Adipose tissue inflammation, characterized by both inflammatory cell infiltration and increased expression and secretion of proinflammatory mediators, occurs in both rodent obesity models and in human obesity (1–3).

Adipose inflammation was shown to be more pronounced in the intra-abdominal fat depot than in subcutaneous fat, particularly in persons with visceral (central) adiposity (4, 5). Macrophages were the first immune cell type to be
reported in adipose tissue, and other hematopoietic cells have been implicated since then (1, 2, 6, 7). However, there are remaining gaps in our understanding of the causative roles of adipose tissue inflammation and its individual components in obesity-associated morbidities. Some reports suggest that manipulating macrophage recruitment to adipose tissues in obese mice has an effect on adipose tissue inflammation and glucose tolerance (8, 9), but the contribution of adipose tissue macrophages (ATMs) to obesity-induced insulin resistance is still questionable (10). At the cellular level, a functional cross-talk between macrophages and adipocytes was proposed to support a metabolic inflammatory vicious cycle, resulting in increased proinflammatory cytokines and elevated basal lipolysis (11).

Questioning the simplistic view of ATMs as negative regulators of adipose tissue in obesity are studies suggesting that ATMs may support cellular and extracellular remodeling of adipose tissue in the adaptation to chronic overnutrition (12). ATMs were suggested to clear dead (overhypertrophied) adipocytes from the tissue by engulfing their remains, mainly large triglyceride droplets (13, 14). Such a process would probably result in lipid accumulation within the macrophages (unless enhanced stored lipid breakdown meets the increased accumulation). Increased lipid accumulation in macrophages resembles the atherosclerosis-promoting process in which lipid-laden macrophages accumulate, developing a foamy-appearing cytosol that inspired the term foam cell (FC). These cells, in atherosclerosis, may contribute to pathogenesis: in early lesion areas, macrophages and FCs may contribute to a low-grade inflammatory state, uptake of lipoproteins, and clearing of apoptotic cells; in advanced lesions, attenuated macrophage phagocytosis and FC death contribute to plaque instability and risk of an acute cardiovascular event (15, 16).

Given that obesity is a major risk factor for atherosclerosis and the putative commonalities between atherogenesis and processes occurring within adipose tissue in obesity, we hypothesized that FCs may develop in human adipose tissue. In this study we used a fluorescence-activated cell sorter (FACS)-based approach to quantify lipid content in human ATMs, morphological studies to demonstrate adipose FCs, and a functional method to study their potential involvement in the pathogenesis of obesity-associated morbidities.

Materials and Methods

Participants were part of the Beer-Sheva and the Leipzig cohorts as described previously (5, 17). In brief, participants were recruited in both centers before undergoing abdominal surgery (primarily bariatric surgery and elective cholecystectomy) after providing written informed consent. Paired abdominal subcutaneous (SC) and omental (Om) adipose tissue biopsy samples were obtained and immediately delivered to the laboratory where they were processed for expression and histological studies as described previously (5, 18), using methodology coordinated between the two centers. All procedures were approved in advance by the ethics committees of the institutions and were conducted in accordance with Declaration of Helsinki guidelines. Clinical characteristics of participants in the present analyses are presented in Tables 1 and 2. Adipocytokines were measured as described in Kloting et al (19).

Animals and treatments

The study was approved in advance by Ben-Gurion University Institutional Animal Care and Use Committee and was conducted according to the Israeli Animal Welfare Act following the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). Male wild-type C57BL/6 mice were purchased from Harlan Laboratories (Rehovot, Israel) and fed from age 6 to 7 weeks with a high-fat diet (60% kcal of fat, D12492; Research Diets, New Brunswick, New Jersey) or normal Chow diet (10% kcal of fat, D12450B; Research Diets). After 16 weeks, mice fed the high-fat diet gained significantly more weight, weighing 43.6 ± 0.9 g compared with 31.57 ± 0.9 g for mice fed normal chow (P < .0001). They were insulin resistant, and after an overnight fast had mean fasting glucose, insulin, and homeostatic assessment of insulin resistance values that were 1.7 ± 0.1–, 3.3 ± 1.0–, and 4.7 ± 1.5-fold, respectively, higher than those for mice fed normal chow. At the end of the experiment, mice were killed with isoflurane, and adipose tissues were removed and processed.

Isolation of adipose stromal-vascular cell fraction (SVF)

Human fat pads were excised and minced in 10 ml DMEM containing 4.5 mM glucose (without phenol red), 10 mM HEPES, pH 7.4, and 1% BSA. Collagenase II (C6885, 1 mg/mL; Sigma-Aldrich, St. Louis, Missouri) was added, and minced tissues were incubated at 37°C for 20 minutes with shaking. Larger particles were removed using 250-μm mesh, and 10 mM EDTA was added, followed by centrifugation at 300g for 5 minutes at 4°C to separate the floating adipocytes from the SVF pellets. EDTA (10 mM) was added to the adipocyte fractions and centrifuged again at 500g for 5 minutes at 4°C.

FACS analysis of human adipose tissue

SVF pellets were washed, pelleted (500g for 5 minutes at 4°C), and resuspended in 300 μL of staining buffer (PBS with 2% fetal bovine serum [FBS]) containing human Fc Block (eBioscience, San Diego, California) and stained with the following conjugated antibodies (10 minutes on ice in the dark): CD45-PE-Cy7, CD64-APC, and CD14-APC-Cy7 (BioLegend, San Diego, California). Samples were washed and stained for 20 minutes on ice with BODIPY 493/503 (3 μg/mL BODIPY for 5 × 107 cells, D3922; Invitrogen, Carlsbad, California). Stained samples were further washed and filtered using 100-μm meshes. Propidium iodide (0.2 μg/mL; Sigma-Aldrich) was added to all samples. Stained samples were analyzed by FACS (FACSCanto II; BD Biosciences, San Jose, California).
Table 1. Clinical and Biochemical Characteristics: Beer-Sheva Cohort (N = 94)

<table>
<thead>
<tr>
<th>Protein</th>
<th>Lean (BMI &lt;25 kg/m²)</th>
<th>Overweight (BMI &gt;25–&lt;30 kg/m²)</th>
<th>Obese (BMI &gt;30 kg/m²)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>15</td>
<td>22</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>9/6</td>
<td>5/17</td>
<td>17/40</td>
<td>NS</td>
</tr>
<tr>
<td>Age, y</td>
<td>40.5 ± 3.3</td>
<td>51.9 ± 4.5</td>
<td>43.7 ± 2.0</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.1 ± 0.7</td>
<td>27.4 ± 0.3</td>
<td>39.7 ± 0.9</td>
<td>&lt;.001 a,b</td>
</tr>
<tr>
<td>No. of positive metabolic syndrome parameters</td>
<td>1.2 ± 0.4</td>
<td>2.1 ± 0.3</td>
<td>2.8 ± 0.2</td>
<td>.001 a</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>116.5 ± 3.5</td>
<td>121.8 ± 2.8</td>
<td>129.3 ± 1.6</td>
<td>.006</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>73.2 ± 2.1</td>
<td>72.1 ± 1.6</td>
<td>77.4 ± 1.2</td>
<td>.050</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>104.0 (81.5, 178.5)</td>
<td>110.5 (90.5, 167.0)</td>
<td>124.0 (99.8, 150.5)</td>
<td>NS</td>
</tr>
<tr>
<td>HDL, mg/dL</td>
<td>Men</td>
<td>Women</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>37.9 ± 3.6</td>
<td>54.6 ± 1.7</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Fasting glucose, mg/dL</td>
<td>93.5 ± 6.2</td>
<td>93.2 ± 5.1</td>
<td>98.3 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>HbA1c, %</td>
<td>5.7 ± 0.3</td>
<td>6.7 ± 0.2</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Diabetes, %</td>
<td>6.7</td>
<td>18.2</td>
<td>17.5</td>
</tr>
<tr>
<td></td>
<td>Fasting insulin</td>
<td>3.2 ± 0.5</td>
<td>6.1 ± 1.3</td>
<td>9.9 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>HOMA-IR</td>
<td>0.8 ± 0.1</td>
<td>1.4 ± 0.3</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>hsCRP</td>
<td>0.2 (0.1, 0.7)</td>
<td>0.3 (0.1, 0.8)</td>
<td>0.6 (0.3, 1.2)</td>
</tr>
</tbody>
</table>

Abbreviations: HbA1c, glycosylated hemoglobin; HOMA-IR, homeostatic assessment of insulin resistance; NS, not significant.

a Significant (P < .05) difference between lean and obese groups by post hoc test.

b Significant (P < .05) difference between overweight and obese groups by post hoc test.

c Median values (25%, 75%).

Histological studies

Tissue samples from Om and SC fat were fixed in 10% buffered formalin, processed, and embedded in paraffin. Sections of 5 μm were stained with hematoxylin and eosin (H&E). Immunohistochemical staining of formalin-fixed paraffin-embedded sections for CD68 (1:100; Dako, Glostrup, Denmark) was performed using the avidin-biotin complex method (iVIEW Dab Detection Kit; Ventana XT Benchmark, Strasbourg, France). Microscopic examination was performed by a certified pathologist (R.S.-L.), who was blinded to the identity of the samples. FCs, defined as lipid-laden macrophages with foamy cytoplasm (20), were counted in high-power fields (HPFs) (×400), and the number of FCs per 5 HPFs was calculated.

Sorting of control ATMs and FCs

To identify mouse ATMs, SVF pellets were stained for CD45-APC, F4/80-PE-Cy7 (E-Bioscience), and CD11b-APC-Cy7 (BD Pharmingen, San Jose, California) followed by BODIPY and propidium iodide staining as indicated above. Stained samples were sorted by FACSaria II (BD Biociences) using a 100-μm nozzle, in sterile conditions. Sorting of FCs from obese mice was performed by collecting cells with >10-fold higher lipid content than macrophages from lean mice. Sorting of control ATMs from lean mice was performed by collecting most of the macrophages containing low lipid content. Sorted populations were validated by light microscopy for the presence (95%) or absence of lipid droplets in the ATM fraction.

Coculture of control ATMs or FCs with adipose tissue explants

Mouse ATM populations were sorted from epididymal fat pads, pooled from 1 to 2 mice fed a high-fat diet or 2 to 3 mice fed normal chow and incubated in sterile conditions for 8 hours with DMEM + 10% FBS. Fat explants (1–3 mm³ pieces of epididymal adipose tissue from lean mice) were incubated in sterile conditions for 8 hours in DMEM + 10% FBS. Subsequently, fat explants were transferred to cell culture inserts with a bottom-permeable (0.4-μm pore size) polyethylene terephtalate track-etched membrane (BD Falcon 353180; BD, Franklin Lakes, New Jersey) and placed for 4 hours above the sorted ATMs in a 12-well plate coated with 10 μg/mL fibronectin (03-090-1; Biological Industries, Beit Haemek, Israel). After coculture, ATMs were tested for viability by flow cytometry after 7-aminoactinomycin D labeling, and only if viability remained greater than 85% were analyses continued. Moreover, adipose explant viability was tested by total protein recovery (explants incubated with control macrophages, 231.2 ± 45.2 μg/well; with FCs, 218.0 ± 22.3 μg/well). Moreover, LDH release into the coculture media was measured and was not significantly different (P = .624) when explants were incubated with either control macrophages or FCs. To determine adipose insulin responsiveness after coculture, fat explants were washed and incubated for 2 hours with DMEM without serum. Explants were then stimulated with insulin (100 nM) for 7 minutes and collected for the generation of protein lysates.

Cell lysates and Western blot analysis

Protein lysates from 100 mg of epididymal adipose tissue were prepared by homogenizing in radioimmunoprecipitation assay (RIPA) lysis buffer as described previously (21). Protein samples were resolved on 10% SDS-PAGE and subjected to Western blot using the following antibodies: anti-pSer473 PKB/Akt, anti-pThr308 PKB/Akt and anti-PKB/Akt, anti-pSer21/9GSK3, and anti–GSK3 (Cell Signaling Technology, Danvers, Massachusetts); anti-mouse anti-phosphotyrosine PY20 (Transduction Laboratories, Lexington, Kentucky); and anti–β-actin (Sigma-Aldrich). Western blot was followed by quantification as described previously using Image Gauge software (version 9.0).
We next investigated whether increased macrophage lipid content resulted in cellular morphology of lipid-laden FCs. For this purpose, adipose tissue sections were stained for the leukocyte marker CD45 (Figure 1A). From the CD45+ cells, a cluster of ATMs was identified as double-positive for CD14 and CD64 (Figure 1B). These ATMs were assessed for intracellular lipid content using the neutral lipid dye BODIPY (Figure 1C). The MFI of the lipids in ATMs was determined from both SC and Om fat biopsy samples. Of the entire study group of 94 persons from the Beer-Sheva cohort whose characteristics are presented in Table 1, the adipose tissue sections were analyzed by Mann-Whitney test for mean fluorescence intensity (MFI) and for 2-group analyses such as FC abundance in SC vs Om fat. The Spearman test was used for evaluating correlations between FC abundance in SC and Om fat and clinical parameters. Finally, we tried to assess which coefficients best predict the values of the dependent variables (SC and Om FCs). Aware of the statistical limitation for a multivariable model within our sample size, we chose the multivariable regression parameters. Finally, we tried to assess which coefficients best predict the values of the dependent variables (SC and Om FCs).

### Results

Because ATMs in obesity have been proposed to engulf the remains of dead adipocytes in adipose tissue, we first used a FACS-based approach to determine the lipid content of ATMs from human fat biopsy samples. After tissue collagenase digestion and collection of the SVF, live cells (e.g., propidium iodide-negative) were stained for the leukocyte marker CD45 (Figure 1A). From the CD45+ cells, a cluster of ATMs was identified as double-positive for CD14 and CD64 (Figure 1B). These ATMs were assessed for intracellular lipid content using the neutral lipid dye BODIPY (Figure 1C). The MFI of the lipids in ATMs was determined from both SC and Om fat biopsy samples. Of the entire study group of 94 persons from the Beer-Sheva cohort whose characteristics are presented in Table 1, the adipose tissue sections were analyzed by Mann-Whitney test for mean fluorescence intensity (MFI) and for 2-group analyses such as FC abundance in SC vs Om fat. The Spearman test was used for evaluating correlations between FC abundance in SC and Om fat and clinical parameters. Finally, we tried to assess which coefficients best predict the values of the dependent variables (SC and Om FCs). Aware of the statistical limitation for a multivariable model within our sample size, we chose the multivariable regression parameters. Finally, we tried to assess which coefficients best predict the values of the dependent variables (SC and Om FCs).
examined microscopically. Indeed, FCs were more abundant in Om tissues of obese patients, present as clusters both in between adipocytes and in crown-like structures surrounding adipocytes/lipid droplets (Figure 2, A and B, respectively). Their identity as macrophages was confirmed by CD68 immunohistochemical staining in serial sections (Figure 2A, right panel). The total number of macrophages and FCs in histological sections of SC and Om fat was counted in a subgroup of 57 participants (10 lean, 13 overweight, and 34 obese). Consistent with other and our previous studies (4, 5), obesity was associated with increased ATMs in Om fat, being 1.30- and 1.72-fold higher in overweight and obese subjects, respectively, than in lean persons. FC counts exhibited large variability notable in both depots and in all groups. However, obese patients had a significantly higher number of FCs in Om fat than in SC fat compared with lean persons (Figure 2C). Similarly, the median percentage of FCs from total ATMs was significantly higher only in Om fat, with a median of 0.0 ± 0.0, 0.24 ± 0.14, and 0.92 ± 0.21 in lean, overweight, and obese persons, respectively (P = .024 and .063 in lean and overweight persons, respectively, compared with obese patients). Correlation analyses revealed a statistically significant (P < .001) association between BMI and Om FC counts (Figure 2D) but not with SC FC counts.

To verify the presence of adipose tissue FCs in another patient population, which allows us to assess the contribution of fat distribution to adipose tissue FCs, we analyzed histological sections of Om fat biopsy samples from 40 obese participants in the Leipzig adipose tissue biobank (characteristics are presented in Table 2). In this cohort, patients were classified as having predominantly subcutaneous or intra-abdominal adiposity based on the measurement of abdominal fat distribution by magnetic resonance imaging scans, as described previously (5). In the Leipzig cohort, adipose FCs could be observed in many patients, with mean FC counts at least 2-fold higher than those in the Beer-Sheva cohort (Figure 2, E vs D), consistent with our previous findings that ATM counts are significantly different between the two cohorts (5). Importantly, the mean number of FCs was higher in the group of patients with predominantly intra-abdominal compared with subcutaneous adiposity (Figure 2E). Interestingly, we did not detect significant correlation between Om FC counts and the levels of adiponectin, leptin, IL-6, progranulin, chemerin, vaspin, monocyte chemotactic protein-1, or retinol-binding protein 4 (Table 2). Collectively, FCs can be identified in human adipose tissue, their...
numbers are higher in Om than in SC fat in obese persons, and they seem to be more abundant in Om fat in persons with abdominal fat distribution that is predominantly intra-abdominal (ie, visceral adiposity).

To gain further insight into clinical parameters that associate with adipose FCs in both depots, we first performed correlation analyses (Figure 3). Systemic markers of impaired glucose and lipid metabolism (increased fasting glucose and lower high-density lipoprotein [HDL] levels) tended to be associated with higher FC counts in SC tissue. Conversely, in Om tissue, FC counts are associated with systemic markers of insulin resistance (fasting insulin) and local or systemic inflammation such as the total number of ATMs and circulating high-sensitivity C-reactive protein (hsCRP). Accordingly, the number of positive criteria for the metabolic syndrome significantly correlated with FC counts in the Om but not the SC depot (Figure 3). To further estimate which of these factor(s) independently associate with FCs, we performed stepwise multivariate regression analysis. We used models that included univariate parameters found to significantly associate with FCs (HDL, fasting glucose, insulin, and hsCRP) and age, the BMI group, and number of total macrophages in either tissue. Fasting glucose remained independently associated with FC counts in SC fat ($\beta = 0.667, P < .001$) with borderline negative association with HDL ($\beta = -0.359, P = .066$). In Om fat, fasting insulin ($\beta = 0.413, P = .006$) and total ATM count ($\beta = 0.310, P = .034$) remained independently associated with FC.

To functionally characterize adipose FCs and assess their possible contribution to insulin resistance, we used a common mouse model for diet-induced obesity. C57BL6 mice were fed a high-fat diet for 16 weeks, gained weight, and developed insulin resistance, as described previously (23) and detailed in Materials and Methods. Adipose tissue FCs were isolated by a FACS-based assay similar to the one in humans described earlier, using the mouse-specific macrophage markers CD11b and F4/80. ATMs from lean mice had low lipid content and were collected and defined as control macrophages, whereas ATMs from obese mice with lipid content 10-fold higher than control macrophages were collected and defined as FCs. Light microscopy confirmed that >95% of sorted cells in the latter fraction exhibited FC morphology with abundant lipid droplets (Figure 4A). FCs or control macrophages were cocultured with adipose tissue fragments of lean mice, and
Atherosclerotic plaques. Surprisingly, the presence of FCs in Om
reminiscent of lipid-laden macrophages previously described in
a small subpopulation of ATMs that adopt FC morphology
some patients this excessive ATM lipid accumulation results
increases several fold. Furthermore, we demonstrate that in
human obesity, particularly in Om fat, ATM lipid content
into the ATMs (13). In this study, we demonstrate that in
graphs suggestive of engulfment of lipids from such droplets
viable (perilipin-negative) adipocytes and electron micro-
"crown-like structures" surrounding lipid droplets of non-
accumulate in adipose tissue to eliminate dead adipocytes
largely unknown. A main proposition is that macrophages
participating in some aspects of adipose tissue remodeling is
is still enigmatic. In particular, sorting out whether their activity in adipose tissue
is protective by par-
pathophysiology such as insulin resistance or, conversely,
sort extracellular mediator(s) responsible for this effect awaits further studies.

What are the processes promoting FC biogenesis within adipose tissue? It is important to realize that although adipose FCs were morphologically similar to FCs of atherosclerotic plaques, their stored lipids are probably different. In atherosclerosis, the lipid retained in the macrophage largely originates from modified lipoproteins that penetrate the vessel wall, such as oxidized low-density lipoprotein (16). In adipose tissues, the stored lipids in ATMs would predominantly be triglycerides, the major component of the adipocyte lipid droplet (14). It is also possible that the adipose tissue microenvironment would support lipid storage through reesterification of free fatty acids, which may be uniquely increased particularly in Om fat and under conditions of obesity and insulin resistance, the latter consistent with Om FC association with fasting insulin. Finally, it is possible that the inflammatory microenvironment that develops in adipose tissue in obesity, particularly in intra-abdominal fat, may also impair the

Discussion

The exact function of adipose tissue macrophages in obesity is still enigmatic. In particular, sorting out whether their prime function in obesity contributes to obesity-induced pathophysiology such as insulin resistance or, conversely, whether their activity in adipose tissue is protective by partici-
ating in some aspects of adipose tissue remodeling is largely unknown. A main proposition is that macrophages accumulate in adipose tissue to eliminate dead adipocytes remnants generated by excessive hypertrophy (12, 13). This had been supported by ATM distribution in clusters and “crown-like structures” surrounding lipid droplets of non-
viable (perilipin-negative) adipocytes and electron micrographs suggestive of engulfment of lipids from such droplets into the ATMs (13). In this study, we demonstrate that in human obesity, particularly in Om fat, ATM lipid content increases several fold. Furthermore, we demonstrate that in some patients this excessive ATM lipid accumulation results in a small subpopulation of ATMs that adopt FC morphology reminiscent of lipid-laden macrophages previously described in atherosclerotic plaques. Surprisingly, the presence of FCs in Om or SC fat depots uniquely associates with different clinical pa-
rameters of the patients. Finally, we provide functional characterization of adipose FCs from a mouse diet-induced obesity model, demonstrating their capacity to contribute to adipose tissue insulin resistance.

A major question emerging from the identification of the FC subclass of ATMs is whether they are the mark of a unique phenotype in the obese repertoire, whether they have unique functions in adipose tissue, or whether they are simply an extreme type of ATM bearing no unique association or biological activity compared with non-FC ATMs. Several lines of evidence suggest that adipose FCs do indeed have unique significance. First, their presence in Om vs SC fat associates with unique clinical parameters: the abundance of adipose tissue FCs in Om fat associated with BMI, insulin resistance, and inflammatory state, whereas SC FCs associate with fasting glucose. Using multivariate models, we have shown that omental FCs were independently associated with total ATM count and fasting insulin. Intriguingly, Om FC numbers did not correlate with various adipocytokines in obese patients, suggesting that their presence may indicate a unique phenotype not easily identified by conventional or more novel biomarkers. Finally, using a common mouse nutritional model of obesity, we provide evidence for a potentially unique cross-talk of fat tissues with adipose FCs compared with ATMs from lean mice, resulting in diminished adipose insulin responsiveness. Identifying the exact FC-derived mediator(s) responsible for this effect awaits further studies.
capacity of the macrophage to break down the stored lipids by either cytosolic and/or lysosomal lipases (24) or due to inhibition of fatty acid oxidation capacity. Indeed, inflammatory cytokines including IL-1β have been shown to diminish hepatic fatty acid oxidation capacity (25), and our own preliminary results demonstrate increased FC biogenesis when macrophages were incubated with oleic acid in the presence of IL-1β (data not shown). Thus, it is likely that in Om fat, which is characterized by increased lipolytic rate and higher inflammation than SC fat, these two processes may be major drivers of FC biogenesis. In return, FC cross-talk with adipocytes may render adipose tissue more insulin resistant, forming a local metabolic inflammatory vicious cycle. It would be fascinating to assess whether, consistent with their unique association with clinical parameters, FCs in SC fat use different processes than in Om fat, such as de novo lipogenesis fueled by elevated glucose levels. Based on the associations and mechanistic studies provided herein, it would be tempting to propose that Om FCs potentially contribute to obesity-associated insulin resistance and inflammation, whereas SC FCs respond to the downstream consequences of these insulin resistance-induced endocrine/metabolic alterations.

Regarding the activation status of FCs in obesity, it has been proposed that the ATMs undergo a phenotypic switch from M2 to a M1 and that M1 macrophages contribute to progression of insulin resistance (26). However, a mixed M1/M2 phenotype was reported in ATMs in mice (27) and humans (28). Given the pronounced association of FCs with parameters associated with insulin resistance, the mixed M1/M2 phenotype of ATMs and the dispute about the contribution of M1/M2 phenotype to the pathological conditions associated with obesity, we have focused here on assessing the direct contribution of FCs to adipose tissue insulin resistance. Indeed, culture of adipose tissue explants with FCs compared with control ATMs showed a significant reduction in insulin response and thus may contribute to obesity-induced insulin resistance. However, a cautionary note should be raised given the low abundance of FCs, although the abundance of other leu-
kocyte subtypes implicated in adipose tissue dysfunction was also similarly low (such as natural killer or B cells, which are estimated to account for ≤1% of the SVF) (29).

In conclusion, we demonstrate the presence of a small subpopulation of adipose tissue macrophages defined as FCs. They are more abundant in, although not restricted to, intra-abdominal fat, particularly in obesity, and more so if fat distribution is predominantly intra-abdominal. These cells may be engaged in local cross-talk with adipocytes contributing to adipose tissue insulin resistance. It would seem important to determine whether they are signatures of a unique obese phenotype and, if so, whether manipulating their biosignatures could improve obesity-associated morbidities.

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