Intima-Media Thickness in Severe Obesity

Links with BMI and metabolic status but not with systemic or adipose tissue inflammation

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OBJECTIVE—Obesity is associated with cardiovascular risk and a low-grade inflammatory state in both blood and adipose tissue (AT). Whether inflammation contributes to vascular alteration remains an open question. To test this hypothesis, we measured arterial intima-media thickness (IMT), which reflects subclinical atherosclerosis, in severely obese subjects and explored associations with systemic inflammation and AT inflammation.

RESEARCH DESIGN AND METHODS—IMT of the carotid artery (C-IMT) and IMT of the femoral artery (F-IMT) were measured in 132 nonobese (control) subjects (BMI 22.3 kg/m²; mean age 44.8 years) and 232 subjects who were severely obese without diabetes (OB/ND; mean age 49.4 years). In 57 OB/ND subjects, circulating soluble E-selectin, matrix metalloproteinase 9, myeloperoxidase, soluble intracellular adhesion molecule 1, soluble vascular cell adhesion molecule 1, tissue plasminogen activator inhibitor 1, cystatin C, cathepsin S, and soluble CD14 were measured in serum. AT macrophages were quantified by CD68 immunochemistry.

RESULTS—Both C-IMT and F-IMT increased in OB/ND and OB/D patients. In OB/ND patients, age was the sole independent determinant of IMT. No significant association was found with circulating inflammation-related molecules, number of CD68+ cells, or the presence of crown-like structures in visceral or subcutaneous AT of OB/ND patients.

CONCLUSIONS—IMT increased with severe obesity but was not influenced by the degree of systemic inflammation or AT macrophage accumulation.

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Obesity is well-recognized as a major risk factor for the development of metabolic disorders and cardiovascular disease (CVD), such as heart failure, myocardial infarction, and stroke (1–4). Two recent studies have identified an increased risk of cardiovascular events in subjects with extreme BMI (4,5), and the number of these subjects is increasing rapidly (6,7). As shown in the Prospective Studies Collaboration (4), cardiovascular risk factors (CV-RFs) increase with BMI.

In a French study conducted using the general population (6), the proportion of subjects treated for three CV-RFs (dyslipidemia, diabetes, and hypertension) was 14-fold higher for obese subjects compared with subjects of normal weight. In addition to BMI, altered body-fat distribution and ectopic fat deposition are strongly associated with mortality and morbidity attributable to CVD (8,9). It is well-established that visceral fat accumulation is associated with CV-RFs, such as hypertension, hypertriglyceridemia, or low HDL cholesterol (HDL-c), and with insulin resistance, type 2 diabetes, prothrombotic and proinflammatory states, sleep apnea, and cardiac hypertrophy, which can have potentially deleterious effects on the cardiovascular system.

Although the association between obesity and increased CV-RFs is recognized, the pathophysiological pathways that link expansion of fat mass (FM) to atherosclerosis are less clearly established. Growing evidence attributes a major role to the altered biology of adipose tissue (AT) as a cause of comorbidities in obesity. Part of the systemic inflammation that characterizes obesity originates from AT, where inflammatory cells, mainly macrophages, accumulate and create local inflammation (10,11). Adipose-derived inflammatory factors produced by enlarged adipocytes or AT macrophages (both) are often increased in the serum of obese subjects and thought to contribute to the metabolic complications of obesity, including insulin resistance and liver disease (12). In this context, it is tempting to hypothesize that products released by AT impinge on vascular cells to promote the development of atherosclerotic lesions in obesity. In line with this hypothesis, recent studies of humans report a positive association between AT inflammation, as assessed by the presence of macrophages in crown-like structures (CLS), and endothelial dysfunction, as evaluated by brachial artery flow-mediated dilatation in obese subjects (13,14).
The measure of intima-media thickness (IMT) is a noninvasive marker for subclinical atherosclerosis and provides a reliable and predictive value for later cardiovascular events (15–17). Previous studies of humans have shown correlations between BMI and increased IMT (18–22). However, only one previous study (23) has investigated IMT in morbidly obese subjects and only in a limited sample.

The objectives of our study were to describe the relationships between obesity-related phenotypes and IMT values at two arterial sites in a large group of massively obese subjects and to assess the potential links between systemic inflammation and AT inflammation.

RESEARCH DESIGN AND METHODS

Subjects
The first group of 232 subjects (42 men and 190 women) included obese subjects without diabetes (OB/ND) and obese subjects with diabetes (OB/D). The subjects were involved in a bariatric surgery program and prospectively recruited between 2008 and 2010 while they attended the Department of Nutrition at Pitié-Salpêtrière University Hospital (reference center for the Medical and Surgical Care of Obesity, Paris, France).

Following international and national guidelines, all patients met the criteria for needing obesity surgery. These criteria included BMI ≥40 or ≥35 kg/m² with at least one comorbidity (hypertension, type 2 diabetes, dyslipidemia, or obstructive sleep apnea syndrome). Preoperative evaluation included a detailed medical history, physical examination, and assessment of nutritional, metabolic, cardiopulmonary, vascular, and psychological factors. The weight of the subjects had been stable (variation less than ±2 kg) for at least 3 months before surgery. Eighty-six subjects had type 2 diabetes based on fasting glycaemia of >7 mmol/L or the use of antidiabetic drugs or insulin. These 86 subjects (referred to as the OB/D group) were treated with metformin, and 13 subjects were additionally treated with insulin. Forty-two subjects were treated with hypolipidemic drugs (35 with statins, 4 with fibrates, and 3 with ezetimibe). Six OB/ND and two OB/D patients were treated with aspirin.

A second population (control group) included a total of 132 normal-weight, nondiabetic subjects (51 men and 81 women) without a history of CVD. All were included from the Cardiovascular Prevention Unit in the Department of Endocrinology and Metabolism (Pitié-Salpêtrière Hospital). Inclusion criteria were BMI <25 kg/m² and the absence of diabetes. Forty-three subjects were treated with statins, three were treated with fibrates, and nine were treated with ezetimibe. No subjects were receiving aspirin.

None of the OB/ND, OB/D, or control subjects had experienced a cardiovascular event. The subjects did not show evidence of acute or chronic inflammatory disease, infectious disease, or cancer. Estimated daily alcohol consumption was recorded by a registered diettitian and represented an exclusion criteria when consumption was >20 g/day. Patients with aspartate aminotransferase/alanine aminotransferase (ASAT/ALAT) >2.5 N were excluded. All subjects gave their written informed consent to participate in the study. The Ethics Committee of the Comité de Protection des Personnes Ile de France I approved the clinical investigation of both obese and lean individuals. The study was conducted in accordance with the Helsinki Declaration and registered in a public trials registry. Body composition was assessed in obese subjects using a whole-body fan-beam dual-energy X-ray absorptiometry scan (Hologic Discovery W software version 12.6.2; Hologic, Bedford, MA) as previously described (24). Body regions (arms, legs, trunk, and head) were delineated with the use of specific anatomical landmarks as described elsewhere (25). Variables from dual-energy X-ray absorptiometry used in the analyses were total lean body mass (LBM), trunk LBM, and appendicular LBM (all in kilograms) and total, trunk, and appendicular FM (all in kilograms). Appendicular LBM (or FM) was calculated as the sum of LBM and appendicular LBM (all in kilograms) and total, trunk, and appendicular FM (all in kilograms). Appendicular LBM (or FM) was calculated as the sum of LBM (or FM) from the arms and legs. LBM, either total or appendicular, was defined as bone-free, lean soft tissue. Body-fat partition between the trunk and extremities was assessed by computing the trunk FM to appendicular FM ratio (24).

Risk factors for CVD
The CV-RFs were defined following the criteria of the European guidelines on cardiovascular prevention. These criteria were age (65 years for women and 55 years for men), arterial hypertension, dyslipidemia, current smoker status, and a familial history of premature CVD (60 years for women and 50 years for men) (26). Arterial hypertension was considered present when brachial blood pressure exceeded 140 mmHg for systolic blood pressure or 90 mmHg for diastolic blood pressure (or both) on at least two different occasions or if the patient was receiving antihypertensive medication. Low HDL-c was defined as a serum HDL-c level <1.0 mmol/L. High LDL-cholesterol (LDL-c) was defined as daily intake of statins or ezetimibe or fribate (or a combination of these) or having LDL-c >4 mmol/L. Smoking-habit categories were divided into either nonsmoker (never or former [i.e., tobacco consumption stopped for at least 3 years]) or current smoker (defined as at least one cigarette per day).

In the OB/ND group, we used the biological definition of metabolic syndrome, established by the International Diabetes Federation (2005), to identify subjects who were considered healthy because they had no features of metabolic syndrome. OB/ND subjects were included in the healthy group if they did not have any two of the following four factors: increased triglyceride level (≥1.7 mmol/L or specific treatment for increased levels); decreased HDL-c (<0.93 mmol/L in men and <1.29 mmol/L in women) or specific treatment for this lipid abnormality; increased blood pressure (systolic blood pressure ≥130 or diastolic blood pressure ≥85 mmHg) or treatment of previously diagnosed hypertension; and increased fasting plasma glucose (≥5.6 mmol/L) or previously diagnosed type 2 diabetes. To take into account insulin sensitivity, we added the criteria of insulinemia <20 μU/mL to include OB/ND subjects within the healthy group.

Metabolic and inflammatory blood parameters
Venous blood samples were collected in the fasting state for routine determination of glycemia, HbA1c, lipids, and high-sensitivity C-reactive protein (hsCRP). In the obese groups (OB/ND and OB/D), additional measures included circulating insulin, leptin, adiponectin, and interleukin-6 (IL-6), which were determined during routine evaluations at our clinical department (27).

A subset of 57 OB/ND subjects was further explored for circulatory factors related to inflammation and CVD. All the samples were obtained at the same time during the fasting state, between 7:00 a.m. and 8:00 a.m., to avoid diurnal variations in the tested molecules. We verified that these subjects did not differ significantly from the entire obese group with regard to
age, BMI, and sex ratio. In this group, a cytometric bead array (Human CVD1 Kit; Millipore, Billerica, MA) was used to assess serum concentration of soluble E-selectin (sE-selectin), matrix metalloproteinase 9 (MMP-9), myeloperoxidase (MPO), soluble intracellular adhesion molecule 1 (sICAM-1), soluble vascular cell adhesion molecule 1 (sVCAM-1), and tissue plasminogen activator inhibitor 1 (tPAI-1). Multiplex assays were performed according to the manufacturer’s instructions. Multi-analytical profiling was performed on the Luminex-200 system and the Xmap Platform (Luminex Corporation, Austin, TX).

Replicate fluorescence data were analyzed using Xponent software. Standard curves were obtained from serial dilutions of standard cytokine mixtures.

Additionally, cathepsin S and cystatin C concentrations were determined as described by Naour et al. (28). Soluble CD14 (sCD14) was assayed using a commercial kit for human sCD14 (Quantikine; R&D Systems, Oxford, U.K.). Using these data, we calculated the geometric mean of circulating concentrations of 11 inflammation-related factors, including sE-selectin, MMP-9, MPO, sICAM-1, sVCAM-1, tPAI-1, cathepsin S, cystatin C, sCD14, IL-6, and hsCRP. This variable was used as an index for overall systemic inflammation.

Macrophage quantification in AT

Paired biopsy specimens from omental and subcutaneous AT were obtained during gastric surgery in the 57 OB/ND and subcutaneous AT were obtained during gastric surgery in the 57 OB/ND and OB/D groups. In each group, we obtained paired biopsy specimens from omental and subcutaneous AT. Omental AT biopsy specimens were processed and embedded in paraffin. Sections (5 μm) were stained with hematoxylin and eosin to determine adipocyte diameter using PerfectImage Software (Claravislon). Macrophages were detected using the CD68 antibody (DakoCytomation, Trappes, France). Slides were observed under a Nikon Eclipse 20 AXiostar Plus microscope (Carl Zeiss). Digital images were captured by a Sony tri-CCD camera (Sony). Adipocytes and CD68+ cells were counted in 10 different randomly chosen areas at ×40 original magnification. The number of CD68+ cells per 100 adipocytes was considered to be the number of infiltrating AT macrophages.

Measuring the IMT of carotid and femoral arteries

B-mode ultrasound imaging of the carotid and femoral arteries was performed using a 7-MHz linear array transducer to clearly display both the blood–intima and media–adventitia boundaries on the near wall of the arteries. The lumen of the arteries was maximized using gain settings to optimize image quality. The protocol for measuring carotid artery IMT (C-IMT) consisted of scanning the right and left common carotid arteries longitudinally in the 5- to 20-mm segment proximal to the carotid bulb and in areas free of plaques. Similarly, measurements of femoral artery IMT (F-IMT) were performed from longitudinal scans of the right and left common femoral arteries in the 5- to 20-mm segment proximal to the bifurcation and in areas free of plaques. IMT measurements were performed offline on a personal computer and automated edge-detection software (M’Ath; ICN-METRIS) was used to locate the lumen–intima and media–adventitia echographic boundaries. The presence of plaques was defined as localized echo structures encroaching into the vessel lumen for which the distance between the media–adventitia interface and the internal side of the lesion was >1.5 mm. All scans and IMT measurements were performed by a single experienced technician trained in vascular ultrasound, and the intraobserver coefficient of variation for C-IMT was <3%.

Statistical analyses

Quantitative variables, including clinical and biological parameters, were expressed as mean ± SEM or median and range. The Shapiro-Wilk test was used to test the Gaussian distribution of the biological parameters. Skewed variables were log-transformed to normalize their distribution before statistical analyses. Categorical data were analyzed using the χ² test or Fisher exact test, as appropriate. The Kruskal-Wallis test was used to assess the statistical significance among the different groups (control, OB/ND, and OB/D). When this procedure revealed significant differences, the Tukey test was used for post hoc comparisons between each group. Variables related to C-IMT and F-IMT were studied using univariate and multivariate analyses. In univariate analyses, Spearman correlations were used. Regression analysis was performed to analyze relationships between C-IMT and F-IMT and bioclinical variables. In all models, the dependent variable was C-IMT or F-IMT. The model’s explanatory power was assessed by using adjusted R². All P values were two-sided, and P < 0.05 was considered statistically significant. Statistical analyses were performed using JMP Start Statistics (SAS, Cary, NC).

RESULTS

IMT and obesity

To determine the effect of obesity on IMT values, we explored 232 massively obese subjects, including 146 nondiabetic subjects (OB/ND group; BMI range 35.4–78.7 kg/m²) and 86 subjects with type 2 diabetes (OB/D group; BMI range 33.7–63.5 kg/m²). These obese subjects were compared with 132 normal-weight subjects (control group; BMI range 18.0–24.9 kg/m²). The bioclinical characteristics of all the participants are shown in Table 1 according to sex.

As expected, obese subjects in the OB/ND and OB/D groups showed deterioration of metabolic parameters, such as HbA1c, triglycerides, and HDL-c values. The hsCRP levels were markedly higher in OB/ND and OB/D subjects compared with control subjects. Because age, sex ratio, and the percentage of subjects treated with hypolipidemic drugs varied among the different groups, we adjusted the multivariate analyses according to these factors. The C-IMT and F-IMT arteries were measured in control, OB/ND, and OB/D subjects and then compared in men and women separately, taking into account the impact of sex on IMT values. Both C-IMT and F-IMT were increased in OB/ND and OB/D subjects compared with control subjects, regardless of sex (P < 0.05 for all comparisons except for OB/ND men compared with controls [P = 0.10]) (Fig. 1A and B). A significant worsening effect of type 2 diabetes on C-IMT was observed in women (Fig. 1A). Regarding the presence of plaques, no control or OB/ND subjects displayed significant plaques (>15%) on carotid or femoral arteries. Subjects with plaques were detected only in the OB/D group, and they occurred in 5.9% of women and in 11.1% of men at the carotid site and in 10.3% of women and in 22.2% of men at the femoral site. These data show that elevation of BMI is a major determinant of increased IMT and that type 2 diabetes aggravates the occurrence of thick plaques at two distinct arterial sites in this severely obese population.

IMT and CV-RFs in obesity

Considering type 2 diabetes as a major risk factor, we excluded the OB/D subjects...
from further analyses. CV-RFs were determined in the participants to assess whether the effect of obesity was dependent on the number of CV-RFs. We compared IMT in control and OB/ND individuals classified according to number of CV-RFs (Fig. IC and D). In the low-risk categories, with no or one CV-RF, OB/ND women were ∼10 years younger than control subjects; however, despite being younger, they displayed significantly higher C-IMT values. The limited number of OB/ND men within these categories precluded accurate comparison of their data. In the presence of at least two CV-RFs, a category in which control and OB/ND subjects had the same age range, C-IMT was markedly higher in OB/ND individuals than in control individuals of the same sex (Fig. IC). With regard to F-IMT, the obesity-induced elevation was significant for both men and women in the high-risk category only (Fig. 1D).

Of note, the distribution of CV-RFs, including low HDL-c, high LDL-c, arterial hypertension, familial history of premature CVD, and smoking, was different in our population of massively obese subjects compared with the control group (Fig. 2). The most striking differences

**Table 1—Bioclinical characteristics of nonobese control, OB/ND, and OB/D subjects**

|                  | Control                  | OB/ND                  | OB/D                   |
|------------------|--------------------------|------------------------|------------------------|
|                  | Women | Men  | Women | Men  | Women | Men  |
| n                | 81    | 51   | 122   | 24   | 68    | 18   |
| Age (years)      | 47.9 ± 1.5 | 39.8 ± 1.4 | 38.2 ± 1.0^* | 38.5 ± 2.1 | 49.0 ± 1.2‡ | 50.8 ± 2.3^† |
| BMI (kg/m²)      | 51.0 (20–65) | 38.0 (22–64) | 37.0 (18–64) | 39.0 (22–62) | 51.0 (26–68) | 49.5 (33–67) |
| Menopausal [n (%)] | 38 (47) | —    | 22 (18) | —    | 35 (52) | —    |
| HRT [n (%)]      | 7 (18.4) | —    | 2 (9)   | —    | 0 (0)   | —    |
| Weight (kg)      | 57.7 ± 0.7 | 72.0 ± 1.0 | 128.4 ± 2.0^* | 161.3 ± 5.1^* | 125.0 ± 2.7^* | 142.7 ± 5.2^† |
| BMI (kg/m²)      | 58.7 (44–74) | 71.3 (55–92) | 125.0 (89–217) | 153.1 (123–220) | 120.5 (82–173) | 142.3 (108–203) |
| Glycemia (mmol/L)| 4.8 ± 0.1 | 4.8 ± 0.1 | 5.1 ± 0.0 | 5.4 ± 0.1 | 7.9 ± 0.3^† | 7.9 ± 0.6^‡ |
| Insulin (µU/mL)  | ND    | ND   | 17.1 ± 1.0 | 27.5 ± 2.9 | 21.4 ± 4.7 | 19.0 ± 3.0‡ |
| Total cholesterol (mmol/L) | 5.8 ± 0.1 | 5.6 ± 0.2 | 4.8 ± 0.1 | 4.4 ± 0.2 | 4.6 ± 0.1 | 4.5 ± 0.2^* |
| TGs (mmol/L)     | 5.8 (3.9–8.7) | 5.4 (3.3–10.3) | 4.8 (2.6–7.9) | 4.3 (3.1–6.1) | 4.6 (2.7–6) | 4.5 (3.4–5.6) |
| HDL-c (mmol/L)   | 1.0 ± 0.1 | 1.3 ± 0.1 | 1.4 ± 0.1^* | 1.6 ± 0.1 | 1.7 ± 0.1^† | 2.0 ± 0.2^* |
| LDL-c (mmol/L)   | 0.8 (0.3–5.5) | 0.9 (0.4–3.9) | 1.2 (0.5–4.2) | 1.5 (0.8–3.3) | 1.2 (0.5–4.2) | 2.0 (0.4–3.9) |
| HbA1c (%)        | 5.5 ± 0.1 | 5.5 ± 0.1 | 5.7 ± 0.0^* | 5.7 ± 0.0^* | 7.4 ± 0.2^* | 7.2 ± 0.2^‡ |
| Total cholesterol (mmol/L) | 5.8 ± 0.1 | 5.6 ± 0.2 | 4.8 ± 0.1 | 4.4 ± 0.2 | 4.6 ± 0.1 | 4.5 ± 0.2^* |
| Statin treatment [n (%)] | 26 (32.1) | 17 (33.3) | 7 (5.5)^* | 1 (4.2)^* | 26 (38.2)^† | 9 (50.0)^† |

Data are expressed as mean ± SEM and median (minimum–maximum) unless otherwise indicated. Comparison among the control, OB, and OB/D groups was performed using Tukey test for quantitative variables and Fisher exact test for qualitative variables. DBP, diastolic blood pressure; HOMA-IR, homeostasis model assessment of insulin resistance; HRT, hormone replacement therapy for menopausal women; ND, not determined; SBP, systolic blood pressure; TG, triglyceride. *P < 0.05 vs. control. †P < 0.05 vs. obese. ‡Only determined in subjects without insulin and sulfamides. §Extremile or fibrates.
were in systemic lipid profiles, with higher prevalence and lower prevalence of low HDL-c and high LDL-c, respectively, in OB/ND subjects versus control subjects of the same sex. Smoking status was comparable between the control and OB/ND groups, but it was more frequent in men than in women. We also performed multiple regression analyses for the OB/ND and control groups in a model that took into account the aforementioned CV-RFs of age, sex, and statin treatment and showed that both C-IMT and F-IMT were independently associated with BMI and age ($P < 0.05$; $R^2$ of the model = 0.46 and 0.19, respectively, for C-IMT and F-IMT).

In univariate correlations performed in the cohort of OB/ND and control groups, we showed that C-IMT and F-IMT were positively correlated with age, BMI, glycemic status (glycemia and HbA1c), blood pressure (systolic and diastolic), and hsCRP (Table 2). C-IMT also was correlated with triglycerides. In multiple regression analyses, taking into account all these parameters, both C-IMT and F-IMT were independently associated with BMI ($P < 0.05$; $R^2$ of the model = 0.46 and 0.19, respectively, for C-IMT and F-IMT).

These results were not modified when menopausal status, the use of hormone replacement therapy, and aspirin were assessed in the model. Of note, adding OB/D subjects to the analyses did not...
Obesity and early atherosclerosis

Table 2—Correlations between bioclinical obesity-associated phenotypes and IMT on carotid and femoral sites in OB/ND and control subjects

| Phenotype          | C-IMT ρ | C-IMT P value | F-IMT ρ | F-IMT P value |
|--------------------|---------|---------------|---------|---------------|
| Age                | 0.47    | <10⁻⁴         | 0.32    | <10⁻⁴         |
| BMI                | 0.41    | <10⁻⁴         | 0.20    | 0.001         |
| Glycemia           | 0.34    | <10⁻⁴         | 0.23    | <10⁻⁴         |
| HbA1c              | 0.27    | <10⁻⁴         | 0.16    | 0.002         |
| Total cholesterol  | −0.09   | 0.10          | −0.06   | 0.27          |
| TGs                | 0.18    | 0.02          | 0.07    | 0.24          |
| HDL                | −0.05   | 0.35          | −0.06   | 0.29          |
| LDL                | −0.11   | 0.11          | −0.04   | 0.51          |
| SBP                | 0.25    | <10⁻⁴         | 0.31    | <10⁻⁴         |
| DBP                | 0.24    | <10⁻⁴         | 0.27    | <10⁻⁴         |
| hsCRP              | 0.32    | <10⁻⁴         | 0.16    | 0.01          |

DBP, diastolic blood pressure; SBP, systolic blood pressure; TG, triglyceride.

change the results (Supplementary Table 1 shows the univariate correlations).

Altogether, these data suggest that morbid obesity per se increases C-IMT and F-IMT, with a more marked effect at the carotid arterial site, independently of age, number of CV-RFs, and metabolic status.

IMT and obesity-associated phenotypes

We next searched for associations between IMT and phenotypes related to body composition and metabolic alterations in the OB/ND group. In univariate correlation analyses, age was correlated with C-IMT and F-IMT. F-IMT was positively correlated with BMI, trunk FM, and the ratio of trunk FM to appendicular FM (Table 3). The same tendency was observed for C-IMT, although it did not reach statistical significance. Positive correlations also were found between C-IMT and glycemia, HbA1c, total cholesterol, LDL-c, systolic blood pressure, and diastolic blood pressure. F-IMT was correlated with glycemia, LDL-c, and systolic blood pressure. In multiple regression analyses, taking into account sex and all of these parameters (age, glycemia or HbA1c, total cholesterol, LDL-c, blood pressure, BMI, trunk FM, and ratio of trunk FM to appendicular FM), age remained the sole factor that was significantly associated with C-IMT and F-IMT (P < 10⁻⁴; R² of the model = 0.34 and 0.17, respectively, for C-IMT and F-IMT). These correlations were not significant in OB/D, except for age (ρ = 0.62 and 0.45, respectively, for C-IMT and F-IMT; P < 10⁻⁴).

To obtain further insight into the relationships between IMT and metabolic status in massively obese subjects, we identified a group of healthy subjects, defined as patients without metabolic syndrome (International Diabetes Federation criteria) and with insulinemia <20 μU/mL. Among the 147 OB/ND individuals, 47 (5 men and 42 women) matched these criteria. The healthy OB/ND subjects had similar BMI as nonhealthy OB/ND but, on average, were 8 years younger (36.3 ± 1.5 vs. 44.3 ± 0.9 years; P < 0.001). We found that both C-IMT and F-IMT were significantly lower in healthy than in nonhealthy OB/ND subjects (C-IMT: 0.594 ± 0.014 vs. 0.637 ± 0.007 mm; P = 0.03; F-IMT: 0.486 ± 0.007 vs. 0.514 ± 0.006 mm; P = 0.05). Multiple regression analyses revealed that both C-IMT and F-IMT were predicted by age (P < 10⁻⁷) but not by sex or being healthy.

IMT and obesity-induced inflammation

To test the hypothesis that obesity-induced low-grade inflammation impacts IMT values, we evaluated systemic inflammation and AT in a subset of 57 individuals representative of the whole OB/ND group for age, BMI, and sex ratio. In this subgroup, we performed univariate correlations between IMT values and the bioclinical variables used in

Table 3—Correlations between bioclinical obesity-associated phenotypes and IMT on carotid and femoral sites in OB/ND subjects

| Phenotype          | C-IMT ρ | C-IMT P value | F-IMT ρ | F-IMT P value |
|--------------------|---------|---------------|---------|---------------|
| Age                | 0.65    | <10⁻⁴         | 0.39    | <10⁻⁴         |
| BMI                | −0.02   | 0.79          | 0.15    | 0.05          |
| Glycemia           | 0.16    | 0.05          | 0.24    | 0.004         |
| Insulinemia        | 0.02    | 0.84          | −0.03   | 0.69          |
| HOMA               | 0.06    | 0.48          | 0.01    | 0.89          |
| HbA1c              | 0.17    | 0.03          | 0.08    | 0.20          |
| Total cholesterol  | 0.26    | 0.002         | 0.11    | 0.2137        |
| TGs                | 0.13    | 0.11          | −0.05   | 0.58          |
| HDL                | 0.25    | 0.003         | 0.17    | 0.05          |
| LDL                | 0.18    | 0.11          | <0.05   | 0.23          |
| DBP                | 0.20    | 0.02          | 0.12    | 0.21          |
| Ratio of trunk FM to appendicular FM | 0.16 | 0.07 | 0.25 | 0.004 |
| hsCRP              | −0.09   | 0.20          | −0.07   | 0.38          |
| Leptin             | −0.12   | 0.44          | 0.13    | 0.11          |
| IL-6               | −0.12   | 0.14          | −0.05   | 0.52          |

DBP, diastolic blood pressure; HOMA, homeostasis model assessment; SBP, systolic blood pressure; TG, triglyceride.
Table 3. We found the same statistical associations as in the entire OB/ND group (data not shown). Systemic inflammation was assessed from the serum concentrations of 11 factors known to be related to inflammation or cardiovascular risk (or both), including IL-6, hsCRP, sE-selectin, MMP-9, MPO, sICAM-1, sVCAM-1, tPAI-1, cathepsin S, cystatin C, and sCD14. Using an independent group of nonobese subjects explored elsewhere (11), we verified increased circulating levels of these factors in our OB/ND group (data not shown). However, we did not find any significant correlations between C-IMT or F-IMT and these circulating molecules when assessed individually (Supplementary Table 2) or together in an index of overall systemic inflammation (i.e., the geometric mean of the circulating concentrations of the 11 aforementioned factors) (Fig. 3A). Additionally, leptin and adiponectin levels were not correlated with IMT in this severely obese population (Supplementary Table 2).

AT inflammation was next explored through quantification of CD68⁺ cells and the presence of CD68⁺ cells organized in CLS in visceral and subcutaneous biopsy specimens. We verified that the numbers of CD68⁺ cells and CLS were higher in the OB/ND group compared with a group of six nonobese subjects explored elsewhere (11) (data not shown). Adipocyte diameter was measured in fat deposits, and no significant correlation was found with either C-IMT or F-IMT (data not shown). When the OB/ND subjects were classified by their CLS status, C-IMT or F-IMT values did not significantly differ between individuals with (CLS⁺) or without (CLS⁻) structures in their AT, whatever the deposit (Fig. 3B). Of note, none of the metabolic or inflammatory parameters tested were significantly altered in subjects with CLS in either of the fat depots as compared with the corresponding CLS⁻ groups (data not shown). Moreover, no

**Figure 3**—No relationship between IMT and systemic inflammation or AT inflammation in obese subjects. A: C-IMT (○) and F-IMT (●) values related to systemic inflammation in 57 subjects who were representative of the whole OB/ND group. The index, expressed in arbitrary units (AU), is defined as the geometric mean of circulating concentrations of hsCRP, IL-6, sE-selectin, MMP-9, MPO, sICAM-1, sVCAM-1, tPAI-1, cathepsin S, cystatin C, and sCD14. B: C-IMT and F-IMT values in obese subjects with or without CD68⁺ cells in CLS in visceral and subcutaneous white AT (WAT). CLS⁺ in visceral AT: n = 21; CLS⁺ in subcutaneous AT: n = 43. C and D: C-IMT (○) and F-IMT (●) values related to the amount of CD68⁺ cells in visceral (A) or subcutaneous (B) AT. In A, C, and D, the nonparametric Spearman rank correlation ρ and P values are indicated for an association with C-IMT or F-IMT. Scut, subcutaneous; Vis, visceral.
association was found between C-IMT or F-IMT values and CD68⁺ cell counts in AT (Fig. 3C and D). Similarly, in a multivariate model, taking into account subcutaneous AT CD68⁺ cell number, age, sex, BMI, glycemia, total cholesterol, LDL-c, and blood pressure, age remained the only dependent factor that was significantly associated with C-IMT and F-IMT \( (P < 10^{-4};\ R^2 \text{ of the model } = 0.45 \text{ and } 0.07, \text{ respectively, for C-IMT and F-IMT}).\) The same results were found in a model taking into account the same parameters and visceral AT CD68⁺ cell number \( (R^2 \text{ of the model } = 0.59 \text{ and } 0.16, \text{ respectively, for C-IMT and F-IMT}).\)

In 15 subjects, we were able to perform a surgical biopsy at the subcutaneous AT site 3 months after surgery. For these subjects, we also evaluated C-IMT and F-IMT. BMI was reduced by 17 ± 1.3%, C-IMT was reduced by 4.5 ± 1.9%, F-IMT was reduced by 4.2 ± 2.4%, and CD68⁺ cell number was reduced by 11.7 ± 10.2%. However, we did not find a significant correlation between IMT reduction at either site and variations in macrophage accumulation (C-IMT: \( \rho = 0.13, P = 0.65;\ F-IMT: \rho = 0.36, P = 0.22)).\) No correlation was found between changes in IMT and changes in circulating levels of IL-6, hsCRP, sE-selectin, MMP-9, MPO, sICAM-1, sVCAM-1, tPAI-1, cathepsin S, IL-6, hsCRP, sE-selectin, cystatin C, and sCD14.

CONCLUSIONS—In this study, we sought to evaluate the effect of obesity and obesity-related phenotypes (body composition, metabolic features, and inflammation features) on subclinical atherosclerosis assessed by IMT values at two arterial locations. Comparing large groups of nonobese and massively obese subjects, we showed that C-IMT and F-IMT increased with BMI, independently of age and other classical risk factors. Within the OB/ND group, being healthy with regard to metabolic profile was associated with lower IMT values. However, contrary to our initial hypothesis, neither C-IMT nor F-IMT was significantly associated with systemic inflammation or AT inflammation in the severely obese population explored in this study before and after weight loss.

It is well-known that obesity is associated with CV-RFs, including dyslipidemia, hyperinsulinemia, and hypertension, and that all of these impact IMT measures \((19,21,29)).\) We have shown that C-IMT values are increased in morbidly obese subjects regardless of the presence of none, one, or more than two CV-RFs. The same was true for F-IMT, specifically in the high-risk group, suggesting that the femoral artery might be less sensitive to obesity-induced alterations than the carotid artery. Thus, in line with other studies \((18–21),\) our data support a prominent role for increased BMI to induce IMT alterations.

When compared with the nonobese women in low CV-RF categories, OB/ND women displayed strikingly higher C-IMT values despite the fact that they were >10 years younger. Thus, increased IMT might be a sign of early vessel aging in subjects with severe obesity. Obesity-linked elevation of arterial IMT also was observed in the high-risk groups in which OB/ND and control subjects were approximately the same age. These observations suggest that increased BMI contributes to aggravated arterial alteration, independently of age. Nevertheless, within the OB/ND group and after exclusion of diabetic patients, age remained a major determinant of both metabolic health and IMT values in these severely obese subjects.

Massive obesity is a model of low-grade inflammation, characterized by increased circulating inflammation-related molecules and inflammation of AT \((12)).\) Previous studies of overweight and obese adults or children reported a link between IMT and systemic inflammation \((30–32)).\) We could not confirm this deleterious association in our study population in which neither C-IMT nor F-IMT correlated with circulating factors related to cardiovascular risks or inflammation. Several caveats might account for these discrepant observations. First, we cannot exclude that multiple measurements did not correlate with single assays for some of the circulating factors. Second, the level of systemic metabolic inflammation in our group of massively obese subjects might be high enough to erase the links usually found between low-grade inflammation and early atherosclerotic changes. Third, other factors (such as free fatty acid or growth factors), including vascular endothelial growth factor, known to be increased in obese subjects and not measured in the current study might contribute to the increased IMT.

Our data support the major impact of abdominal adiposity on IMT modulation, as previously reported in less severe obesity \((22,33,34)).\) Faced with these results, it is tempting to suppose that enlarged AT, especially viscerally, plays a role in vascular phenotype. Beyond the quantity of FM, cellular alterations of AT, including infiltration with various immune cell types, influence obesity-related complications \((12,35)).\) At the tissue level, Apovian et al. \((13))\) have shown that obese subjects with subcutaneous AT inflammation, determined by the presence of macrophages organized in CLS, displayed worse metabolic features and reduced flow-mediated dilatation of the brachial artery \((14)).\) In our experience, however, the presence of CLS in subcutaneous or visceral AT did not significantly impinge on insulinemia, homeostasis model assessment of insulin resistance, systemic inflammation, or IMT values. Currently, we do not have an obvious explanation for this discrepancy. The lack of relationship between AT macrophage counts and IMT values in our obese population further supports the idea that subcutaneous or visceral AT inflammation does not significantly influence subclinical atherosclerosis. This does not preclude that vascular parameters other than IMT, not explored in the current study, might show an association with the inflammatory state of AT. Moreover, a more detailed evaluation of macrophage phenotype or quantification of other immune cells (lymphocytes, mast cells, and natural killer T cells) within selected fat deposits could reveal relationships with vascular alterations. Because IMT is clearly not linked to the degree of visceral or subcutaneous AT inflammation, it is tempting to speculate that epicardial or perivascular AT could have a greater impact on arterial structure \((9,36)).\) However, it remains to be determined if the quality of fat deposits in close vicinity to the arterial wall are linked to the development of atherosclerosis.

There were several limitations in our study. The first was that the prevalence of individual CV-RFs was different between subjects of normal weight and obese subjects, making matching of these factors impossible. The second limitation was the failure to take into account the presence of sleep apnea, known to be involved in vascular alterations \((37,38));\) unfortunately, we did not have this information for the control group. Data regarding the evaluation of insulin resistance and systemic inflammation and AT inflammation were lacking for the nonobese control group; also, in this same group of control subjects, we did not have information regarding IMT exploration or quantification of AT macrophages. These data would have allowed us to make comparisons between the groups and increase the range of measures for the correlation study. Finally, the lack of association between
inflammation and IMT in the group of 57 OB/ND subjects could be attributable to a lack of statistical power.

In conclusion, in morbidly obese subjects, subclinical atherosclerosis is associated with FM distribution and altered metabolic features and might reflect precocious vessel aging. In this population, we did not demonstrate convincing links between increased IMT and the degree of systemic inflammation or AT inflammation, for which the level could hide the links usually found between low-grade inflammation and early atherosclerotic changes.

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E.D. conducted the research, analyzed the data, and wrote the manuscript. J.F.K. conducted the research. P.G. provided the control cohort. M.A. analyzed the data. J.-L.B. provided the AT. S.F. performed the cytokine measurements. J.-M.O. contributed to discussion and reviewed and edited the manuscript. K.C. designed the research, contributed to discussion, and reviewed and edited the manuscript. M.G.-M. designed the research, analyzed the data, and wrote the manuscript. C.P. designed the research, analyzed the data, and wrote the manuscript. All authors read and approved the final manuscript. C.P. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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