Low-grade adipose tissue inflammation in patients with mild-to-moderate chronic obstructive pulmonary disease

Bram van den Borst, Harry R. Gosker, Geertjan Wesseling, Wilco de Jager, Valéry ACV Hellwig, Frank J Snepvangers, and Annemie MWJ Schols

ABSTRACT
Background: Low-grade systemic inflammation is common in chronic obstructive pulmonary disease (COPD), but its source remains unclear. Adipose tissue is a potent producer of inflammatory mediators and may contribute to systemic inflammation in COPD, possibly via hypoxia.

Objective: We studied the influence of COPD and exercise-induced oxygen desaturation on adipose tissue inflammation (ATI) and its contribution to systemic inflammation.

Design: Subcutaneous adipose tissue biopsies were investigated in 28 clinically stable COPD patients [forced expiratory volume in 1 s: 58 ± 16% predicted; BMI (in kg/m²): 24.9 ± 2.9] and 15 age-, sex-, and body composition–matched healthy control subjects. Fat mass was measured with dual-energy X-ray absorptiometry. Patients were prestratified by oxygen desaturation assessed by incremental cycle ergometry. The adipocyte size and adipose tissue expression of 19 inflammatory and hypoxia-related genes were measured, and adipose tissue macrophages (ATMs) were histologically quantified. Systemic inflammatory markers included C-reactive protein (CRP) and a panel of 20 adipokines.

Results: COPD patients had comparable fat mass but higher CRP and HOMA-IR than did control subjects. COPD patients and control subjects had comparable adipose tissue gene expression, adipocyte size, ATM infiltration, and systemic adipokine concentrations. Desaturating COPD patients had no different ATI status than did non-desaturating COPD patients. COPD patients with high CRP had significantly greater ATM infiltration than did patients with low CRP, which was independent of BMI and fat mass.

Conclusions: In COPD patients, mild-to-moderate COPD, per se, does not enhance ATI or its contribution to systemic inflammation compared with in well-matched healthy control subjects. However, to our knowledge, our study provides a first indication for a possible role of ATMs in the systemic inflammatory response in COPD that requires additional investigation. This trial was registered at www.trialregister.nl as NTR1402. Am J Clin Nutr doi: 10.3945/ajcn.111.023911.

INTRODUCTION
COPD is characterized by low-grade systemic inflammation that has been associated with IR (1). Classically, systemically elevated cytokine concentrations are hypothesized to result from a spillover of the pulmonary compartment. However, there is no convincing evidence to support this theory in clinically stable disease. Alternatively, adipose tissue has been proposed to contribute to systemic inflammation in obese COPD patients (2). In addition to storing energy, adipose tissue is an active producer of mediators involved in inflammation, the so-called adipokines of which leptin and adiponectin are examples (2).

Several mechanisms have been proposed to induce or relate to ATI that we hypothesized could be involved in COPD and result in enhanced systemic inflammation. First, large adipocytes produce more proinflammatory adipokines than do small adipocytes (3), which is indicative of the adipocyte size as a marker of ATI. Most patients with COPD receive chronic β2-agonists that could contribute to the blunted β-adrenergic receptor mediated lipolysis and thermogenesis that results in the maintenance or relative expansion of fat tissue in COPD (4). Second, a major reduction of oxygen supply may lead to ATI and be potentially aggravated via a disbalance between adipocyte size and (neo)vascularization that leads to local hypoxic areas or intraadipocytic hypoxia (5). However, it is unknown whether mild hypoxemia as present in early stages of COPD or intermittent desaturations in some COPD phenotypes could also trigger ATI in COPD. Chronic hypoxemia can occur during advanced disease, but recurrent desaturations also occur in moderate disease during daily physical activities (6). Third, IR has been linked to both systemic inflammation (7) and ATI (8). Finally, although ATMs have been put forward as orchestrators

of ATI (9), they have never been quantified in COPD to our knowledge.

Recently, Tkacova et al (10) showed higher proinflammatory gene expression in adipose tissue of underweight GOLD IV patients compared with overweight GOLD I–III patients (10). Groups were also different in PaO₂ (7.7 compared with 9.4 kPa, respectively), which suggested that hypoxemia may play a role in ATI. Moreover, this study suggested that ATI may not be restricted to the obese state. Subsequently, the same authors (11) showed that with increasing BMI (kg/m²; range: 18–36), a higher prevalence of IR was present in COPD patients, which was associated with higher adipose tissue expression of proinflammatory genes. However, BMI and FM are known correlates of ATI (8, 12, 13). Thus, to answer the question if COPD, per se, is characterized by ATI and to study its relation with systemic inflammation, we studied COPD patients and BMI- and FM–matched healthy control subjects. In addition, we investigated a potential role of exercise-induced desaturation in COPD on ATI and systemic inflammation.

SUBJECTS AND METHODS

Subjects, pulmonary function, and incremental exercise test

This study included 28 clinically stable COPD patients without overt comorbidity and 15 healthy control subjects (see the online supplement under “Supplemental data” in the online issue for a detailed methodology; also see Table S1 under “Supplemental data” in the online issue for a list of pulmonary medications). Pulmonary function testing included forced spirometry and single-breath diffusion-capacity measurement (Masterlab; Jaeger). All subjects performed an incremental cycle ergometry test according to international standards to determine exercise-induced desaturation defined as a fall in oxygen saturation (SaO₂ by pulse oximetry) of ≥4% (14). There were 12 COPDⅢ patients (mean ± SD: decrease in SaO₂ 8.5 ± 2.2%) and 16 COPDⅢⅢ patients (decrease in SaO₂ 2.1 ± 1.7%). Arterial blood gas analysis (Blood Gas Analyzer 865; Chiron Diagnostics) was performed at rest and at peak exercise with a radial artery puncture. Written informed consent was obtained from all subjects, and the ethical review board of the University of California, San Francisco, CA, 20 January 2011). The trial was registered at www.trialregister.nl as NTR1402.

Anthropometric measurements and body composition

Height was measured with a wall-mounted stadiometer. Body weight was assessed to the nearest 0.1 kg with a standard balance beam scale. BMI was calculated as weight divided by the square of height. Whole-body dual-energy X-ray absorptiometry (Hologic Discovery (Hologic) or Lunar Prodigy (GE Healthcare)) was applied to retrieve whole-body BMI and FFMI. Comparison of Lunar Prodigy (GE Healthcare) and Hologic Discovery (Hologic) dual-energy X-ray absorptiometry–retrieved measures was enabled through the application of cross-calibration equations (unpublished equations, provided by JA Shepherd, University of California, San Francisco, CA, 20 January 2011). The FFMI and FMI were calculated as FFMI divided by the square of height and FM divided by the square of height, respectively.

Adipose tissue biopsy

SAT was obtained paraumbilically after an overnight fast through a needle biopsy. One specimen was snap frozen in liquid nitrogen and stored at −80°C until further analysis, and another specimen was processed in 4% formalin for paraffin embedding.

Real-time quantitative polymerase chain reaction

Total RNA from SAT was extracted with a kit (RNasy Lipid Tissue Mini Kit; Qiagen) and reverse-transcribed into cDNA with a Transcripter cDNA synthesis kit (Roche Applied Sciences). cDNA was amplified with SYBR Green Fluorescein Mix (Westburg) on a quantitative polymerase chain reaction machine (Bio-Rad Laboratories BV). We measured a panel of genes that consisted of adipokines (ie, leptin, adiponectin, plasminogen activator inhibitor-1, chemerin, osteoprotegerin, adrenomedullin, and visfatin), markers of hypoxia and (neo)vascularization (glucose transporter-1, hypoxia inducible factor-1z, vascular endothelial growth factor-A, and CD34), markers of macrophages (CD68, CD163, CD206, and CD11b and monocyte chemotactic protein-1), markers of inflammation (IL-8 and -10), and peroxisome proliferator-activated receptor-γ as a marker of adipocyte differentiation. GeNorm software (Primerdesign) was applied to calculate a normalization factor on the basis of expression levels of 3 housekeeping genes (15). See Table S2 under “Supplemental data” in the online issue for primer sequences. Gene expression was quantified and expressed as arbitrary units.

Adipocyte size

Adipocyte size was determined on hematoxylin and eosin–stained paraffin sections by quantifying the actual area of ≥400 unique adipocytes per individual through computer image analysis software (Lucia GF, version 4.81).

Quantification of ATMs

ATMs were quantified on adipose tissue paraffin sections that were incubated with anti-CD68 and visualized (EnVision FLEX/FLEX+ System; DakoCytomation) and counterstained with hematoxylin. Through direct microscopy at magnification ×400, 2 blinded raters systematically quantified the number of solitary ATMs and CLSs (Figure 1), and their densities were expressed as the number of solitary ATMs and CLSs per square millimeter of analyzed tissue.

Cardiometabolic and systemic inflammatory profile

Fasting plasma concentrations of 19 adipokcytines were analyzed with a multiplex immunoassay as previously described in detail (16). See Table S3 under “Supplemental data” in the online issue for a presentation of the lower limits of detection. Additional measurements included CRP, N-terminal pro-brain natriuretic peptide, glucose, and insulin (see online supplement under “Supplemental data” in the online issue for more details). Homeostatic model assessment was applied to estimate IR, whereby
ADIPOSE TISSUE INFLAMMATION IN COPD?

Statistics

Differences in descriptive characteristics between COPD patients and healthy control subjects were tested by using independent-samples *t* test for continuous variables and chi-square test for categorical variables. In case of a significantly skewed distribution according to the Shapiro-Wilk test, a natural logarithmic transformation was applied, and for these variables, the geometrical mean and 95% CIs are presented. Analyses of adipose tissue mRNA expression and systemic adipocytokines were corrected for multiple comparisons by using the false discovery rate method (19). Correlations were tested by using Pearson’s *r* and Spearman’s *ρ*. Analyses were performed in PASW Statistics 17.0 software (SPSS Inc). *P* < 0.05 was considered statistically significant.

### TABLE 1

Main characteristics of study population

<table>
<thead>
<tr>
<th></th>
<th>Healthy control subjects (<em>n</em> = 15)</th>
<th>Total COPD group (<em>n</em> = 28)</th>
<th>COPD&lt;sub&gt;D&lt;/sub&gt; patients (<em>n</em> = 12)</th>
<th>COPD&lt;sub&gt;ND&lt;/sub&gt; patients (<em>n</em> = 16)</th>
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<tr>
<td><strong>Demographics</strong></td>
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<tr>
<td>Sex (M/F)</td>
<td>9/6</td>
<td>17/11</td>
<td>7/5</td>
<td>10/6</td>
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<tr>
<td>Age (y)</td>
<td>65 ± 6&lt;sup&gt;2&lt;/sup&gt;</td>
<td>65 ± 7</td>
<td>65 ± 6</td>
<td>66 ± 7</td>
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<tr>
<td>Smoking status</td>
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<tr>
<td>Current smoker [n [%]]</td>
<td>1 (6.7)</td>
<td>10 (36)****</td>
<td>2 (20)</td>
<td>8 (50)</td>
</tr>
<tr>
<td>Former smoker [n [%]]</td>
<td>7 (46.7)</td>
<td>18 (64)****</td>
<td>10 (80)</td>
<td>8 (50)</td>
</tr>
<tr>
<td>Never-smoker [n [%]]</td>
<td>7 (46.7)</td>
<td>0 (0)****</td>
<td>0 (0)</td>
<td>0 (0)</td>
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<td><strong>Pulmonary function</strong></td>
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<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;, (percentage predicted)</td>
<td>113 ± 15</td>
<td>58 ± 16***</td>
<td>57 ± 19</td>
<td>59 ± 15</td>
</tr>
<tr>
<td>FVC, (percentage predicted)</td>
<td>120 ± 17</td>
<td>105 ± 22*</td>
<td>114 ± 21</td>
<td>98 ± 17</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;/FVC (%)</td>
<td>74 ± 5</td>
<td>45 ± 12***</td>
<td>39 ± 9&lt;sup&gt;f&lt;/sup&gt;</td>
<td>49 ± 12</td>
</tr>
<tr>
<td>DLCO, (percentage predicted)</td>
<td>95 ± 19</td>
<td>51 ± 16***</td>
<td>48 ± 16</td>
<td>54 ± 17</td>
</tr>
<tr>
<td>SaO&lt;sub&gt;2&lt;/sub&gt; at rest (%)</td>
<td>99 ± 1</td>
<td>96 ± 2***</td>
<td>96 ± 2</td>
<td>97 ± 1</td>
</tr>
<tr>
<td>SaO&lt;sub&gt;2&lt;/sub&gt; at V&lt;sub&gt;O2&lt;/sub&gt;max (%)</td>
<td>98 ± 1</td>
<td>91 ± 4***</td>
<td>87 ± 3**&lt;sup&gt;**&lt;/sup&gt;</td>
<td>94 ± 2</td>
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<tr>
<td>PaO&lt;sub&gt;2&lt;/sub&gt; at rest (kPa)&lt;sup&gt;6&lt;/sup&gt;</td>
<td>12.1 ± 1.1</td>
<td>9.4 ± 1.0***</td>
<td>9.2 ± 1.2</td>
<td>9.6 ± 0.8</td>
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<tr>
<td>PaO&lt;sub&gt;2&lt;/sub&gt; at V&lt;sub&gt;O2&lt;/sub&gt;max (kPa)&lt;sup&gt;6&lt;/sup&gt;</td>
<td>13.1 ± 1.3</td>
<td>9.1 ± 1.4***</td>
<td>7.9 ± 1.2**&lt;sup&gt;**&lt;/sup&gt;</td>
<td>9.8 ± 1.0</td>
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<td><strong>Body composition</strong></td>
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<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)&lt;sup&gt;4&lt;/sup&gt;</td>
<td>24.9 ± 3.3 (20.4–31.4)</td>
<td>24.9 ± 2.9 (19.6–31.0)</td>
<td>23.2 ± 2.2 (19.6–27.8)<strong>&lt;sup&gt;</strong>&lt;/sup&gt;</td>
<td>26.1 ± 2.7 (20.9–31.0)</td>
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<tr>
<td>Fat-free mass index (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>18.0 ± 1.9</td>
<td>17.6 ± 1.7</td>
<td>17.1 ± 1.4</td>
<td>17.9 ± 1.9</td>
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<tr>
<td>Fat mass index (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>7.0 ± 3.3</td>
<td>7.2 ± 2.6</td>
<td>6.0 ± 2.1&lt;sup&gt;5&lt;/sup&gt;</td>
<td>8.1 ± 2.7</td>
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<tr>
<td>Total body fat (%)</td>
<td>28.1 ± 10.3</td>
<td>29.2 ± 8.2</td>
<td>26.5 ± 7.6</td>
<td>31.3 ± 8.1</td>
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<tr>
<td><strong>Cardiometabolic profile</strong>&lt;sup&gt;2,6&lt;/sup&gt;</td>
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<tr>
<td>Fasting glucose (mmol/L)</td>
<td>5.33 (5.11, 5.57)</td>
<td>5.85 (5.62, 6.09)<strong>&lt;sup&gt;</strong>&lt;/sup&gt;</td>
<td>5.68 (5.32, 6.06)</td>
<td>5.99 (5.68, 6.32)</td>
</tr>
<tr>
<td>Fasting insulin (pmol/L)</td>
<td>29.0 (23.0, 36.5)</td>
<td>47.7 (35.6, 63.9)*&lt;sup&gt;**&lt;/sup&gt;</td>
<td>29.5 (23.3, 37.3)<strong>&lt;sup&gt;</strong>&lt;/sup&gt;</td>
<td>70.1 (46.4, 105.8)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.75 (0.50, 1.13)</td>
<td>1.53 (1.03, 2.27)*</td>
<td>0.80 (0.50, 1.28)<strong>&lt;sup&gt;</strong>&lt;/sup&gt;</td>
<td>2.56 (1.56, 4.21)</td>
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<tr>
<td>NT-proBNP (pmol/L)</td>
<td>6.6 (4.4, 9.8)</td>
<td>9.6 (6.8, 13.5)</td>
<td>15.2 (10.4, 22.2)<strong>&lt;sup&gt;</strong>&lt;/sup&gt;</td>
<td>6.6 (4.1, 10.8)</td>
</tr>
<tr>
<td><strong>Systemic inflammation</strong>&lt;sup&gt;3&lt;/sup&gt;</td>
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<tr>
<td>CRP (mg/L)</td>
<td>0.95 (0.57, 1.59)</td>
<td>2.05 (1.31, 3.21)*&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1.62 (0.95, 2.76)</td>
<td>2.48 (1.19, 5.15)</td>
</tr>
</tbody>
</table>

<sup>1</sup> Continuous variables were tested by using the independent-samples *t* test, and categorical variables were tested by using the chi-square test.

<sup>2</sup> Compared with healthy control subjects, *P* < 0.05, **P* < 0.01, ***P* < 0.001.

<sup>3</sup> Compared with COPD<sub>ND</sub> patients: *P* < 0.05, **P* < 0.01, ***P* < 0.001.

<sup>4</sup> Geometric mean; 95% CI in parentheses.

<sup>5</sup> No plasma available for one COPD<sub>ND</sub> patient.

<sup>6</sup> Geometric mean; 95% CI in parentheses.

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**Figure 1.** CD68-staining of ATMs. A: Solitary ATM. B: CLS, with a well-organized collection of macrophages surrounding an adipocyte, which is a marker of adipocyte death (17). CD68 is a lysosomal glycoprotein that is implicated in endocytosis and lysosomal migration of macrophages. Note the difference in macrophage size and granulomatous aspect between a solitary ATM and ATMs that formed a CLS. See Figure S1 under ‘Supplemental data’ in the online issue for a color version. ATM, adipose tissue macrophage; CLS, crown-like structure.
RESULTS

COPD patients had mild-to-moderate airflow obstruction (Table 1). FFMI and FMI were not different between COPD patients and healthy control subjects. COPD patients had slightly higher fasting plasma glucose, insulin, and HOMA-IR scores than healthy control subjects ($P \leq 0.021$) and CRP was also modestly but significantly higher in COPD patients ($P = 0.031$), which reflected a low-grade systemic inflammatory state. CRP and HOMA-IR were positively correlated (Figure 2).

Comparisons between healthy control subjects and total COPD group

Adipocyte size was not different between COPD patients and control subjects (Figure 3A). The correlation between adipocyte size and FM was similar in COPD patients and healthy control subjects (Figure 3B), as illustrated in Figure 3C. None of the measured gene-expression levels were significantly different between COPD patients and healthy control subjects after adjustment for multiple testing (Table 2). The mean (95% CI) solitary ATM density in COPD patients was not different from in healthy control subjects [30.4 ATMs/mm² (23.9, 38.6) compared with 23.7 ATMs/mm² (18.0, 31.3); $P = 0.19$]. CLS were shown in 12 COPD patients (43%) and 5 healthy control subjects (33%) ($P = 0.75$). In this subset, the CLS density was not different between COPD patients and healthy control subjects [0.15 CLS/mm² (0.08, 0.28) compared with 0.09 CLS/mm² (0.04, 0.19); $P = 0.27$]. The CLS density was positively correlated with CD68 gene expression (Spearman’s $\rho = 0.59$, $P = 0.014$, $n = 17$), whereas the ATM density was not (see Figure S2 under “Supplemental data” in the online issue).

None of the measured systemic adipokine concentrations were significantly different between COPD patients and healthy control subjects (Table 3). Systemic IL-6 concentrations were near the detection limit in both COPD patients and healthy control subjects, and no differences were shown between these 2 groups. Measures above the lower limits of detection of systemic IL-1β, -8, and -10, IFN-γ, and TNF-α were shown in only 1, 24, 4, 15, and 2 subjects, respectively, without clear differences between COPD patients and healthy control sub-

No significant correlations were shown in COPD patients or healthy control subjects between FM and the remaining systemic adipokines (all $P > 0.05$; data not shown).

FIGURE 2. Correlation between CRP and HOMA-IR. Closed circles represent COPD patients, and open circles represent healthy control subjects (Spearman’s $\rho = 0.36$, $P = 0.018$; $n = 44$). COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein.

FIGURE 3. Mean (±SE) adipocyte size in COPD patients and healthy control subjects. A: Comparison of adipocyte size between COPD patients ($n = 28$; black bars) and healthy control subjects ($n = 15$, gray bars). No differences were observed in group size categories between COPD patients and healthy control subjects by using independent-samples $t$ test (all $P > 0.05$). B: Similar correlations were shown between fat mass and median adipocyte size in COPD patients (closed circles, solid line; Pearson’s $r = 0.41$, $P = 0.031$; $n = 28$) and healthy control subjects (open circles, dashed line; Pearson’s $r = 0.63$, $P = 0.012$; $n = 15$). C: Hematoxylin and eosin–stained adipose tissue sections at magnification ×200 that illustrate the similarity in adipocyte size between lean and obese COPD patients and healthy control subjects. COPD, chronic obstructive pulmonary disease.
Comparisons between COPD and COPDD patients

COPDD patients had a higher FEV1: BMI (P = 0.007) and FMI (P = 0.040) were significantly lower in COPDND patients. COPDND patients had a higher FEV1:

Comparisons between COPD_{D} and COPD_{ND} patients

BMI (P = 0.007) and FMI (P = 0.040) were significantly lower in COPD_{D} patients. COPD_{ND} patients had a higher FEV1:

<table>
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<tr>
<th>TABLE 3</th>
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<tr>
<td>Comparison of systemic adipokine concentrations between COPD patients and healthy control subjects</td>
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| Adipose tissue mRNA amounts (arbitrary units) between COPD patients and healthy control subjects |

<table>
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<th>Table 2</th>
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<td>Comparison of adipose tissue mRNA amounts (arbitrary units) between COPD patients and healthy control subjects</td>
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<table>
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<tr>
<th>TABLE 2</th>
<th>Comparison of adipose tissue mRNA amounts (arbitrary units) between COPD patients and healthy control subjects</th>
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<tbody>
<tr>
<td>Healthy control subjects</td>
<td>Total COPD group</td>
</tr>
<tr>
<td>(n = 15)</td>
<td>(n = 28)</td>
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<tr>
<td>GLUT1</td>
<td>0.17 (0.12, 0.23)</td>
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<tr>
<td>HIF1z</td>
<td>0.18 (0.16, 0.20)</td>
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<tr>
<td>VEGF-A</td>
<td>0.16 (0.11, 0.22)</td>
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<tr>
<td>CD34</td>
<td>0.14 (0.07, 0.27)</td>
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<tr>
<td>PAI-1</td>
<td>0.14 (0.11, 0.18)</td>
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<tr>
<td>Adrenomedullin</td>
<td>0.16 (0.10, 0.26)</td>
</tr>
<tr>
<td>Visfatin</td>
<td>0.19 (0.09, 0.37)</td>
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<tr>
<td>Osteoprotegerin</td>
<td>0.16 (0.09, 0.29)</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.14 (0.08, 0.26)</td>
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<tr>
<td>MCP-1</td>
<td>0.11 (0.08, 0.14)</td>
</tr>
<tr>
<td>CD68</td>
<td>0.15 (0.11, 0.19)</td>
</tr>
<tr>
<td>CD163</td>
<td>0.11 (0.05, 0.21)</td>
</tr>
<tr>
<td>CD206</td>
<td>0.13 (0.09, 0.21)</td>
</tr>
<tr>
<td>CD11b</td>
<td>0.16 (0.10, 0.26)</td>
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<tr>
<td>Leptin</td>
<td>0.16 ± 0.11</td>
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<tr>
<td>Adiponectin</td>
<td>0.19 ± 0.08</td>
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<tr>
<td>PPAR-γ</td>
<td>0.19 ± 0.09</td>
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<tr>
<td>Chemerin</td>
<td>0.20 ± 0.10</td>
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<tr>
<td>IL-10</td>
<td>0.30 ± 0.11</td>
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Note: Independent-samples T tests were used to analyze data, and P values were interpreted by applying false discovery rate correction for multiple testing. None of the differences were significant. COPD, chronic obstructive pulmonary disease; COPD_{D}, desaturating chronic obstructive pulmonary disease; COPD_{ND}, nondesaturating chronic obstructive pulmonary disease; GLUT1, glucose transporter 1; HIF1z, hypoxia inducible factor 1z; MCP-1, monocyte chemotactic protein-1; mRNA, messenger RNA; PAI-1, plasminogen activator inhibitor-1; PPAR-γ, peroxisome proliferator-activated receptor-γ; VEGF-A, vascular endothelial growth factor-A.

1. Independent-samples T tests were used to analyze data, and P values were interpreted by applying false discovery rate correction for multiple testing. None of the differences were significant. COPD, chronic obstructive pulmonary disease; COPD_{D}, desaturating chronic obstructive pulmonary disease; COPD_{ND}, nondesaturating chronic obstructive pulmonary disease; CXCL10, C-X-C motif chemokine 1; IL-1RA, IL-1 receptor antagonist; MCP-1, monocyte chemotactic protein-1; MIF, macrophage migration inhibiting factor; MIP-1α, macrophage inflammatory protein-1α; OPG, osteoprotegerin; PAI-1, plasminogen activator inhibitor-1; SAA-1, serum amyloid A1.

2. Geometric mean; 95% CI in parentheses (all such values).

3. No plasma was available for one COPD patient. Independent-samples T tests were used to analyze the data, and P values were interpreted by applying false discovery rate correction for multiple testing. None of the differences were significant. COPD, chronic obstructive pulmonary disease; COPD_{D}, desaturating chronic obstructive pulmonary disease; COPD_{ND}, nondesaturating chronic obstructive pulmonary disease; IL-8, interleukin-8; MCP-1, monocyte chemotactic protein-1; MIP-1α, macrophage inflammatory protein-1α; OPG, osteoprotegerin; PAI-1, plasminogen activator inhibitor-1; SAA-1, serum amyloid A1.

4. Geometric mean; 95% CI in parentheses (all such values).

5. Valid measures above the lower limit of detection were available for 22 COPD patients and 13 healthy control subjects.

6. Mean ± SD (all such values).

7. Mean ± SD (all such values).
COPD patients (data not shown). We showed no significant differences between COPD and COPDND patients for all measured SAT gene expressions (Table 2) and systemic adipokine concentrations (Table 3). Also, the mean (95% CI) ATM density [30.6 ATMs/mm² (21.3, 43.8) compared with 30.2 ATMs/mm² (21.2, 43.2); \(P = 0.96\)] and CLS density [0.10 CLS/mm² (0.05, 0.20) compared with 0.20 CLS/mm² (0.07, 0.56); \(P = 0.22\)] were not different between COPD and COPDND patients, respectively.

On the basis of the median CRP concentration in COPD patients, we performed a post-hoc comparison between patients with high CRP concentrations \([n = 14; \text{mean: } 4.38 \text{ mg/L (95\% CI: } 2.33, 8.24 \text{ mg/L)}\) and low CRP concentrations \([n = 13, 0.90 \text{ mg/L (0.75, 1.08 mg/L)}\) (see Tables S3–S5 under “Supplemental data” in the online issue), which showed that patients with high CRP concentrations had higher ATM densities \((P = 0.021); \text{Figure 6}\). Linear regression analysis further revealed that the ATM density (ln transformed) was significantly predicted by CRP status (low CRP: 1; high CRP: 2; \(\hat{\beta} \pm SE: 0.61 \pm 0.23; P = 0.013\) independent of BMI (\(\hat{\beta} \pm SE: -0.04 \pm 0.04; P = 0.294\)). In a separate model, CRP status (\(\hat{\beta} \pm SE: 0.63 \pm 0.22; P = 0.008\)) also predicted ATM density independent of FM (\(\hat{\beta} \pm SE: -0.03 \pm 0.02; P = 0.068\)).

**DISCUSSION**

We investigated whether ATI was enhanced in patients with stable mild-to-moderate COPD independent of age, sex, BMI, and body composition. The second aim was to investigate whether ATI was more pronounced in COPD patients with a desaturation phenotype. Knowledge of potential adipose tissue dysfunction in COPD may increase our understanding of the origin of enhanced systemic inflammation in COPD and contribute to tailoring interventions. COPD patients were characterized by a slightly higher CRP and higher HOMA-IR that could be interrelated (as supported by a positive correlation). COPD patients tended to have higher adipose tissue gene expression of 2 macrophage markers (monocyte chemotactic protein-1 and CD206). We showed that COPD patients with high CRP had greater ATM infiltration and tended to have higher CD206 expression than did patients with low CRP. However, other markers of ATI were not different between COPD and COPDND patients, respectively.

As larger adipocytes have been shown to be more proinflammatory than are smaller adipocytes (3), we explored whether COPD patients had enlarged adipocytes, for which we did not find any evidence. In mild-to-moderate COPD patients, Skyba et al (11) reported a positive association between adipocyte size and BMI that was not associated with different gene expressions of apoptosis markers, which suggested that fat wasting is not associated with adipocyte death but rather with adipocyte atrophy. In line with this result, we report a positive relation between FM and adipocyte size in COPD, which was similar in healthy control subjects. This result suggested that the behavior of...
adipocyte atrophy and hypertrophy with changing FM follows a physiologic pattern in COPD patients.

ATMs are believed to be mediators of ATI, and thus, a quantification of ATMs would provide an indication of ATI. Although we showed no differences in ATM infiltration between COPD patients and healthy control subjects, the ATM infiltration was higher in COPD patients with high CRP than in COPD patients with low CRP, which suggested a relation between low-grade systemic inflammation and ATI. We showed that CD68 mRNA concentrations were not correlated with the density of solitary ATMs, but a correlation was shown with the density of CLS. Because CD68 is a lysosomal glycoprotein that is implicated in endocytosis and lysosomal migration of macrophages, our findings suggested that ATMs involved in a CLS express considerably more CD68 than do residing or resting solitary ATMs (Figure 1). In line with previous publications (11, 20), we showed that FM was positively correlated with markers of ATMs, which was highly comparable between COPD patients and healthy control subjects. This finding has been well accepted to be a characteristic of obesity and has been linked to the development of IR via ATI (20). Despite the absence of a greater ATM density, COPD patients were characterized by a higher HOMA-IR than were healthy control subjects, which suggested that IR in COPD patients is not necessarily associated with greater ATM infiltration. Likewise, COPDND patients had considerably higher insulin and HOMA-IR than did COPDD patients, but no differences in ATM infiltration were shown. It must, however, be mentioned that we did not perform a hyperinsulinemic euglycemic clamp to verify IR.

Animal and in vitro studies indicated that hypoxia may lead to ATI. For example, hypoxia increased the activity of the proinflammatory transcription factor nuclear transcription factor κB

![FIGURE 5](image1.png)

*FIGURE 5.* Correlations between fat mass and markers of adipose tissue macrophages in COPD patients (closed circles) and healthy control subjects (open circles) A: Correlation between fat mass and adipose tissue CD163 mRNA expression (COPD patients: Spearman’s $\rho = 0.36$, $P = 0.060$, $n = 28$; healthy control subjects: Spearman’s $\rho = 0.66$, $P = 0.007$, $n = 15$) B: Correlation between fat mass and adipose tissue CD68 mRNA expression (COPD patients: Spearman’s $\rho = 0.42$, $P = 0.025$, $n = 28$; healthy control subjects: Spearman’s $\rho = 0.66$, $P = 0.007$, $n = 15$). C: Correlation between fat mass and adipose tissue CD206 mRNA expression (COPD patients: Spearman’s $\rho = 0.60$, $P = 0.001$, $n = 28$; healthy control subjects: Spearman’s $\rho = 0.64$, $P = 0.010$, $n = 15$). D: Correlation between fat mass and adipose tissue MCP-1 mRNA expression (COPD patients: Spearman’s $\rho = 0.41$, $P = 0.033$, $n = 28$; healthy control subjects: Spearman’s $\rho = 0.30$, $P = 0.277$, $n = 15$). A.U., arbitrary units; COPD, chronic obstructive pulmonary disease; MCP-1, monocyte chemotactic protein-1; mRNA, messenger RNA.

![FIGURE 6](image2.png)

*FIGURE 6.* Geometric mean (95% CI) ATM density in COPD patients with low compared with high CRP. Data points denote geometrical means, and error bars represent 95% CIs. Data were tested by using an independent-samples t test. ATM, adipose tissue macrophage; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein.
and the TNF-α gene promoter in murine adipocytes (21), and hypoxia deregulates the production of adiponectin and plasminogen activator inhibitor-1 (22). In obesity, it has been pos-
tulated that adipocyte hypertrophy combined with impaired neovascularization may ultimately lead to an unbridgeable dif-
fusion distance for oxygen both on the tissue and cellular levels (5). Indeed, obese mice had hypoxic patches in the adipose
tissue that was associated with ATI (21, 23). However, human
data on adipose tissue hypoxia as underlying ATI is scarce. 
Recently, Goossens et al (24) challenged the concept of adipose
tissue hypoxia in humans by showing that obese insulin resistant
men were characterized by adipose tissue hyperoxia rather than
hypoxia. In the presence of a mild hypoxemia in COPD patients
relative to in healthy control subjects (ie, a mean difference in
PaO₂ of 2.7 kPa), we showed that no clear differences were
observed with regard to markers of adipose tissue hypoxia or
ATI. With the assumption that COPD patients would also de-
saturate during daily living activities (6), we showed that the
COPD phenotype was not associated with either of these out-
comes. In addition, adipocyte size and (neo)vascularization
markers were comparable across the current study groups. 
Collectively, our data suggested that neither mild hypoxemia
nor a desaturation phenotype is associated with ATI in COPD
patients.

Several authors have claimed disturbances in systemic adi-
pokine concentrations in COPD patients (25–30). Takabatake
et al (29) showed lower systemic leptin concentrations in un-
derweight COPD patients than in normal-weight non-COPD
control subjects, and Eker et al (31) showed lower systemic leptin
concentrations in COPD patients with decreased FFM than in
BMI- and sex-matched healthy control subjects. In our study,
one of the measured systemic adipokines (including leptin and
adiponectin) revealed different concentrations between COPD
patients and matched healthy control subjects, which indicated
the importance of adjustment for FM. Indeed, we showed a strong
positive correlation between FM and circulating leptin concen-
trations, which was shown to be similar in COPD patients and
healthy control subjects. In addition, adipose tissue expression
of leptin and adiponectin was positively correlated with systemic
leptin and adiponectin concentrations, respectively, in a similar
fashion in COPD patients and healthy control subjects.

Recently, important distinct functions and characteristics of
SAT compared with VAT have been reported. For example, the
relative contribution of SAT compared with that of VAT to
systemic adipokine concentrations may be limited (32), and
differences exist in neovascularization with expanding FM that
may exert different metabolic effects in SAT and VAT (33). Also,
expanding VAT is generally associated with increased cardio-
vascular risk, whereas expanding SAT may even lower cardio-
vascular risk, especially in women (34). Therefore, it would
be interesting to explore a potential VAT dysfunction in COPD
patients in future studies and to compare it to SAT function. In
addition, the study of potential adipose tissue dysfunction in
patients with more-severe COPD and during acute exacer-
bations may add to the optimization of nutritional and lifestyle
interventions.

In conclusion, mild-to-moderate COPD, per se, does not en-
chance ATI or its contribution to systemic inflammation in COPD
patients compared with in well-matched healthy control subjects.
However, to our knowledge, our study provides the first in-
dication of a possible role of ATMs in the systemic inflammatory
response in COPD that requires additional investigation.

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designed the study; BvdB, Wdl, VACVIH, and FJS: conducted the research;
GW: involved in patient inclusion; BvdB: analyzed data and wrote the first
draft of the manuscript; HRG and AMWJS: had significant contributions to
the writing of the manuscript; AMWJS: had primary responsibility for the
final content of the manuscript; and all authors: approved the manuscript
for submission. Top Institute Pharma and participants in the project approved
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