Increased Adipose Tissue Expression of Proinflammatory CD40, MKK4 and JNK in Patients with Very Severe Chronic Obstructive Pulmonary Disease

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Key Words

Adipose tissue · CD40 · Chronic obstructive pulmonary disease · Hypoxia · JNK · MKK4 · Systemic effects

Abstract

Background: CD40, a transmembrane receptor of the tumor necrosis factor gene superfamily, is activated in response to cellular stress, including hypoxia, and orchestrates the process of inflammation via secondary messengers such as mitogen-activated protein kinase kinase 4 (MKK4) and c-Jun NH₂-terminal kinases (JNK). **Objectives:** We hypothesized that CD40, MKK4 and JNK expression is increased in the adipose tissue of patients with very severe chronic obstructive pulmonary disease (COPD). **Methods:** In 20 patients with stable COPD, lung function was assessed using body plethysmography, and samples of subcutaneous adipose tissue were analyzed using real-time PCR. Body composition, including fat mass index (FMI), was assessed by bioelectrical impedance. **Results:** 12 patients in GOLD stage I–III (age 61.6 ± 8.6 years, 4 females, mean partial pressure of oxygen in arterial blood, PaO₂, 9.38 ± 0.21 kPa) were compared to 8 patients in GOLD stage IV (age 62.6 ± 6.3 years, all male,

mean PaO₂ 7.70 ± 0.37 kPa). Compared to patients in GOLD stage I–III, patients in GOLD stage IV had lower FMI ($p = 0.004$), being associated with significantly higher adipose tissue expression of CD40, MKK4 and JNK [$\Delta\Delta Ct$: 2.55 (1.99, 4.40) vs. 1.87 (1.63, 2.23), $p = 0.013$; 5.19 (3.13, 5.96) vs. 2.98 (2.82, 3.86), $p = 0.002$; 9.01 (5.12, 11.41) vs. 4.65 (4.42, 6.26), $p = 0.001$, respectively]. Log-transformed CD40, MKK4 and JNK expression was significantly inversely related to PaO₂, respectively. **Conclusions:** Upregulation of proinflammatory CD40, MKK4 and JNK gene expression in adipose tissue in very severe COPD raises the possibility of a role of chronic systemic hypoxia in the pathogenesis of adipose tissue inflammation in COPD.

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Introduction

According to the WHO, chronic obstructive pulmonary disease (COPD) is one of the most prevalent diseases, expected to rise to the third leading cause of mortality in 2020 [1]. The international Global Initiative for Chronic Obstructive Lung Disease (GOLD) has defined COPD as

a preventable and treatable disease with some significant extrapulmonary effects that may contribute to disease severity [2]. Systemic inflammation has been implicated as a key putative mechanism involved in the development and progression of systemic consequences of COPD [3–5].

Recently, Franssen et al. [6] suggested that adipose tissue may represent a possible source of systemic inflammation in COPD. Adipose tissue is considered a highly dynamic endocrine organ that synthesizes large numbers of different proteins that are involved in a wide range of physiologic processes including inflammation [7–9]. In obesity, relative tissue hypoxia represents one of the key pathologic mechanisms resulting in dysregulation of adipocyte function and increased expression of proinflammatory adipokines [10–14]. Nevertheless, until now no studies have focused on the potential link between systemic hypoxia from reduced pulmonary function and adipose tissue inflammation in patients with advanced COPD.

CD40, a transmembrane receptor of the tumor necrosis factor (TNF) gene superfamily, plays a central role in the onset and maintenance of inflammatory reactions in a variety of cells, including adipocytes [15, 16]. Hypoxia and oxidative stress enhance CD40-CD40 ligand-mediated inflammation [17, 18], and stimulation of proinflammatory mediator production via induction of mitogen-activated protein (MAP) kinase activation has been reported in adipocytes [16]. Within the MAP kinase family, the MAP kinase kinase 4 (MKK4) is a direct activator of MAP kinases, including the c-Jun NH₂-terminal kinases (JNK) in response to cellular stress, including hypoxia [19]. Therefore, in the present study, we examined adipose tissue expression of proteins involved in the CD40-CD40 ligand-associated inflammatory pathway, and hypothesized that expression of key signaling molecules, i.e. CD40, MKK4 and JNK, are increased in adipose tissue of hypoxic patients with advanced COPD.

Patients and Methods

Study Design

This study was a prospective cohort study at the L. Pasteur Teaching Hospital, a tertiary teaching center. The primary endpoint of the study was to compare CD40, MKK4 and JNK mRNA expression in adipose tissue between patients with COPD in GOLD stage I–III and those with very severe COPD (GOLD stage IV). COPD severity was evaluated on the basis of GOLD recommendations [2].

Subjects

Patients with a diagnosis of COPD according to the American Thoracic Society/European Respiratory Society guidelines [20]

recruited from two outpatient clinics participated in the study, which was conducted in a university hospital setting. Patients were free from exacerbation for ≥ 8 weeks. Exclusion criteria were: use of systemic corticosteroids, treatment with long-term domiciliary oxygen therapy, respiratory disorders other than COPD, known diabetes or other metabolic or endocrine disorders, chronic autoimmune, hepatic or renal disease, malignancies, overt cardiac failure, blood coagulation disorders or therapy with warfarin. To evaluate dyspnea severity, the Modified Medical Research Council scale was used [21]. The distance travelled within 6 min was recorded without supplemental oxygen used during the test. Information concerning the number of exacerbations in the year preceding the study and medication use was retrieved by two physicians recruiting the patients from their outpatient clinics. The study had Institutional Review Board approval, and all subjects gave written consent to the study.

Pulmonary Function Tests and Body Composition

Pulmonary function tests were evaluated with the use of body plethysmography (Ganshorn, Germany). All pulmonary function testing was performed according to the European Respiratory Society standards with the patients in a sitting position by the same technician in order to ensure consistency of the technique. Three technically acceptable measurements were performed in each patient, and the highest value was included in the analyses.

Anthropometric measurements were taken after overnight fasting. Body composition was estimated using bioelectrical impedance analysis technology (Bodystat 1500; Bodystat, UK), as described previously [22]. Body weight, fat-free mass and fat mass were adjusted for height squared as the fat-free mass and fat mass indices (BMI, FFMI and FMI, respectively).

Biochemical Analyses

In all patients, peripheral venous blood samples from the antecubital vein were collected between 6.00 and 8.00 a.m. after 10 h of fasting, within the first 36 h after admission. Arterial blood gas parameters were analyzed in samples obtained by puncture of the radial artery with the patient seated and breathing room air. High-sensitivity serum C-reactive protein (CRP) levels were assessed by the immunoturbidimetric method (Randox, UK). The analytical sensitivity of this CRP assay is 0.1 mg/l. Serum interleukin (IL)-6 levels were measured using commercially available enzyme-linked immunosorbent assay kits (Beckman-Coulter Immunotech, USA).

Adipose Tissue Biopsy

Abdominal subcutaneous adipose tissue was taken by aspiration with a bioptic needle (Medin, Czech Republic) under local intracutaneous anesthesia with 1% mesocain after an overnight fast. The samples were quickly washed in saline to eliminate blood and other connective tissue, immediately frozen in liquid nitrogen and stored at -80°C until analysis.

CD40, MKK4 and JNK Expression and Adipocyte Diameter

To determine gene expression of CD40, MKK4 and JNK in subcutaneous adipose tissue, the RT2 ProfilerTM PCR Array (PAHS-063; SABiosciences, USA) that contains genes of interest and 6 housekeeping genes was used. Real-time PCR was performed on ABI 7900HT (Applied Biosystems, USA) using the standard procedures described in our previous studies [23]. In the

Table 1. Baseline characteristics, body composition and circulating inflammatory markers in patients with COPD stratified by GOLD stage

Parameters	Group 1 GOLD stage I–III	Group 2 GOLD stage IV	p value
Patients	12	8	
Gender			
Male	8	8	0.117
Female	4	0	
Age, years	61.6 ± 8.6	62.6 ± 6.3	0.775
Pack-years	17.7 ± 13.3	38.6 ± 14.1	0.005
BMI, kg/m ²	29.4 ± 4.8	19.3 ± 3.3	<0.001
FFMI, kg/m ²	19.2 ± 2.9	13.4 ± 1.6	<0.001
FMI, kg/m ²	10.4 ± 3.9	5.1 ± 1.8	0.004
Leukocytes, × 10 ⁹ /l	6.91 ± 1.41	6.87 ± 2.00	0.961
Neutrophils, × 10 ⁹ /l	3.85 ± 0.80	4.55 ± 1.61	0.222
hsCRP, mg/l	2.80 (1.95–6.40)	6.15 (2.05–11.60)	0.280
IL-6, pg/ml	4.15 (2.45–8.05)	4.30 (3.50–5.30)	0.671

Values are given as numbers, means ± SD or medians (interquartile ranges). hsCRP = High-sensitivity CRP.

present work, a comparative quantification method ($\Delta\Delta C_t$) was used to calculate relative gene expression, a method that is considered both reproducible and accurate [24].

Adipocyte diameter was assessed histomorphologically on 5- μ m-thick sections of adipose tissue stained with hematoxylin and eosin. Cell diameter was determined using the ImageJ free-ware (University of Texas Health Science Center at San Antonio, USA). Average diameter of at least 100 cells from each adipose tissue section was calculated.

Statistical Analyses

Statistical analyses were performed using SPSS software (version 14.0; SPSS, USA). Power calculations were performed based on published TNF- α and IL-6 mRNA expression in human subcutaneous adipose tissue [25] since analogical reports on CD40 mRNA have not been published previously. For a standard deviation of 0.6, a power calculation indicated that we would need at least 7 patients per group to detect a difference of 1 in any mRNA expression with a power of 80% at a 0.05 significance level.

The Kolmogorov-Smirnov test of normality was applied. Levels of inflammatory markers in the circulation and adipose tissue mRNA expression were not normally distributed, and were rendered so by log₁₀ transformation. Differences between groups in normally distributed variables were tested by Student's two-tailed unpaired t test and in non-normally distributed variables by non-parametric Mann-Whitney rank sum test. Fisher's exact test was used to compare the proportion of categorical variables between groups. To assess the relationships between selected variables, linear regression analysis was used. Because the distribution of mRNA expression was skewed, we used the log-transformed values of these variables in regression analyses. Continuous variables are shown as means ± SD and non-normally distributed variables as medians (interquartile ranges).

Table 2. Pulmonary function parameters and arterial blood gases in patients with COPD stratified by GOLD stage

Parameters	Group 1 GOLD stage I–III	Group 2 GOLD stage IV	p value
FEV ₁ , % pred.	63.2 ± 18.4	23.6 ± 4.3	<0.001
FEV ₁ , liters	1.79 ± 0.52	0.75 ± 0.14	<0.001
FEV ₁ /FVC, % pred.	60.9 ± 13.8	29.4 ± 9.5	<0.001
RV, % pred.	136.2 ± 26.9	257.9 ± 34.4	<0.001
RV, liters	3.00 ± 0.56	6.17 ± 0.85	<0.001
TLC, % pred.	100.6 ± 10.2	131.0 ± 5.8	<0.001
TLC, liters	6.00 ± 0.90	8.79 ± 0.98	<0.001
RV/TLC, %	49.9 ± 5.1	70.1 ± 5.8	<0.001
DLCO, % pred.	75.5 ± 17.3	22.5 ± 4.8	<0.001
PaO ₂ , mm Hg	70.4 ± 1.6	57.8 ± 2.8	<0.001
PaCO ₂ , mm Hg	37.4 ± 6.9	43.4 ± 8.2	0.095
pH	7.42 ± 0.03	7.41 ± 0.03	0.475

Means ± SD. FEV₁ = Forced expiratory volume in 1 s; pred. = predicted; FVC = forced vital capacity; RV = residual volume; TLC = total lung capacity; DLCO = diffuse lung capacity for carbon monoxide; PaCO₂ = arterial carbon dioxide partial pressure.

Results

Patient Characteristics

Twenty patients with COPD (16 men and 4 women) were enrolled in this study. They were generally of late middle age (mean age 62.0 ± 1.7 years) with a mean history of smoking of 26.0 ± 3.6 pack-years. Since our primary goal was to compare CD40, MKK4 and JNK expression in adipose tissue of hypoxic patients with advanced COPD to those with less severe forms of the disease, patients were divided into two groups: the first group consisted of 12 COPD patients in GOLD stage I–III and the second of 8 COPD patients in GOLD stage IV. All patients were current smokers or ex-smokers, had COPD diagnosed before study entry by their respirologist, and were using short-acting bronchodilators to relieve their symptoms.

Table 1 displays demographic data, body composition parameters and circulatory inflammatory markers in the two groups stratified by GOLD stage. Although both groups did not differ regarding mean age ($p = 0.775$), patients in stage IV had a significantly higher number of pack-years ($p = 0.005$). In addition, patients in GOLD stage IV experienced more frequent exacerbations during the year preceding the study (1.38 ± 0.91 vs. 0.33 ± 0.65 events, $p = 0.008$) and had a higher dyspnea score (Modified Medical Research Council scale: 2.50 ± 0.76 vs. 1.42 ± 0.79 , $p = 0.007$), and 6-min walking distance

Table 3. Expression of proteins involved in the CD40-CD40 ligand-associated inflammatory pathway in patients with COPD stratified by GOLD stage (medians and interquartile ranges)

Gene symbol	Description	Alternative gene name	Group 1 GOLD stage I–III	Group 2 GOLD stage IV	p value
CD40	CD40 molecule, TNF receptor superfamily member 5	Bp50/CDW40	1.86 (1.60–2.23)	2.81 (2.11–5.28)	0.021
CD40LG	CD40 ligand (TNF superfamily, member 5, hyper-IgM syndrome)	CD154/CD40L	0.41 (0.27–0.58)	0.45 (0.36–0.51)	0.589
IKBKAP	inhibitor of κ light polypeptide gene enhancer in B cells, kinase complex-associated protein	DKFZp781H1425/DYS	5.52 (4.90–6.75)	9.79 (6.51–14.19)	0.014
IKBKB	inhibitor of κ light polypeptide gene enhancer in B cells, kinase β	IKK-beta/IKK2	3.78 (3.31–4.66)	3.88 (3.10–6.04)	0.758
IKBKG	inhibitor of κ light polypeptide gene enhancer in B cells, kinase γ	AMCBX1/FIP-3	3.11 (2.42–4.08)	4.66 (2.92–6.77)	0.076
MADD	MAP kinase-activating death domain	DENN/IG20	1.50 (1.35–2.30)	2.66 (1.94–3.40)	0.011
MAP2K4	MAP kinase kinase 4	JNKK/JNKK1	2.97 (2.82–3.87)	5.19 (3.67–6.12)	0.017
MAP3K1	MAP kinase kinase kinase 1	MAPKKK1/MEKK	5.22 (4.35–6.13)	5.04 (4.23–6.53)	0.817
MAP3K7	MAP kinase kinase kinase 7	TAK1/TGF1a	3.42 (3.13–3.99)	5.69 (3.75–8.31)	0.045
MAPK8	MAP kinase 8	JNK/JNK1	4.64 (4.24–6.26)	9.06 (5.93–11.69)	0.002
NFKB1	nuclear factor of κ light polypeptide gene enhancer in B-cells 1 (p105)	DKFZp686C01211/EBP-1	12.94 (10.59–16.73)	16.72 (12.81–25.35)	0.165
NFKBIA	nuclear factor of κ light polypeptide gene enhancer in B-cell inhibitor, α	IKBA/MAD-3	36.18 (30.38–45.86)	49.43 (43.32–59.36)	0.021
TRAF1	TNF receptor-associated factor 1	EBI6/MGC:10353	1.01 (0.93–1.23)	1.61 (1.07–3.41)	0.076
TRAF2	TNF receptor-associated factor 2	MGC:45012/TRAP	0.73 (0.63–1.05)	1.28 (0.85–1.56)	0.017
TRAF3	TNF receptor-associated factor 3	CAP-1/CD40bp	4.14 (3.22–5.13)	4.73 (3.34–9.77)	0.316

Table 4. Linear relationships [R (p value)] between CD40, MKK4 and JNK expression in white adipose tissue of patients with COPD, and PaO₂, BMI and adipocyte diameter

Adipose tissue expression	PaO ₂ , mm Hg	BMI	Adipocyte diameter, μ m
CD40 (log $\Delta\Delta$ Ct)	–0.745 (<0.001)	–0.470 (0.036)	–0.451 (0.052)
MKK4 (log $\Delta\Delta$ Ct)	–0.534 (0.015)	–0.571 (0.009)	–0.491 (0.033)
JNK (log $\Delta\Delta$ Ct)	–0.653 (0.002)	–0.566 (0.009)	–0.474 (0.041)

was significantly shorter (266 ± 56 vs. 382 ± 51 m, $p < 0.001$). Compared to patients in GOLD stage I–III, patients in stage IV had a lower BMI ($p < 0.001$), FFMI ($p < 0.001$) and FMI ($p = 0.004$; table 1). Nevertheless, no differences were observed between the two groups in the circulatory inflammatory markers such as leukocyte and neutrophil counts, and high-sensitivity CRP or IL-6 levels.

Arterial blood gases and pulmonary function parameters in the two groups are listed in table 2. By definition, patients in GOLD stage IV had more severe airflow obstruction and also suffered from significantly higher pulmonary hyperinflation, in association with significantly lower partial pressure of oxygen in arterial blood (PaO₂; $p < 0.001$ for all variables; table 2). In 8 patients in

GOLD stage IV, partial pressure of CO₂ in arterial blood was >42.0 mm Hg, nevertheless no patient suffered from acidosis.

Adipose Tissue Expression of CD40, MKK4 and JNK

CD40, MKK4 and JNK gene expression in subcutaneous adipose tissue was significantly higher in GOLD stage IV patients than in stage I–III [$\Delta\Delta$ Ct: 2.55 (1.99–4.40) vs. 1.87 (1.63–2.23), $p = 0.013$; 5.19 (3.13–5.96) vs. 2.98 (2.82–3.86), $p = 0.002$, and 9.01 (5.12–11.41) vs. 4.65 (4.42–6.26), $p = 0.001$, respectively; fig. 1]. In addition, among further 12 genes related to the CD40 signaling pathway, for 5 genes expression was significantly higher in GOLD stage IV compared with stage I–III patients (table 3). Adipocyte diameter was significantly lower in pa-

Fig. 1. Relative expression of CD40, MKK4 and JNK in adipose tissue of COPD patients with GOLD stage I–III and GOLD stage IV ($p = 0.013$; $p = 0.002$, and $p = 0.001$, respectively). Relative mRNA expression was calculated using comparative quantification method ($\Delta\Delta C_t$).

tients with stage IV than in those with stage I–III COPD (51.6 ± 12.9 vs. $80.9 \pm 18.6 \mu\text{m}$, $p = 0.002$).

Significant positive linear relationships were observed between log-transformed CD40 expression and expression of both MKK4 and JNK in the whole group ($r = 0.540$, $p = 0.014$, and $r = 0.832$, $p < 0.001$, respectively). In addition, log-transformed CD40, MKK4 and JNK expression was significantly inversely related to PaO_2 , respectively; inverse relationships between the respective mRNA expression, BMI and adipocyte diameter were also observed (table 4). Figure 2 illustrates the inverse relationship between log-transformed CD40 and PaO_2 .

Fig. 2. Linear relationship between PaO_2 and adipose tissue expression of CD40. See legend of figure 1 for the calculation of relative mRNA expression. $R = -0.745$, $p < 0.001$.

Discussion

The present study provides novel insights into the mechanism of adipose tissue inflammation in patients with very severe COPD. Our data demonstrate that in COPD patients in GOLD stage IV, expression of proinflammatory CD40, MKK4 and JNK in white adipose tissue was increased compared with those in GOLD stage I–III. In addition, log-transformed adipose tissue expression of CD40, MKK4 and JNK was inversely related to PaO_2 . In previous studies, relative hypoxia resulting from the abundance of adipose mass has been linked to tissue inflammation in clinical conditions associated with obesity [11–14, 26]. In contrast, however, extensive search of electronic databases revealed no original studies that would analyze potential associations between systemic hypoxia in COPD and expression of inflammatory mediators within the adipose tissue despite recent evidence linking circulatory levels of adipokines, such as leptin, adiponectin and visfatin, with the COPD phenotype [27, 28] and/or to circulatory inflammatory biomarkers [29–31]. Therefore, to the best of our knowledge, our data are the first to suggest that chronic systemic hypoxia is related to upregulation of proinflammatory signaling molecules in adipose tissue in patients with advanced COPD.

In the last two decades, the concept of adipose tissue as a passive lipid reservoir for energy storage only has been dramatically changed: adipose tissue is currently

viewed as a highly dynamic organ that is involved in a wide range of physiologic processes [7–9]. In obesity, relative adipose tissue hypoxia is present due to reductions in blood flow per unit adipose tissue mass and an increased distance of large adipocytes from the vasculature [32]. Moreover, it was shown to be related to chronic low-grade adipose tissue inflammation reflected by a shift to upregulation of proinflammatory molecules, including those involved in TNF- α signaling [9, 33–35]. The hypothesis that chronic systemic hypoxia resulting from impaired pulmonary function may also be involved in the upregulation of proinflammatory signaling molecules in the TNF- α signaling cascade within adipose tissue arose. Indeed, our findings of increased adipose tissue CD40, MKK4 and JNK expression in hypoxemic patients with very severe COPD are in favor of this hypothesis. An alternative mechanism, i.e. stimulation of gene expression within adipose tissue by circulating inflammatory factors was unlikely since circulating high-sensitivity CRP or IL-6 levels did not differ between both groups, and, in addition, there were no relationships between these circulating inflammatory markers and adipose tissue expression of CD40, MKK4 and JNK. The inflammatory CD40 is a membrane glycoprotein that belongs to the TNF receptor superfamily and is constitutively expressed on a wide variety of cells [18]. Recently, Poggi et al. [16] reported that CD40 is expressed on human adipocytes, and CD40 mRNA levels in subcutaneous adipose tissue are higher in adipocytes than in the stromal-vascular fraction. Importantly, the CD40-CD40 ligand interactions play a role in the onset and maintenance of inflammatory processes by activation of several transcription factors and stimulation of nuclear factor κ B [15, 36]. In various cell types, CD40-mediated signaling upregulates TNF receptor-associated factor and MAP kinase pathways [37–39]. In addition, hypoxia enhances the inflammatory effects of CD40 activation [17]. In the present study, PaO₂ was highly significantly inversely related to adipose tissue CD40 expression. Furthermore, our observation of significant positive linear relationships between adipose tissue CD40 expression and both MKK4 and JNK expression support the role of CD40 expression in MAP kinase pathway activation in human adipose tissue. Another possible interpretation is the existence of a common regulatory pathway of this gene expression.

Three MAP kinase cascades constitute the major mechanisms of relaying extracellular signals to specific responses and gene induction in mammalian cells [40–42]. The MAP kinase MKK4 directly phosphorylates and activates JNK in response to cellular stress and proin-

flammatory cytokines. JNK is a member of the MAP kinase family and a key component of stress-activated protein kinase signaling [19, 43]. The phosphorylation and activation of JNK leads to the phosphorylation and activation of transcription factors such as activating transcription factor 2 or c-Jun, which leads to the activation of the transcriptional complex of activator protein-1, with subsequent downstream upregulation of proinflammatory proteins [44]. Therefore, the MKK4/JNK pathway has been recognized as a key mechanism involved in inflammation [19]. In addition, acidosis and hypoxia/ischemia activate multiple proinflammatory pathways including MAP kinase cascades, and reversal of such activation seems to be a promising target to prevent ischemia-reperfusion injury or apoptosis [45–47]. In the present study, MKK4 and JNK were upregulated in adipose tissue of hypoxemic patients with very severe COPD who were stable at the time of investigation and had no signs of acidosis. In addition, expression of both MKK4 and JNK was significantly inversely related to BMI and adipocyte diameter. Although CD40, MKK4 and JNK have been implicated in apoptosis in various cell types under different experimental conditions [48] it remains unclear whether the small adipocyte cell size observed in the present study in patients with very severe COPD reflects deprivation of lipid vacuoles, enhanced apoptosis of adipocytes or both. A detailed study on the potential activation of proapoptotic signaling pathways in adipose tissue by systemic hypoxia in patients with COPD remains a challenge for future research.

In COPD, two main sources of inflammatory mediators in the circulation may be considered: lungs and peripheral organs including adipose tissue [6]. Although our study was not designed to provide conclusive evidence regarding the origin of increased circulatory inflammatory proteins, our results suggest that adipose tissue may, indeed, contribute to the chronic systemic inflammatory syndrome in COPD. Future studies are urgently needed to analyze potential links between adipose tissue inflammation in COPD and systemic manifestation of the disease, such as accelerated atherosclerosis, cachexia, muscle loss, osteoporosis and anemia.

There are several limitations to this study. First, since adipose tissue hypoxia was not directly measured, we cannot exclude the possibility that the relationships between PaO₂ and expression of the respective adipose tissue mRNA are only an epiphenomenon, albeit an interesting and thought-provoking one. However, this seems unlikely in the light of the previously discussed extensive body of data suggesting an important role of hypoxia in

the upregulation of proinflammatory processes within adipose tissue [11–14, 32]. Second, our analyses are based on single measurements of PaO₂ and expression of the respective adipose tissue mRNA, which may not reflect these relationships over time. Therefore, this study does not provide information about the time-course relationship between chronic systemic hypoxia and the activation of proinflammatory gene expression in adipose tissue. Third, the small study cohort is a clear limitation of this study. However, compared to COPD patients with GOLD stage I–III, in patients with GOLD stage IV, median values of adipose tissue expression of proinflammatory markers were increased by 36 (CD40), 74 (MKK4) and 94% (JNK). We therefore feel that the sample size of this study, although limited, helps to enlarge our understanding of adipose tissue inflammation in COPD patients with very severe COPD and hypoxia. Also, no control group of cachectic but otherwise healthy subjects with normal lung function was studied. Nevertheless, in adults residing in developed countries, cachexia is almost exclusively associated with various chronic diseases and is unlikely to occur in their absence [49]. The question of whether hypoxemia and/or increased CD40, MKK4 and JNK expression is related to enhanced apoptosis in adipocytes remains unclear, and was beyond the scope of the present study. Taken together, in view of the cross-sectional character of our data in a limited number of patients, our results should be considered as hypothesis generating.

In conclusion, our study showed upregulation of proinflammatory CD40, MKK4 and JNK gene expression in adipose tissue of COPD patients with GOLD stage IV in association with inverse relationships between the respective mRNA expression and PaO₂. Therefore, the present results raise the possibility of a pathogenetic role of chronic systemic hypoxia in the pathogenesis of adipose tissue inflammation in very severe COPD. Further investigations are needed to elucidate the exact mechanisms underlying increases in proinflammatory gene expression in adipose tissue, and their potential relationship to the development of cachexia in patients with COPD.

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