent when considering precursors cells. This finding is in keeping with a recent study, which reported that progenitor cells from VAT and SAT paired biopsies share similar morphological, ultrastructural, electrophysiological, and immunophenotypical properties, as well as similar cytokine/chemokine expression profiles.[37] We cannot exclude the possibility that EPC found in the SVF of the adipose tissue arise from the microvasculature given that EPC circulate in the whole body. The level of CD34+/KDR+/CD45- cells in adipose tissue did not correlate with the level of circulating EPC either before or after weight loss. These findings suggest that circulating EPC level is mainly determined by progenitor cells originating from bone marrow rather than those originating from adipose tissue. Thus, it is not possible to deduce from the present results whether the correlations between EPC level and indexes of adiposity are mainly mediated by bone marrow or by adipose tissue-derived EPC.

Study Limitations

We did not carry out a power calculation because the present study was based mainly on correlations and, although the total number of patients was 100, there were relatively few DM patients, and this could be responsible for the loss of correlations in this subset of patients. In particular, the group at follow-up was too small to allow the drawing of definite conclusions on the relationship between insulin secretion and EPC number.

The best EPC definition on flow cytometry is still a matter of debate. In 2009 we proposed the stringent definition for circulating EPC of CD34+/KDR+/CD45- cells. Nevertheless, very recently Schmidt-Lucke et al adapted the ISHAGE protocol for EPC assessment on flow cytometry, defining EPC as CD45dim, CD34+, KDR+ cells.[38] They were able to significantly increase intra- and inter-observer reproducibility, confirming also correlations with clinical findings previously described using the old definition of EPC. Unfortunately, that study had not been published when we designed and started the present study, in which we used the CD34+/KDR+/CD45- cell definition. Nevertheless we found a significant direct correlation between CD45dim/CD34+/KDR+ cells and CD34+/KDR+/CD45- cells in a relatively large series of patients with different cardiac syndromes assessed at the Catholic University of the Sacred Heart in Rome (Leone et al, unpubl. data, 2010).

Although we excluded cells expressing the surface marker CD45, thus excluding cells pertaining to the mature hematopoietic lineage, we cannot avoid the fact that EPC are mesenchymal stem cells, given that both express the surface cell marker CD34+. The very small number of these cells, however, would have made their further characterization difficult, beyond the scope of the present study, which was to assess the correlation between indexes of adiposity and circulation and adipose tissue EPC level.

Moreover, it would be ideal to provide the cell number/μl together with the percentage of EPC, as we did in other previous papers. In the present case, however, we used percentage for the sake of simplicity and to be able to compare circulating EPC to cells found within the adipose tissue.

Conclusions

In the present study, carried out in a large group of patients with morbid obesity, EPC were significantly and directly related to different indexes of adiposity and to insulin level. We also found cells similar to true circulating EPC in adipose tissue and that significant weight loss was associated with decrease in circulating EPC level. EPC were inversely correlated with IMT, confirming their putative protective role against atherosclerosis also in the context of severe obesity, as already shown in other settings.[11] We also found that the correlations between circulating EPC level, indexes of adiposity and IMT were confined to DM(−) patients, thus suggesting that DM could offset the potential beneficial effect of fatness per se.[39]

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