CD14^{dim}CD16^{+} and CD14^{+}CD16^{+} Monocytes in Obesity and During Weight Loss: Relationships With Fat Mass and Subclinical Atherosclerosis

Christine Poitou, Elise Dalmas, Mariana Renovato, Vanessa Benhamo, Froogh Hajduch, Meriem Abdennour, Jean-François Kahn, Nicolas Veyrie, Salwa Rizkalla, Wolf-Hervé Fridman, Catherine Sautès-Fridman, Karine Clément, Isabelle Cremer

Objective—Studies suggest the implication of CD16^{+} subpopulations (CD14^{+}CD16^{+}, CD14^{dim}CD16^{+}) in inflammatory diseases. We aimed to determine the frequency of these subpopulations during weight loss in obesity and diabetes, conditions associated with changes in systemic inflammation, and we tested the link with subclinical atherosclerosis.

Methods and Results—CD14^{dim}CD16^{+} and CD14^{+}CD16^{+} frequencies were measured by flow cytometry in lean subjects, obese subjects before and after a hypocaloric diet or gastric surgery, and obese diabetic subjects before and after gastric surgery. Both monocyte subsets were increased in obese subjects, with a significant enrichment of the CD14^{dim}CD16^{+} subpopulation in obese diabetic patients. Multivariate analysis demonstrated a link between the percentages of CD14^{dim}CD16^{+} monocytes and glycemia, independent of fat mass. Drastic weight loss led to a sharp decrease of this subset, the variations of which were strongly related to fat mass changes. A reduction of at least 5% of fat mass was sufficient to observe a significant decrease of CD14^{dim}CD16^{+} monocytes. A diminution of the CD14^{+}CD16^{+} subset was also observed during weight loss and was associated with a decrease in intima-media thickness.

Conclusion—This work demonstrates a major impact of fat mass variations on CD14^{dim}CD16^{+} monocyte subsets and that the decrease in the CD14^{+}CD16^{+} subpopulation is linked to a reduction of subclinical atherosclerosis.

Clinical Trial Registration—URL: http://clinicaltrials.gov. Unique identifier: NCT00476658.

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Key Words: atherosclerosis ■ blood cells ■ diabetes mellitus ■ nutrition ■ obesity

Human obesity is associated with the development of cardiometabolic diseases, such as insulin resistance, dyslipidemia, diabetes, and cardiovascular injury. Obesity is characterized by the modulation of innate immunity. Obese subjects have increased systemic levels of inflammatory markers, such as acute-phase proteins (C-reactive protein [CRP] and serum amyloid A), cytokines, and interleukins.\(^1\) Obese subjects before and after compared to lean subjects, with a significant enrichment of the CD14^{dim}CD16^{+} subpopulation in obese diabetic subjects. Multivariate analysis demonstrated a link between the percentages of CD14^{dim}CD16^{+} monocytes and glycemia, independent of fat mass. Drastic weight loss led to a sharp decrease of this subset, the variations of which were strongly related to fat mass changes. A reduction of at least 5% of fat mass was sufficient to observe a significant decrease of CD14^{dim}CD16^{+} monocytes. A diminution of the CD14^{+}CD16^{+} subset was also observed during weight loss and was associated with a decrease in intima-media thickness.

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Correspondence to Christine Poitou, INSERM U872, Team 7, Centre de Recherche des Cordeliers, 15 rue de l’Ecole de Médecine, 75006 Paris, France. E-mail bernert@pslapph.fr

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2322
chemokine receptor-1 (the fractalkine receptor). On the contrary, the CD16⁺ subpopulation is CCR2 negative but expresses high levels of CX3 chemokine receptor-1 and CCR5 receptors.¹³ These CD16⁺ cells exhibit a macrophage-like phenotype with enhanced antigen-presenting capacities and higher endothelial affinity, and they are potent producers of proinflammatory cytokines. They express higher levels of tumor necrosis factor-α and major histocompatibility class II and lower levels of interleukin-10, as compared with CD16⁻ monocytes. The CD16⁺ monocyte population is increased in inflammatory situations, such as sepsis, rheumatoid arthritis, and infections.¹¹ A significant increase in the CD16⁺ subset has also been described in human chronic pathologies with low-grade inflammation components, such as in obesity¹²,¹³ and related cardiovascular diseases.¹⁴ CD16⁺ monocyte frequency is related to intima-media thickness (IMT), a marker of subclinical atherosclerosis¹⁵,¹⁶ in patients with chronic kidney disease characterized by high cardiovascular risk, and in healthy volunteers as well.¹⁷ Based on these observations, it is considered that CD16⁺ monocytes could be cellular players, mediating the pathophysiological relationships between metabolic and cardiovascular diseases.

The cell population of CD16⁺ per se also exhibits phenotype heterogeneity, with 2 described subsets that express either low levels of CD14 (CD14dimCD16⁺) or high levels of CD14 (CD14⁺CD16⁺). These cell subsets display distinct phenotypic and functional properties.¹⁸,¹⁹ Whether these monocyte subsets show different pathogenic roles in cardiometabolic diseases has not been clearly established. A recent study conducted in 622 volunteers as well.¹⁷ Based on these observations, it is considered that CD16⁺ monocytes could be cellular players, mediating the pathophysiological relationships between metabolic and cardiovascular diseases.

In Poitou et al.²¹ Homeostasis model assessment (HOMA) insulin resistance (IR) was determined using the HOMA Calculator version 2.2.²⁴

In this study, we explored the hypothesis that both the CD14dimCD16⁺ and the CD14⁺CD16⁺ monocyte subsets could be modulated in obesity and during weight loss and could associate with metabolic phenotypes and subclinical atherosclerosis. Thus, we studied both CD16⁺ subsets in different conditions of obesity and in response to different programs inducing fat mass variations, induced by either diet or surgery. In this latter condition, metabolic and inflammatory parameters and vascular phenotypes obtained in morbidly obese subjects were analyzed in parallel to CD16⁺ monocyte subset frequencies. We demonstrate for the first time a relationship between changes in the CD14dimCD16⁺ subset and reduction of fat mass.

**Materials and Methods**

**Subjects**

Three groups of subjects were included in this study. The first population (the obese [OB] and obese diabetic [OB/D] groups) included 105 obese subjects involved in a gastric surgery program, prospectively recruited between 2008 and 2009 in the Department of Nutrition of Pitié-Salpêtrière Hospital (reference center for the medical and surgical care of obesity, Paris, France). Patients meeting the criteria for obesity surgery included those with a BMI ≥40 kg/m² or ≥35 kg/m² with at least 1 comorbidity (hypertension, type 2 diabetes, dyslipidemia, or obstructive sleep apnea syndrome). The preoperative evaluation included a detailed medical history and physical, nutritional, metabolic, cardiopulmonary, vascular, and psychological assessments. The weight of the included subjects had been stable (variation of less than ±2 kg) for at least 3 months before surgery. Subjects did not demonstrate evidence of acute or chronic inflammatory disease, infectious diseases, cancer, or known alcohol consumption (>20 g per day). Patients displaying surgical complications during the first year after surgery were subsequently excluded. They did not take any antiinflammatory drugs. Thirty-eight subjects were classified as having type 2 diabetes by registering a fasting glycemia of greater than 7 mmol/L or by their use of an antidiabetic drug. These 38 subjects (the OB/D group) were treated with metformin and hypolipemic drugs (either fibrates or statins). Nine subjects were also treated with insulin. An oral glucose tolerance test was performed before Roux-en-Y gastric bypass (RYGB) and confirmed that all patients in the nondiabetic obese group (the OB group) had glucose levels of less than 11 mmol/L (200 mg/dL) in the 2 hours following a 75-g oral glucose challenge. Clinical and biological parameters and monocyte subpopulations were assessed before RYGB surgery (n=105) and 3 and 6 months (n=36) after surgery.

A second population (the Diet group) included 39 overweight and moderately obese subjects undergoing a weight loss program (1200 kcal daily over 6 weeks).²³ Clinical and biological parameters and monocyte subpopulations were assessed before diet intervention (n=39) and after 6 weeks of caloric restriction (n=20).

The third population (the control [C] group) included 32 lean, healthy, white volunteers living in the same area as the obese subjects.

Total body fat mass was determined by DXA scanning (GE Lunar Prodigy Corp, Madison, WI). The ethics committee of the Hôtel-Dieu Hospital approved the clinical investigations for both obese and lean individuals. All subjects gave written informed consent. The study was conducted in accordance with the Helsinki Declaration and was registered in the ClinicalTrials.gov registry.

**Metabolic and Inflammatory Parameters**

Venous blood samples collected in the fasting state were used to assess lipid, insulin, and glucose values (enabling the determination of insulin-sensitivity parameters) and many others factors, outlined in Poitou et al.²¹ Homeostasis model assessment (HOMA) insulin resistance (IR) was determined using the HOMA Calculator version 2.2.²⁴

**Carotid and Femoral Artery IMT Measurement**

Carotid and femoral B-mode ultrasound imaging was performed using Sequoia 512 ultrasound mainframes (Acuson, Mountain View, CA). A 7-MHz linear array transducer was used for clearly displaying both the blood-intima and media-adventitia boundaries on the far wall of the arteries. The lumen of the arteries was maximized with gain settings to optimize the image quality.

The protocol for measuring carotid IMT (CIMT) consisted of scanning the right and left common carotid arteries longitudinally in the segment 5 to 20 mm proximal to the carotid bulb and on a site free of plaques. Similarly, measurements of femoral IMT (FIMT) were obtained from longitudinal scans of the right and left common femoral arteries in the segment 5 to 20 mm proximal to the bifurcation and on a site 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. All scans and IMT measurements were performed by a single experienced physician trained in vascular ultrasound, and the intraobserver coefficient of variation for CIMT was <3%.
Peripheral Blood Mononuclear Cell Isolation and Flow Cytometry Analysis

Peripheral blood mononuclear cells were isolated from blood by centrifugation on a Ficoll/Hypaque gradient (PAA Laboratories), and were counted by trypan blue exclusion for each patient. Single-cell suspensions were analyzed by 3-color flow cytometry. Cells were incubated with fluororescin isothiocyanate–conjugated anti-CD14 (clone MOP9 from Becton Dickinson), phycoerythrin-conjugated anti-CD16 (clone MOP9 from BD Biosciences), and fluorescein isothiocyanate–conjugated anti-CD14 (clone 48607 from Becton Dickinson) or 647–conjugated anti-CCR2 (clone 48607 from Becton Dickinson). Flow cytometry data were analyzed using Cellquest Pro software (BD Biosciences). Flow cytometry data were counted by trypan blue exclusion for each patient. Single-cell side-scatter profiles and then defined as CD14dimCD16+ Monocyte cells were first gated according to their forward- and

Statistics

The normal distribution of the data were tested using the Shapiro-Wilk test. Data were log-transformed when required. Quantitative variables, including clinical and biological parameters as well as monocyte subset percentages, were expressed as mean±SEM values. All analyses were adjusted for age. ANOVA was used to assess the statistical significance of the differences in clinical and biological parameters, as well as in monocyte subsets between the different groups at baseline. When the ANOVA procedure revealed significant differences, Bonferroni multiple tests were used for post hoc comparisons. Data were analyzed using Cellquest Pro software (BD Biosciences). Flow cytometry data were counted by trypan blue exclusion for each patient. Single-cell side-scatter profiles and then defined as CD14dimCD16+ Monocyte cells were first gated according to their forward- and side-scatter profiles and then defined as CD14+ cells.

CD14dimCD16+ and CD14+CD16+ Monocyte Distributions in Obesity and Diabetes

Table 1 presents the bioclinical characteristics of lean subjects (the C group, BMI range 17.6 to 23.8 kg/m²), overweight and moderately obese subjects from the Diet group (BMI range 25.3 to 35.5 kg/m²), obese subjects (the OB group) (BMI range 35.4 to 66.1 kg/m²), and diabetic obese subjects (the OB/D group) (BMI range 35.3 to 68.6 kg/m²) before RYGB. As expected, obese subjects in the Diet, OB, and OB/D groups showed higher fat mass; deterioration of metabolic parameters, such as glucose, insulin, and lipid

| Table 1. Bioclinical Characteristics in the Different Cohorts: Lean Controls (C), Overweight and Moderately Obese Subjects (Diet), Obese (OB), and Obese Diabetic (OB/D). |
|-------------------------------|---------------|------------|--------------|-------------|-------------|
| C                            | Diet          | OB         | OB/D         | Overall P Value |
| No.                          | 32            | 39         | 67           | 38          |
| Sex ratio, F/M               | 25/7          | 31/8       | 57/10        | 26/12       |
| Age, y                       | 33.9±1.6**    | 43.1±1.9C  | 37.6±1.5B    | 49.8±1.8A   | <10-4       |
| Weight, kg                   | 58.7±1.3C     | 90.1±2.4A  | 127.2±2.4A   | 135.0±3.9A  | <10-4       |
| BMI, kg/m²                   | 21.5±0.2C     | 32.3±0.6B  | 46.4±0.1A    | 48.8±1.3A   | <10-4       |
| Fat mass, kg                 | 14.7±1.3C     | 33.2±1.5B  | 58.5±1.7A    | 61.3±2.6B   | <10-4       |
| Fat mass, %                  | 25.1±1.9C     | 38.1±1.2B  | 46.8±0.6A    | 46.3±0.9A   | <10-4       |
| Glycemia, mmol/L             | 4.7±0.1B      | 5.1±0.1A   | 5.1±0.08B    | 8.1±0.3A    | <10-4       |
| HbA1c, μU/mL                 | ND            | ND         | 5.7±0.04A    | 7.5±0.3A    | ND          |
| Insulinemia, μU/mL           | 6.0±0.9C      | 9.6±0.8C   | 17.2±1.5B    | 24.8±3.5A   | <10-4       |
| HOMA-IR, %                   | 0.62±0.08C    | 1.08±0.09C | 1.90±0.16B   | 2.95±0.40A  | <10-4       |
| Total cholesterol, mmol/L    | 4.6±0.2       | 5.3±0.4    | 5.0±0.1      | 4.7±0.1     | NS          |
| Triglycerides, mmol/L        | 1.03±0.10     | 1.44±0.14C | 1.41±0.08B   | 2.04±0.15A  | <10-4       |
| HDL-c, mmol/L                | 1.30±0.07B    | 1.33±0.07B | 1.18±0.04B   | 1.11±0.07A  | 0.05        |
| Leptin, ng/mL                | ND            | 43.1±3.8   | 46.3±2.1     | 39.8±3.7    | NS          |
| Adiponectin, μg/mL           | ND            | 13.7±1.3B  | 6.8±0.5A     | 5.8±0.4A    | <10-4       |
| CRP, mg/L                    | 2.3±0.4B      | 4.4±0.7A   | 10.4±1.0A    | 8.1±0.9A    | <10-4       |
| IL-6, pg/mL                  | ND            | ND         | 3.46±0.22    | 4.22±0.74   | NS          |

All values are expressed as mean±SEM. Data are expressed as mean±SEM. Overall P values were obtained by 1-way ANOVA on log-transformed data adjusted for age. When the ANOVA procedure revealed significant differences, Bonferroni multiple tests were used for post hoc comparisons. BMI indicates body mass index; CRP, C-reactive protein; F, female; HDL-c, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment insulin resistance; IL, interleukin; M, male; ND, not determined; NS, nonsignificant data.

*Data not sharing the same letter (A, B, or C) within a horizontal line are significantly different (P<0.05).
values; and changes in leptin and adiponectin concentrations. The hsCRP levels were significantly higher in obese groups, in agreement with obesity-associated low-grade inflammation.

The absolute count for peripheral blood mononuclear cells was not different among the 4 groups (2.94×10^3±0.96, 3.14×10^3±1.20, 2.96×10^3±1.01, and 3.22×10^3±1.84 cells/µL for the C, Diet, OB, and OB/D groups, respectively). The distribution of CD14<sup>dim</sup>CD16<sup>+</sup> and CD14<sup>+</sup>CD16<sup>+</sup> monocytes was determined by flow cytometry. We observed 2 CD16<sup>+</sup> monocyte subpopulations, according to CD14, CD16 and CCR2 level expression (Supplemental Figure 1A, available online at http://atvb.ahajournals.org). We confirmed the presence of a first population characterized by a low expression of CD14, high expression of CD16, and no or low expression of CCR2 (CD14<sup>dim</sup>CD16<sup>+</sup>CCR2) and of a second one characterized by high expression of CD14 and CD16 and moderate expression of CCR2 (CD14<sup>+</sup>CD16<sup>+</sup>CCR2+).<sup>19</sup>

A representative staining pattern of the surface expression of CD14 and CD16 on monocytes from patients of the C, OB, and OB/D groups is shown in Figure 1A.

Having observed some differences in the mean age of the groups, we adjusted all analyses for age. The mean percentage of CD14<sup>dim</sup>CD16<sup>+</sup> was significantly different among the 4 groups (Figure 1B, 3.7±0.3%, 4.8±0.2%, 7.2±0.4%, and 9.6±0.6% for the C, Diet, OB, and OB/D groups, respectively, P<10<sup>−4</sup>). This cell population was 2-fold increased in the OB group compared with lean subjects with significant additional effects of diabetes. Similar findings were observed with absolute counts of CD14<sup>dim</sup>CD16<sup>+</sup> (Figure 1C). The mean percentage of CD14<sup>+</sup>CD16<sup>+</sup> was higher in the OB and OB/D groups, compared with the Diet and C groups, but without additional effects of diabetes (Figure 1D; 3.2±0.4%, 2.4±0.2%, 5.4±0.4% and 5.8±0.6% for the C, Diet, OB, and OB/D groups, respectively, P<10<sup>−4</sup>). Similar findings were observed with absolute counts of CD14<sup>dim</sup>CD16<sup>+</sup> (Figure 1E). Conversely, the mean of CD14<sup>+</sup>CD16<sup>+</sup> subset was significantly different among the 4 groups, with a decrease of CD14<sup>+</sup>CD16<sup>+</sup> frequencies in the obese subjects (93.1±0.6%, 92.8±0.4%, 87.4±0.6%, and 85.0±1.1% for the C, Diet, OB, and OB/D groups, respectively, P<10<sup>−4</sup>). Similar findings were observed with absolute counts of CD14<sup>+</sup>CD16<sup>+</sup>. Obesity is thus associated with an increase in both CD16<sup>+</sup> monocyte subsets, with a selective and significant enrichment of the CD14<sup>dim</sup>CD16<sup>+</sup> subset in obese patients with type 2 diabetes.

### CD16<sup>+</sup> Monocyte Subsets and Obesity-Associated Phenotypes

To evaluate the clinical relevance of increased CD16<sup>+</sup> monocytes, we searched for associations between the CD14<sup>dim</sup>CD16<sup>+</sup> and CD14<sup>+</sup>CD16<sup>+</sup> subsets and phenotypes related to corpulence and metabolic status in 166 subjects (the C, Diet, OB, and OB/D groups). We found strong associations between CD14<sup>dim</sup>CD16<sup>+</sup> and CD14<sup>+</sup>CD16<sup>+</sup> subpopulations and parameters of corpulence (BMI, fat mass), glucose tolerance and insulin sensitivity (glycemia, insulin, HOMA-IR), and inflammation markers, such as hsCRP (Table 2). In the Diet, OB, and OB/D groups,

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*n*=166 subjects. Data show Spearman correlation coefficient. BMI indicates body mass index; CRP, C-reactive protein; HDL-c, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment insulin resistance; NS, nonsignificant data.

*HbA1c was only measured in the obese and obese diabetic groups.
in which adiponectin was measured, high percentages of CD14<sup>dim</sup>CD16<sup>+</sup> and CD14<sup>-</sup>CD16<sup>+</sup> subpopulations were associated with lower adiponectin concentrations. In the OB and OB/D groups, in which glycosylated hemoglobin (HbA1c) was systematically determined, a positive association was found with monocyte subset frequencies. Furthermore, the percentage of CD14<sup>dim</sup>CD16<sup>+</sup> monocytes was positively correlated with age and TG and negatively with high-density lipoprotein cholesterol (Table 2), unlike the CD14<sup>-</sup>CD16<sup>+</sup> subset.

In multivariate analysis, taking into account age, gender, BMI, fat mass (kg), HOMA-IR, TG, and glycemia, only BMI (or fat mass) and fasting glycemia were independently associated with the percentage of CD14<sup>dim</sup>CD16<sup>+</sup> monocytes (β=0.11, P<10<sup>-4</sup>, and β=0.67, P<10<sup>-4</sup>, respectively). On the other hand, the relationships among CD14<sup>-</sup>CD16<sup>+</sup> percentage and other quantitative variables (Table 2) were not independent of BMI (or fat mass) because BMI was the only significant regressor determined from multivariate regression analysis (β=0.2, P=8.10<sup>-7</sup>).

These results strongly suggest that the CD14<sup>dim</sup>CD16<sup>+</sup> population is linked to glycemia modifications, an observation not made with the CD14<sup>-</sup>CD16<sup>+</sup> population, which is mainly linked with corpulence parameters. We further examined the changes of these monocyte subsets in nutritional situations known to modulate fat mass and related changes in inflammatory and metabolic parameters differently.

### CD16<sup>+</sup> Monocytes Subsets and Diet-Induced Weight Changes

The dietary intervention consisted of a hypocaloric diet over 6 weeks, leading to a mean decrease of 5.9±0.6% in body weight and BMI (Supplemental Table I). As anticipated, caloric restriction led to a decrease in fat mass (−8.3%±1.3) and improvement of metabolic parameters, such as circulating insulin, HOMA-IR (−37.4%±3.8), and lipid profile, whereas it had no significant impact on inflammatory parameters (hsCRP and interleukin-6).

Despite significant improvements in fat mass and insulin sensitivity, the dietary challenge was not accompanied by a significant modification of CD16<sup>+</sup> monocyte populations. Indeed, CD14<sup>dim</sup>CD16<sup>+</sup> percentages were 4.3±0.3% and 3.9±0.3%, and CD14<sup>-</sup>CD16<sup>+</sup> percentages were 2.3±0.2% and 2.5±0.3% at baseline and after the dietary intervention, respectively. Similar findings were observed with absolute counts of CD14<sup>dim</sup>CD16<sup>+</sup> (14.7±2.9 versus 13.4±1.4 cells/μL) and CD14<sup>-</sup>CD16<sup>+</sup> (6.8±0.8 versus 7.8±2.7 cells/μL). However, in 11 of 20 patients, a significant decrease in CD14<sup>dim</sup>CD16<sup>+</sup> monocytes could be observed after the diet compared with baseline. To explore the relationships between the kinetic variations of CD14<sup>dim</sup>CD16<sup>+</sup> monocytes and changes in bioclinical markers, we performed an LME model (n=20). In a multivariate analysis associating age, BMI or fat mass (kg), TG, and glycemia as fixed effects in a combined LME model, we found a positive relationship between the changes in CD14<sup>dim</sup>CD16<sup>+</sup> frequencies and the variations in fat mass (P=0.04).

### CD16<sup>+</sup> Monocyte Subsets and RYGB-Induced Weight Loss

We further examined the kinetic evolution of CD16<sup>+</sup> monocyte subsets in 36 obese subjects before and after RYGB. Of these subjects, 15 were diabetic. RYGB resulted in significant decreases in BMI (−16.8±0.9% and −23.9±1.2% from baseline at 3 and 6 months, respectively) and in fat mass (kg) (−19.0±0.9% and −32.4±1.1% from baseline at 3 and 6 months, respectively) (Table 3). As expected, this RYGB-induced weight loss was associated with major improvements in blood lipids and glucose homeostasis, with a 49% decrease in the insulin resistance surrogate HOMA-IR and a reduction in low-grade inflammation (−32.9±0.8% diminution of hsCRP) at 3 months. These metabolic and inflammatory changes were also observed in both the OB and OB/D groups when considered separately (Supplemental Figure II).

RYGB-induced weight loss was associated with a drastic reduction of both CD14<sup>dim</sup>CD16<sup>+</sup> and CD14<sup>-</sup>CD16<sup>+</sup> monocytes, with a sharper decrease in CD14<sup>dim</sup>CD16<sup>+</sup> 3 months (−36±4% versus −17±6% for CD14<sup>-</sup>CD16<sup>+</sup>) (Table 3 and Figure 2A). At 6 months postsurgery, the percentages of both monocyte subsets increased slightly but remained significantly lower than presurgical levels (Figure 2A). The kinetic profiles were similar in the OB and OB/D groups, with a greater decrease at 3 months in OB/D patients (Figure 2B and 2C). Considering the total monocyte counts in whole blood, similar changes in the absolute numbers of CD14<sup>dim</sup>CD16<sup>+</sup> and CD14<sup>-</sup>CD16<sup>+</sup> were observed (Table 3).

The major improvement in corpulence-related parameters and blood TG was associated with variations in the CD16<sup>+</sup> subpopulation following RYGB. Through univariate analyses, we observed a positive correlation between BMI, fat mass (kg), and TG changes and variations in the percentages of CD14<sup>dim</sup>CD16<sup>+</sup> and CD14<sup>-</sup>CD16<sup>+</sup> monocytes (P<0.05). On the contrary, we found no significant association with blood glucose and insulin-resistance markers. A trend in correlation was only found between CD14<sup>dim</sup>CD16<sup>+</sup> and HbA1c (P=0.09).

The multivariate models confirmed that variations of monocyte subsets after surgery were mainly related to variations in adiposity and blood lipids but were not independent of glucose tolerance and insulin-sensitivity markers. Indeed, the analysis associating diabetic status, age, gender, BMI (or fat mass [kg]), TG, and HbA1c as fixed effects in a combined LME model confirmed the positive relationship between changes in CD14<sup>dim</sup>CD16<sup>+</sup> or CD14<sup>-</sup>CD16<sup>+</sup> monocyte percentages and changes in BMI (or fat mass) (P=0.05) and TG (P=0.01).

We examined the individual responses of subjects to RYGB and separated the 36 subjects into 2 groups based on BMI reduction at 6 months after RYGB (Figure 2D). Before surgery, no significant difference was found in anthropometric, metabolic, or inflammatory variables or in the concentrations of serum factors between the 2 groups (data not shown). The subjects with higher weight loss displayed greater decreases in CD14<sup>dim</sup>CD16<sup>+</sup> and CD14<sup>-</sup>CD16<sup>+</sup> subsets at 6 months (Figure 2E).

Combining values of fat mass variations from the diet and surgery groups, we showed that changes in fat mass were
strongly related to variations in CD14\textsuperscript{dim}CD16\textsuperscript{+} (Figure 3A). Results show that a decrease of at least 5% in fat mass is necessary to observe a significant reduction in the percentages of CD14\textsuperscript{dim}CD16\textsuperscript{+} monocyte subpopulation. However, variations in CD14\textsuperscript{+}CD16\textsuperscript{+} subset percentage were not significantly correlated with changes in fat mass ($R=0.22$, $P=0.1$) (Figure 3B).

The results of this series of analysis confirmed the strong dependence between the amount of adiposity reduction and changes in CD16\textsuperscript{+} monocyte subpopulations, as well as the association with blood TG, but they did not show strong evidence of a link with the improvement of glucose metabolism or with insulin sensitivity.

### Improvement of Vascular Phenotype Associated With Variation of CD14\textsuperscript{+}CD16\textsuperscript{+} Monocytes Subsets During Weight Loss

The data suggested that CD16\textsuperscript{+} monocytes could be important cellular actors in atherosclerosis development.\textsuperscript{14–17,20,25–27}

We evaluated whether a relationships exists between CD16\textsuperscript{+} monocyte subsets and subclinical indicators of early atherosclerosis. We measured FIMT and CIMT in OB and OB/D patients who had never experienced cardiovascular events. No differences in FIMT and CIMT were found between the 2 groups (0.51±0.04 versus 0.54±0.04 mm and 0.63±0.05 versus 0.66±0.05 mm in the OB group versus the OB/D group for FIMT and CIMT, respectively). At baseline, neither CD14\textsuperscript{dim}CD16\textsuperscript{+} nor CD14\textsuperscript{+}CD16\textsuperscript{+} percentages were correlated with FIMT or CIMT in the univariate analysis.

We found that age, BMI, fat mass, leptin, and CRP were significantly correlated with FIMT and CIMT in the OB and OB/D groups together (data not shown). In the multivariate analysis, taking into account age, gender, BMI, fat mass (kg), leptin, and CRP, only BMI and fat mass were independently associated with CIMT ($P=0.02$ and $P=5.10^{-3}$, respectively, for BMI and fat mass), whereas the only significant relationship with age persisted with FIMT. Thus, in this population of morbidly obese subjects, no statistical link was found between the CD16\textsuperscript{+} monocyte subunit and subclinical atherosclerosis.

We further analyzed the variations of subclinical atherosclerosis indicators 3 months after RYGB. Mean FIMT decreased from 0.51±0.01 mm to 0.48±0.01 mm ($P=0.08$), whereas CIMT decreased from 0.62±0.02 to 0.59±0.02 mm ($P=0.07$) at 3 months.

In the univariate analysis, variations in CD14\textsuperscript{+}CD16\textsuperscript{+} monocytes were correlated with variations in CIMT ($P=0.02$), with a trend toward a similar relationship with FIMT ($P=0.08$). However, no relationship was found with changes in CD14\textsuperscript{dim}CD16\textsuperscript{+} monocytes. In the multivariate

### Table 3. Changes in Bioclinical Characteristics and CD14\textsuperscript{dim}CD16\textsuperscript{+} and CD14\textsuperscript{+}CD16\textsuperscript{+} Monocyte Subpopulations in 36 Subjects Following RYGB

<table>
<thead>
<tr>
<th>Value</th>
<th>Preoperative Baseline</th>
<th>3 Mo</th>
<th>6 Mo</th>
<th>Overall $P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>133.9±4.2</td>
<td>111.6±3.7*</td>
<td>101.9±3.5†</td>
<td>$&lt;10^{-4}$</td>
</tr>
<tr>
<td>BMI, kg/m\textsuperscript{2}</td>
<td>49.1±1.4</td>
<td>40.9±1.2*</td>
<td>37.3±1.2†</td>
<td>$&lt;10^{-4}$</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>61.7±2.6</td>
<td>49.9±2.2*</td>
<td>41.6±2.0†</td>
<td>$&lt;10^{-4}$</td>
</tr>
<tr>
<td>Fat mass, %</td>
<td>47.5±0.7</td>
<td>44.5±0.7*</td>
<td>41.0±0.8†</td>
<td>$&lt;10^{-4}$</td>
</tr>
<tr>
<td>Glycemia, mmol/L</td>
<td>6.4±0.3</td>
<td>5.6±0.2*</td>
<td>5.1±0.1†</td>
<td>$&lt;10^{-4}$</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>6.4±0.2</td>
<td>5.9±0.1*</td>
<td>5.8±0.1†</td>
<td>6.10\textsuperscript{-4}</td>
</tr>
<tr>
<td>Insulinemia, µU/mL</td>
<td>20.1±2.2</td>
<td>11.6±2.0*</td>
<td>10.7±2.2†</td>
<td>$&lt;10^{-4}$</td>
</tr>
<tr>
<td>HOMA-IR, %</td>
<td>2.32±0.23</td>
<td>1.36±0.23*</td>
<td>1.18±0.24*</td>
<td>2.10\textsuperscript{-4}</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.9±0.2</td>
<td>4.4±0.2*</td>
<td>4.2±0.1†</td>
<td>8.10\textsuperscript{-4}</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.6±0.11</td>
<td>1.4±0.07*</td>
<td>1.2±0.08*</td>
<td>2.10\textsuperscript{-3}</td>
</tr>
<tr>
<td>HDL-c, mmol/L</td>
<td>1.24±0.07</td>
<td>1.17±0.06</td>
<td>1.22±0.06</td>
<td>NS</td>
</tr>
<tr>
<td>Leptin, ng/mL</td>
<td>44.1±3.1</td>
<td>24.7±2.3*</td>
<td>21.7±2.3†</td>
<td>$&lt;10^{-4}$</td>
</tr>
<tr>
<td>Adiponectin, µg/mL</td>
<td>5.9±0.4</td>
<td>6.7±0.6</td>
<td>6.3±0.6</td>
<td>NS</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>10.6±1.5</td>
<td>6.7±1.0*</td>
<td>5.8±1.4*</td>
<td>$&lt;10^{-4}$</td>
</tr>
<tr>
<td>IL6, pg/mL</td>
<td>4.2±0.7</td>
<td>4.7±0.8</td>
<td>3.2±0.4</td>
<td>NS</td>
</tr>
<tr>
<td>PBMC, 10\textsuperscript{9} cells/µL</td>
<td>3.0±0.3</td>
<td>2.5±0.3</td>
<td>3.0±0.6</td>
<td>NS</td>
</tr>
<tr>
<td>CD14\textsuperscript{dim}CD16\textsuperscript{+}, %</td>
<td>8.3±0.6</td>
<td>5.0±0.3*</td>
<td>5.3±0.6†</td>
<td>$&lt;10^{-4}$</td>
</tr>
<tr>
<td>CD14\textsuperscript{dim}CD16\textsuperscript{+}, cells/µL</td>
<td>20.7±2.9</td>
<td>12.4±1.7*</td>
<td>10.9±2.8†</td>
<td>$&lt;10^{-4}$</td>
</tr>
<tr>
<td>CD14\textsuperscript{+}CD16\textsuperscript{+}, %</td>
<td>5.6±0.8</td>
<td>4.7±0.5*</td>
<td>4.8±0.5†</td>
<td>6.10\textsuperscript{-3}</td>
</tr>
<tr>
<td>CD14\textsuperscript{+}CD16\textsuperscript{+}, cells/µL</td>
<td>15.1±2.1</td>
<td>10.8±1.7*</td>
<td>7.9±4.2†</td>
<td>3.10\textsuperscript{-3}</td>
</tr>
</tbody>
</table>

Data are shown as mean±SEM. Overall $P$ values were obtained using repeated-measures multivariate ANOVA. Comparisons between preoperative baseline and each time point after gastric surgery were obtained by paired Wilcoxon test. $P$ values were Bonferroni corrected. BMI indicates body mass index; CRP, C-reactive protein; HDL-c, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment insulin resistance; IL, interleukin; NS, not significant; PBMC, peripheral blood mononuclear cell; RYGB, Roux-en-Y gastric bypass.  

* $P<0.050$ compared with preoperative value.  
† $P<0.050$ compared with 3 mo value.
In the present study, combining subjects with different levels of corpulence (from moderate to severe obesity) and 2 clinical intervention studies inducing weight loss, we showed strong links between fat mass and the frequencies of CD16+CD14dim monocyte subpopulations in obese subjects. Further, we also observed that diabetes is associated with an increased frequency of CD14dimCD16+ cells, a subtype also characterized by a significant increase in the CD16+ monocyte count. The increased frequency of CD16+ monocytes in obese subjects is likely explained by an increase in fat mass, as observed in patients involved in clinical trials improving insulin sensitivity. In this context, the only association found with metabolic parameters was with fasting TG.

In healthy humans, 3 monocyte subpopulations have been described (CD14+CD16+, CD14+CD16-, and CD14dimCD16+), differing in phenotype and function.19 Human obesity is characterized by a significant increase in the CD16+ subset.12,13 In our study, we demonstrated an increase in the 2 CD14dimCD16+ and CD14+CD16+ monocyte subtypes in obese subjects. Furthermore, we showed strong links between fat mass and the frequencies of CD14+CD16+ monocytes in the population. Indeed, we observed an increase of about twice the percentage of CD16+ monocyte subsets in obesity and a reduction of these cell populations by drastic fat mass loss. A fat mass decrease of at least 5% was sufficient to observe a reduction in the CD16+ monocyte count. On the contrary, we could not demonstrate a convincing link with glucose homeostasis in patients involved in clinical trials improving insulin sensitivity. In this context, the only association found with metabolic parameters was with fasting TG.

Analysis, taking into account age, gender, variations in BMI (or fat mass [kg]) and variations in CD16+ subsets, the CD14+CD16+ monocyte count was not independently associated with IMT measurements, confirming a strong dependence with the level of corpulence.

**Discussion**

In the present study, combining subjects with different levels of corpulence (from moderate to severe obesity) and 2 clinical intervention studies inducing weight loss, we showed strong links between fat mass and the frequencies of CD16+CD14dim monocyte subpopulations in obese subjects. Further, we also observed that diabetes is associated with an increased frequency of CD14dimCD16+ cells, a subtype also...
BMI had greater decreases in CD16
moderate weight reduction (ie,
in obese subjects.28–31 Here, we observed that the percentages
of CD16 subsets decreased with surgery-induced weight loss. Patients displaying a higher diminution of fat mass or
BMI had greater decreases in CD16+ monocyte subsets. A moderate weight reduction (ie, <5%) did not affect monocyte
subset frequencies.

Furthermore, we found that a quantitative variation in fat mass during weight loss was strongly correlated with changes in CD14dimCD16+ monocytes. It is now well established that the enlarged fat mass characterizing obesity is associated with macrophage accumulation and with alteration of adipose-tissue secretions32, which are also potent modulators of monocyte phenotypes. We have observed a significant correlation between the number of macrophages in visceral adipose tissue and the percentages of CD16+ monocytes (Dalmas C, Tordjman J, Clement K, Veyrie N, Guerre-Millo M, Poitou C, personal communication), but an extended analysis is required to study the impact of adipose-tissue secretion on monocyte phenotypes in obesity and during weight loss to find the factors involved in the differentiation of CD16+ monocytes.

Importantly, although both moderate and drastic weight-loss procedures unambiguously improve insulin-sensitivity surrogates, no kinetic association was found with changes in the percentages of CD16+ monocytes. We nevertheless highlighted the association between the frequency of mono-
lymphocyte subsets and TG changes independent of BMI decrease. After RYGB, changes in TG are either related to variations in free fatty acid flux provided from adipose tissue lipolysis or to very-low-density lipoprotein production by the liver in relation to the improvement of insulin resistance. We did not observe any correlation with HOMA-IR or changes in CD16+ subsets, suggesting that a decrease in CD16+ monocytes could not be attributed to an improvement in insulin resistance. The association between macrophages in human adipose tissue and insulin resistance is also debated. Previous studies have shown a negative correlation between whole-body insulin sensitivity and the expression of the macrophage marker CD68 in subcutaneous adipose depots.33,34 Preferential macrophage accumulation into visceral adipose tissue has been observed mainly in subjects with impaired glucose homeostasis,35 and obese subjects with more crown-like structures of macrophages in adipose tissue have been shown to be more insulin-resistant than those subjects lacking these cell aggregates.36 On the contrary and in agreement with this present work on circulating monocytes, no correlation in morbid obesity has been found between adipose tissue mac-
rophages in visceral depots and blood-derived parameters of insulin resistance,37,38 whereas an association was found with fasting TG.39 An overfeeding challenge rapidly installed an insulin-resistant state in healthy subjects, despite the fact that no significant change occurred in the total macrophage accumulation in the adipose tissue and that there was no change in the number of circulating cells.40 Additionally, we found that irrespective of the degree of insulin resistance in morbid obesity, macrophage accumulation in omental adipose tissue was associated with the severity of liver fibroinflammation, a well-known and severe complication of obe-

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Disclosures
None.

References
CD14<sup>dim</sup>CD16<sup>+</sup> and CD14<sup>+</sup>CD16<sup>+</sup> Monocytes in Obesity and During Weight Loss: Relationships With Fat Mass and Subclinical Atherosclerosis

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Supplemental Figure II