MAPK signaling in the quadriceps of patients with chronic obstructive pulmonary disease

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Muscle atrophy in chronic obstructive pulmonary disease; intramuscular signaling; muscle biopsy

PERIPHERAL MUSCLE DYSFUNCTION in chronic obstructive pulmonary disease (COPD) is characterized by fiber-type shifting, metabolic alterations, and atrophy. Among these, muscle atrophy is associated with reduced exercise tolerance, muscle strength, and survival. The molecular mechanisms leading to muscle atrophy in COPD remain elusive. The mitogen-activated protein kinases (MAPKs) such as p38 MAPK and ERK 1/2 can increase levels of MAFbx/Atrogin and MuRF1, which are specifically involved in muscle protein degradation and atrophy. Our aim was to investigate the level of activation of p38 MAPK, ERK 1/2, and JNK in the quadriceps of patients with COPD. A biopsy of the quadriceps was obtained in 18 patients with COPD as well as in 9 healthy controls. We evaluated the phosphorylated as well as total protein levels of p38 MAPK, ERK 1/2, and JNK as well as MAFbx/Atrogin and MuRF1 in these muscle samples. The corresponding mRNA expression was also assessed by RT-PCR. Ratios of phosphorylated to total level of p38 MAPK (P = 0.02) and ERK 1/2 (P = 0.01) were significantly elevated in patients with COPD compared with controls. Moreover, protein levels of MAFbx/Atrogin showed a tendency to be greater in patients with COPD (P = 0.08). mRNA expression of p38 MAPK (P = 0.03), ERK 1/2 (P = 0.02), and MAFbx/Atrogin (P = 0.04) were significantly elevated in patients with COPD. In addition, phosphorylated-to-total p38 MAPK ratio (Pearson’s r = −0.45; P < 0.05) and phosphorylated-to-total ERK 1/2 ratio (Pearson’s r = −0.47; P < 0.05) were negatively associated with the mid-thigh muscle cross-sectional area. These data support the hypothesis that the MAPKs might play a role in the development of muscle atrophy in COPD.

chronic obstructive pulmonary disease; intramuscular signaling; muscle biopsy

regulated kinase 1–2 (ERK 1/2), p38 MAPK, and c-Jun NH2-terminal kinase (JNK), are involved in the muscle atrophy process in cell culture and animal models of immobilization-induced atrophy (19, 21, 37). In C2C12 cells exposed to TNF, ERK activation led to a greater expression of muscle atrophy F-box protein (MAFbx/Atrogin) that is involved in protein degradation, suggesting that ERK could play a crucial role in the pathogenesis of muscle atrophy (27). p38 MAPK also mediates oxidative stress-induced MAFbx/Atrogin gene expression and ubiquitin-conjugating activity in skeletal muscle (18, 21). Lastly, JNK has been shown to potentiate the caspase activity during inflammation, which in itself can lead to loss of muscle proteins, while JNK-deficient mice are protected against the inflammation-induced loss of muscle force (40).

Because the ubiquitination of proteins and the levels of E3 ligases such as MuRF1 and MAFbx/Atrogin have been associated with skeletal muscle atrophy and since the active forms of ERK 1/2, p38 MAPK, and JNK could modulate their expression, we hypothesized that the level of MAPK protein phosphorylation would be increased and the corresponding mRNA overexpressed in the quadriceps of patients with COPD. We also speculate that the quadriceps level of ubiquitinated and polyubiquitinated proteins and of MAFbx/Atrogin and MuRF1 would be upregulated in this context. Lastly, we hypothesized that these biochemical abnormalities would be associated with evidence of inflammation and oxidative stress at the muscle level.

To test these hypotheses, we investigated the phosphorylation levels of ERK 1/2, p38 MAPK, and JNK and of ubiquitinated proteins, MAFbx/Atrogin and MuRF1, in the quadriceps of patients with COPD compared with aged-matched healthy controls. The presence of several key markers of inflammation [tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-8 (IL-8), serum amyloid A1 (SAA1), cluster of differentiation 45 receptor (CD45R)] and of oxidative stress (lipid peroxidation, protein carbonylation, and advance oxidative protein products) was also evaluated in the quadriceps of these individuals.

METHODS

Subjects. Eighteen patients with moderate to severe COPD and nine healthy aged-matched controls subjects participated in this study. The diagnosis of COPD was based on spirometry showing moderate to severe irreversible airflow obstruction [forced expiratory volume in 1 s (FEV1) < 80% predicted value, and FEV1/forced vital capacity (FVC) < 70%]. All patients with COPD were in a stable condition at the time of the study without any acute exacerbation of their disease or exposure to systemic corticosteroids within 2 mo of their participation in the study. None of the patients was receiving long-term oxygen therapy. Patients with COPD and healthy controls were
excluded if they presented any medical condition other than COPD (in the case of patients with COPD) likely to influence muscle and biochemical testing (i.e., cardiovascular, neurological, musculoskeletal, locomotor, or other respiratory diseases). In both groups, only sedentary male exsmokers who had stopped for at least 6 mo were included in the present study to minimize heterogeneity in the measurements. The institutional ethics committee approved the research protocol, and a signed informed consent was obtained from each subject.

Study design. After reviewing medical history and familiarization with the study procedures, subjects filled out a physical activity questionnaire (41). Anthropometric measurements, pulmonary function testing, mid-thigh muscle cross-sectional area measures, and a body composition assessment were performed. Subjects were also given a physical activity monitor for a period of 7 days. Subjects then returned to the laboratory for a quadriceps biopsy at rest.

Pulmonary function testing. Standard pulmonary function tests including spirometry, lung volumes, and carbon monoxide diffusion capacity were obtained in all subjects during the initial evaluation according to previously described guidelines (3). Results were related to previously published normal values (31).

Measurement of mid-thigh muscle cross-sectional area. A computed tomography (CT) of the right thigh halfway between the pubic symphysis and the inferior condyle of the femur was performed using a fourth-generation Toshiba Scanner 900S (Toshiba). Computed tomography was used to quantify muscle cross-sectional area mass in a more specific manner than anthropometric measurements (39).

Muscle biopsy and muscle specimen analyses. After 30 min of rest in the supine position, a needle biopsy of the quadriceps was performed as described by Bergstrom and routinely done in our laboratory (6, 23). Muscle specimens were frozen in liquid nitrogen and stored at −80°C for future analysis.

Protein extraction and Western blotting. Cytoplasmic protein extraction was performed with ≈30 mg of muscle. Muscle samples were then homogenized using a Polytron PowerGen 125 (Omni International, Marietta, GA). Protein content was determined by a Bio-Rad protein assay (BioRad, Mississauga, ON, Canada) based on a modified Lowry method. Electrophoresis was performed using 10–15% SDS PAGE gels. After a 3-h protein transfer in cold (4°C) buffer, nitrocellulose membranes were blocked for 1 h with 5% nonfat dry milk in TBS. The membranes were then incubated overnight at 4°C using the primary antibody phosphorylated-p38 MAPK [monoclonal rabbit anti-human (Thr180/Tyr182) Cell Signaling Technology, Danvers, MA; cat. no. 4511], p38 (polyclonal rabbit anti-human, Cell Signaling Technology; cat. no. 9212), phosphorylated-ERK 1/2 [monoclonal rabbit anti-human (Thr202/Tyr204), Cell Signaling Technology; cat. no. 4376], ERK (monoclonal rabbit anti-human, Cell Signaling Technology; cat. no. 4695), phosphorylated-JNK [monoclonal rabbit anti-human (Thr183/Tyr185), Cell Signaling Technology; cat. no. 4686], and JNK (monoclonal rabbit anti-human, Cell Signaling Technology; cat. no. 9258). MuRF1 (polyclonal rabbit anti-human, GeneTex, Irvine CA; cat. no. GTX110475), ubiquitin (mouse monoclonal anti-human, Cell Signaling Technology; cat. no. 3936), polyubiquitin (polyclonal rabbit anti-human Lysine 48-linkage, Cell Signaling Technology, cat. no. 4289), and 4-HNE [polyclonal rabbit anti-HNE-Michael (4-hydroxynonenal) Adducts, Reduced, Calbiochem, San Diego, CA; cat. no. 393207] For p38, ERK 1/2, and JNK the antibodies that were used detected all protein isoforms (p38, β and γ, Erk1/Erk2, and SAPK/JNK). The antibody against MAf/bx/Atrogin was developed in our center as previously reported (11, 12). The membranes were then washed 3 × 20 min with TBS and incubated for 1 h at room temperature with the secondary antibody and again washed as above. Secondary antibodies used were goat anti-rabbit IgG and horse anti-mouse IgG (Cell Signaling Technology). Finally, the membranes were treated for 5 min with ECL Plus (GE Healthcare, Baie D’Urfé, QC, Canada) to detect chemiluminescence substrates on X-ray exposed over a nitrocellulose membrane. For protein oxidation measurements, the OxyBlot kit was used to detect carbonyl groups according to the manufacturer’s protocol (Millipore, Billerica, MA). All protein contents were normalized to α-tubulin (Sigma, Oakville, ON, Canada) except for polyubiquitin, which was normalized according to the intensity of Amido black staining (2). All proteins were referenced to an optical density quantity-dependent curve at the end of each SDS PAGE gel. Phosphorylated-to-total protein level ratios were individually calculated and averaged per group for comparison.

Real-time PCR. Total RNA extraction was performed from ≈15 mg of muscle using a commercially available preparation (TRizol Reagent) (Invitrogen, Burlington, ON, Canada). One microgram of RNA was reverse transcribed to cDNA using Quantitect Reverse Transcription Kit (Qiagen, Mississauga, ON, Canada). Real-time PCR was performed in a Rotor-Gene 6000 (Corbett Life Science, San Francisco, CA) using Quantitect SYBR Green PCR Kit (Qiagen). At the end of the PCR amplifications, the samples were subjected to a melting curve analysis. The comparative threshold cycles (ΔCT) values for p38, ERK 1/2, JNK, MAf/bx/Atrogin, and MuRF1 were normalized for RPLPO, 18s reference genes, and analyzed using the 2–ΔΔCT method (22). All RT-PCR runs were performed in duplicate to ensure quantitative accuracy. Specific primers are given in Table 1. The p38 primers detect p38α, ERK detects ERK2, and JNK detects JNK1.

Fiber typing. Five (5)-micrometer cryosections were prepared from frozen muscle samples. Sections were fixed for 10 min with acetone/methanol (60/40) at −20°C, washed 10 min in PBS (58 mM

Table 1. Primer sequences used for RT-PCR analyses

<table>
<thead>
<tr>
<th>Target mRNA</th>
<th>PCR Primer Sequence, 5′ → 3′</th>
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<tbody>
<tr>
<td>p38 MAPK</td>
<td>CAGGGCCACGTCCTGGGGGCCT</td>
</tr>
<tr>
<td>ERK 1/2</td>
<td>GCCGGCGCAAGACGAACCAG</td>
</tr>
<tr>
<td>JNK</td>
<td>GACACCCGAGGCTATCCGGGG</td>
</tr>
<tr>
<td>MAf/bx/Atrogin</td>
<td>CAGCGGCTGAGAGGCTGCAC</td>
</tr>
<tr>
<td>MuRF1</td>
<td>CCTAGAACCTGAGCTATCCGG</td>
</tr>
<tr>
<td>18 s</td>
<td>ACCGGGCGCAAGACGAACCAG</td>
</tr>
<tr>
<td>RPLPO</td>
<td>GCCGGCGCAAGACGAACCAG</td>
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NaHPO₄, 17 mM NaH₂PO₄, 68 mM NaCl), blocked for 5 min with 3% hydrogen peroxide, washed for another 10 min in PBS, and then blocked in horse serum for 1 h. Muscle was immunohistochemically stained using monoclonal anti-skeletal myosin fast (Sigma, Oakville, ON, Canada) incubated overnight at 4°C with 1:200 dilution. Cryosections were washed 10 min in PBS just before adding 1:50 dilution of biotinylated antibody IgG from VECTASTAIN Elite ABC system (Vector Laboratories, Burlington, ON, Canada). The immunoreactivity was visualized by AEC chromogen substrate (Dako, Burlington, ON, Canada). All fibers were counted and classified according to the staining intensity: type I (nonstained) and type II (stained). All muscle sections were magnified and transferred to an image analyzing system (Image Pro Plus 4.5 for Windows, Media Cybernetics, Silver Spring, MD). All fibers were counted and classified to obtain the fiber-type composition for each subject.

**Immunochemical assays.** Muscle tissue was cut in 5-µm consecutive sections on a cryostat and prepared like the cryosections for fiber typing. Sections were then incubated overnight at 4°C with monoclonal mouse anti-human SAA1 diluted 1:10 (Calbiochem, San Diego, CA; cat. no. ST1506), polyclonal rabbit anti-human II-6 diluted 1:10,000 (Genetex; cat. no. GTX26672), polyclonal rabbit anti-human II-8 diluted 1:1,000 (Bender Med System, San Diego, CA; cat. no. BMS136), monoclonal rabbit anti-human TNF-α diluted 1:40 (Millipore; cat. no. 04-1114), monoclonal rabbit anti-human CD45R diluted 1:10 (Pharmingen, Mississauga, ON, Canada; cat. no. 555904). Cryosections were then treated exactly like fiber typing immunohistochemistry with VECTASTAIN Elite ABC system (described above) and AEC chromogen substrate. Positive and negative assays were used to ensure specific staining stated in the manufacturer’s protocol. A blinded evaluator randomly selected 50 muscle fibers to be analyzed for each specific antibody described above. Two evaluators then counted all specific stains to get the total stain count, which was then averaged between the two evaluators and divided by the number of fibers to obtain the stains/fiber ratio. Representative examples of the inflammatory immunohistochemical stains are provided in Fig. 1.

**Statistical analysis.** Results are expressed as means ± SE. Between-group comparisons were done using unpaired Student’s t-tests, while normality for all data was assessed with a Shapiro-Wilk test. Possible relationships were evaluated using Pearson’s correlations. Results were considered significant if P values were <0.05.

**RESULTS**

**Subjects’ characteristics.** Anthropometric characteristics and pulmonary function data are provided in Table 2. On average, patients with COPD had moderate to severe airflow obstruction, reduced diffusion capacity, and mild hypoxemia. Total fat-free mass and fat-free mass index were significantly lower in patients with COPD compared with healthy controls.

**Muscle characteristics.** Mid-thigh muscle cross-sectional area was smaller in COPD compared with controls. In COPD, mid-thigh muscle cross-sectional areas ranged from severe atrophy (47 cm²) to normal value (102 cm²). Six patients exhibited a mid-thigh muscle cross-sectional area < 70 cm², a value that is associated with poor survival (24). Patients with COPD also exhibited a smaller proportion of type I fibers with a reciprocal increase in type II fiber proportion (Table 2).

**MAPK pathway: phosphorylated and total protein levels and mRNA levels.** Phosphorylated-to-total protein ratios for p38 MAPK, ERK 1/2, and JNK were significantly lower in patients with COPD compared with controls. However, no statistically significant difference between groups was seen for phosphorylated-to-total JNK ratio (153%; P = 0.23). The mRNA expression of p38 MAPK α (253%; P = 0.04) and ERK2 (384%; P = 0.02) was significantly elevated in patients with COPD compared with controls, while JNK1 (171%; P = 0.52) mRNA levels did not show any differences between groups (Fig. 3, A–C).

Total protein content of MAFbx/Atrogin tended to be elevated in patients with COPD (170%; P = 0.08) (Fig. 4A) while the proportion of MAFbx/Atrogin mRNA was decreased by 10.22 ± 0.32.247 on August 15, 2017 http://jap.physiology.org/ Downloaded from

![Fig. 1. Examples of immunohistochemical staining. Isotypical control (A), positive staining for SAA1 (B), and positive staining for TNF-α (C) on muscle cryosections are provided. Arrows point to area of positive staining.](image)
MuRF total protein levels was similar between groups (Fig. 4B). MAFbx/Atrogin mRNA level was significantly elevated in patients with COPD (364%; \( P < 0.04 \), Fig. 4C). A similar tendency was observed for MuRF1, but this did not reach statistical significance (201%; \( P > 0.28 \), Fig. 4D). Total ubiquitinated proteins were similar between groups (Fig. 4E) while polyubiquitinated proteins levels were significantly elevated in patients with COPD (155%; \( P = 0.02 \), Fig. 4F). In all subjects, there was a negative correlation between the phosphorylated-to-total p38 MAPK ratio (Pearson’s \( r = -0.45; P < 0.05 \)) and the phosphorylated-to-total ERK1/2 ratio (Pearson’s \( r = -0.47; P < 0.05 \)) and mid-thigh muscle cross-sectional area (Fig. 5, A and B). In addition, there were significant correlations between the phosphorylated-to-total p38 MAPK ratio (Pearson’s \( r = 0.44; P < 0.05 \)) and the phosphorylated-to-total ERK1/2 ratio (Pearson’s \( r = 0.57; P < 0.05 \)) with MAFbx/Atrogin (Fig. 5, C and D). Finally, a correlation was observed between the phosphorylated-to-total ERK1/2 ratio (Pearson’s \( r = 0.55; P < 0.05 \)) and the polyubiquitinated proteins levels (Fig. 5E).
Muscle oxidative stress and inflammatory markers. Immunohistochemical assays revealed similar muscle levels for IL-6, IL-8, TNF-α, SAA1, and CD45R in both groups (Table 3). Also depicted in Table 2, lipid peroxidation (4-HNE) and protein carbonylation levels (OxyBlot) measured in muscle samples were similar between groups.

DISCUSSION

In this study, the extent of activation of several MAPKs was assessed in the context of their possible contribution to the development of muscle atrophy in patients with COPD. The main finding was the greater proportion of phosphorylated proteins levels of p38 MAPK and ERK 1/2 in the quadriceps of patients with moderate to severe COPD compared with healthy controls. The presence of an elevated proteolytic activity in the muscles of these patients was supported by the mRNA overexpression of MAFbx1/Atrogin and increased muscle protein polyubiquitination in COPD patients. Moreover, significant correlations were found between quadriceps phosphorylated protein levels of p38 MAPK and ERK 1/2 with mid-thigh muscle cross-sectional area, MAFbx1/Atrogin, and polyubiquitin. Although the origin of this activation of p38 MAPK and ERK 1/2 in these patients could not be confirmed in the absence of evidence for muscle inflammation.
and oxidative stress, our results nevertheless highlight the abnormal regulation of key signaling proteins that could be involved in the regulation of muscle mass in patients with stable COPD.

**MAPK signaling.** While the activity of the MAPKs responds to several distinct stresses, convincing evidence shows that p38 MAPK and ERK are contributors to the development of skeletal muscle atrophy through activation of proteolysis (18, 30, 40). Specifically, MAFbx/Atrogin expression is upregulated by inflammatory stresses in a p38 MAPK-dependent manner in culture myotubes (21). Recent findings indicate that the blockade of MAFbx/Atrogin gene induction can be achieved with the inhibition of p38 MAPK (18). In addition, ERK activation increases the expression of the MAFbx/Atrogin in C2C12 cells, suggesting an upregulation of the proteasome activity and a downregulation of the myogenic process (27). Although the MAPKs (p38 MAPK, ERK, and JNK) induce transcriptional factors such as AP-1, p53, and c-Myc and many other non-transcriptional factors involved in muscle cell degradation, the specific activation of these factors remains elusive (30). Thus our findings of increased levels of activation of p38 MAPK and ERK 1/2, muscle protein polyubiquitination levels, and of MAFbx1/Atrogin mRNA overexpression in patients with COPD are consistent with cell and animal experiments in suggesting a potential contribution of this pathway in the regulation of skeletal mass in this disease. This statement is further supported by the correlations between p38 MAPK and ERK 1/2 with MAFbx1/Atrogin, polyubiquitin, and mid-thigh muscle cross-sectional area.

Apart from its involvement in the regulation of muscle mass, the activation of the various MAPKs signaling pathway may contribute to other muscle phenotypic manifestations commonly seen in COPD. Increased levels of ERK1/2 have been associated with type II fiber predominant muscle phenotype that is commonly seen in COPD (14, 38). For instance, p38

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![Figure 5](http://jap.physiology.org/)

**Fig. 5.** Correlation between phosphorylated-to-total p38 ratio and mid-thigh muscle cross-sectional area (A), phosphorylated-to-total ERK ratio and mid-thigh muscle cross-sectional area (B), phosphorylated-to-total p38 MAPK ratio and MAFbx/Atrogin (C), phosphorylated-to-total ERK ratio and MAFbx/Atrogin (D), and phosphorylated-to-total ERK ratio and polyubiquitinated protein levels (E) in healthy controls and patients with COPD.
showed no significant difference in phosphorylated p38 MAPK because a reduction in GLUT4 transporters and glucose transport/contraction-stimulated glucose transport (17). This is of interest cytokines like TNF-α, the basis that several studies have shown that proinflammatory pathway and to differences in study populations.

Methodologies used to explore the effectors of the MAPK signalization levels compared with healthy controls (34). Discrepancies between these and our results are likely to be related to different levels intrinsic to this type of human experimentation is the descriptive nature of the study. As such, our results do not confirm a causal relationship between the activation of the p38 MAPK and ERK 1/2 and the reduction of muscle mass, although this was suggested from correlational analyses. Future mechanistic experiments or pharmacological manipulations of these MAPKs pathways are necessary to address a possible causality between these observations. Furthermore, several isoforms and expression patterns of the MAPKs exist; however, dissecting of the specific role of each subunit in the COPD-related atrophying process appeared premature and was beyond the scope of this investigation (25, 30). As such, antibodies detecting all protein isoforms were used in the Western blot analyses. Lastly, we acknowledge that some discrepancies exist between our protein and mRNA results. Apart from biological explanations for this phenomenon (see previous paragraph), some methodological issues could contribute to these discrepancies; while the primers that were used for PCR analyses were specific for certain mRNAs, the antibodies that were used for the Western blot analyses covered all the different isoforms of the proteins of interest.

Mechanisms of MAPK signaling pathway upregulation. On the basis that several studies have shown that proinflammatory cytokines like TNF-α can stimulate muscle proteolysis and lead to atrophy, we investigated whether the activation of p38 MAPK and ERK 1/2 was driven by muscle inflammation or oxidative stress. A low level of systemic and/or muscle inflammation has been proposed as a potential trigger of muscle atrophy in COPD (10, 13). For example, patients with COPD who fail to gain weight in response to nutritional support present high circulating levels of TNF-α, which can activate all three major MAPK signaling pathways in skeletal muscle, thus possibly contributing to the muscle protein catabolic state (8).

Oxidative stress is also associated with loss of muscle mass in COPD (5). Oxidative stress within skeletal muscle fibers activates redox-sensitive transcription factors and protein kinases, including p38 MAPK, ERK1/2, and JNK (30). In the present study, we found that p38 MAPK and ERK 1/2 activation as well as MAFbx/Atrogin levels were elevated in patients with COPD, but no apparent evidence of muscle inflammation and oxidative stress could be observed.

There are inconsistencies regarding the presence of muscle inflammation and oxidative stress in patients with COPD across studies (1, 26, 28, 32), including ours. A definitive conclusion about the significance of inflammation and/or oxidative stress to the COPD-related atrophying process cannot be currently reached. Our view is that this process is likely to be multifactorial and that the relative contribution of each individual factor is likely to vary in time and from one patient to the other. One possibility is that intermittent inflammatory or oxidative bursts occurring during an acute COPD exacerbation or during acute immobilization and that cannot be detected during a stable phase of the disease, could be detrimental to skeletal muscle maintenance in patients with COPD, although one report does not support this contention (9).

Even if not all mRNAs translate into proteins, our results of the greater expression of p38 MAPK and ERK 1/2 mRNA show that the cellular environment is prepared to produce a larger amount of those proteins, if necessary. This could be relevant for situations requiring rapid inflammatory or oxidative responses.

Limitations. This study has the merit of reporting on molecular mechanisms that have been convincingly associated with the development of muscle atrophy in cells and hindlimb-immobilized animals (7, 21, 25). However, a number of methodological considerations and potential limitations need to be taken into account regarding its interpretation. First, although patients with COPD clearly exhibited a loss of muscle mass, we cannot ascertain whether they were actively losing muscle mass at the time of experimentation. Another limitation that is intrinsic to this type of human experimentation is the descriptive nature of the study. As such, our results do not confirm a causal relationship between the activation of the p38 MAPK and ERK 1/2 and the reduction of muscle mass, although this was suggested from correlational analyses. Future mechanistic experiments or pharmacological manipulations of these MAPKs pathways are necessary to address a possible causality between these observations. Furthermore, several isoforms and expression patterns of the MAPKs exist; however, dissecting of the specific role of each subunit in the COPD-related atrophying process appeared premature and was beyond the scope of this investigation (25, 30). As such, antibodies detecting all protein isoforms were used in the Western blot analyses. Lastly, we acknowledge that some discrepancies exist between our protein and mRNA results. Apart from biological explanations for this phenomenon (see previous paragraph), some methodological issues could contribute to these discrepancies; while the primers that were used for PCR analyses were specific for certain mRNAs, the antibodies that were used for the Western blot analyses covered all the different isoforms of the proteins of interest.

Conclusions. This study demonstrates elevated phosphorylation levels of p38 MAPK and ERK1/2 in the quadriceps of patients with COPD compared with healthy aged-matched controls. These intracellular signaling events were associated with increased levels of MAFbx/Atrogin and protein polyubiquitination. As such, the present results support the hypothesis that p38 MAPK and ERK1/2 could be potentially involved in the regulation of muscle mass in COPD.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: B.B.L. and F.M. conception and design of research; B.B.L. and A.D. performed experiments; B.B.L., A.D., and M.-E.T. analyzed Table 3. Muscle oxidative stress and inflammatory markers

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 9)</th>
<th>COPD (n = 18)</th>
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<tbody>
<tr>
<td>Inflammatory markers, stains/fiber</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>41.0 ± 4.7</td>
<td>40.6 ± 2.1</td>
</tr>
<tr>
<td>IL-6</td>
<td>7.0 ± 2.3</td>
<td>6.0 ± 1.8</td>
</tr>
<tr>
<td>IL-8</td>
<td>9.8 ± 2.8</td>
<td>8.2 ± 1.5</td>
</tr>
<tr>
<td>SAA1</td>
<td>55.8 ± 7.3</td>
<td>46.1 ± 5.1</td>
</tr>
<tr>
<td>CD45R</td>
<td>3.5 ± 0.8</td>
<td>3.6 ± 0.4</td>
</tr>
<tr>
<td>Oxidative stress markers, fold increase from controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxyblot</td>
<td>1.00 ± 0.03</td>
<td>1.03 ± 0.03</td>
</tr>
<tr>
<td>4-HNE</td>
<td>1.00 ± 0.09</td>
<td>1.02 ± 0.04</td>
</tr>
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Values are mean ± SE. TNF-α, tumor necrosis factor alpha, IL-6, interleukin 6; IL-8, interleukin 8; SAA1, serum amyloid A1; CD45R, cluster of differentiation 45 antigen receptor; Oxyblot, protein carbonylation; 4-HNE, trans-4-hydroxy-2-nonenal.
data: B.B.L., R.D., M.-E.T., C.H.C., and F.M. interpreted results of experiments; B.B.L. prepared figures; B.B.L. drafted manuscript; B.B.L., R.D., A.D., M.-E.T., C.H.C., and F.M. edited and revised manuscript; R.D. and F.M. approved final version of manuscript.

REFERENCES