**HIGHLIGHTED TOPIC** | *Muscle Dysfunction in COPD*

Impaired exercise capacity and skeletal muscle function in a mouse model of pulmonary inflammation


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CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) is the fourth leading cause of death in North America and Europe (21). In addition to the primary pathology that develops in the lung, patients with COPD exhibit comorbidities or extrapulmonary abnormalities in heart, bone, and skeletal muscle that may contribute to physical inactivity (9). For instance, a decrease in lean muscle mass has been reported in both smokers with normal lung function and those with obstructive lung disease. Inflammation and/or oxidative stress triggered by cigarette smoke have been suggested as one mechanism that could interfere with regeneration and/or atrophy pathways in skeletal muscle (44, 58). This is supported by the finding of Remels et al. (43) that a subgroup of COPD patients with higher expression of the inflammatory cytokine TNFα in skeletal muscle exhibit a reduced body mass index (BMI).

TNFα is also upregulated in lung and skeletal muscle of mice exposed to cigarette smoke (23, 54). TNFα overexpression in the mouse lung results in a phenotype with many similarities to human COPD. SP-C/TNFα transgenic mice exhibit robust lymphocytic inflammation, severe emphysema, and pulmonary hypertension (18, 19, 41). Smoke exposure also leads to early changes in body and skeletal muscle mass, an oxidative to glycolytic fiber transition, and loss of oxidative metabolic capacity that correlates with downregulation of the transcriptional coactivator PGC-1α (23, 54). Muscle wasting or a loss of muscle mass has been reported to occur in SP-C/TNFα transgenic mice (23, 54). Muscle wasting or a loss of muscle mass has been reported to occur in SP-C/TNFα transgenic mice in part due to an impaired differentiation of myoblasts (33).

We hypothesized that overexpression of TNFα in the lung leads to comorbidities in both the heart and skeletal muscle, in addition to the primary pulmonary pathology, that could contribute to exercise limitation. Therefore, exercise capacity in mice that overexpress TNFα in the lung under the control of the surfactant protein C promoter (SP-C/TNFα mice) was assessed in relation to the degree of emphysema, pulmonary hypertension, cardiac and skeletal muscle function and maintenance of body weight. We found that in SP-C/TNFα mice, exercise capacity was selectively impaired in male, but not female mice, and was accompanied by pulmonary hypertension, reduced body weight, and downregulation of the oxidative muscle phenotype, but not by physiological evidence of left ventricular dysfunction. In oxidative skeletal muscle, PGC-1α expression was reduced and the atrophy-related genes, atrogin and MurF1, upregulated. Together, these data suggest that estrogen may play a role in protecting against TNFα-induced myopathy in part by preventing the downregulation of the transcriptional co-activator, PGC-1α.

**MATERIALS AND METHODS**

**Experimental Design**

The study was designed to evaluate the role of TNFα-induced loss of exercise capacity in relation to pulmonary, cardiac, and musculoskeletal function, as well as sex. Male and female SP-C/TNFα mice and their sex-specific littermate controls (WT) were first evaluated each month for a change in body weight up to 10–15 mo of age. Subsequently, SP-C/TNFα and WT mice at 6–8 mo of age were studied to determine if there was a sex-specific change in exercise...
capacity (maximal treadmill speed, submaximal endurance time), pulmonary structure, and function (lung volume, airspace size, arterial \( O_2 \) saturation), cardiac function (direct micromanometer right and left ventricular pressures and echocardiography) and skeletal muscle structure and function (in situ time to fatigue, in vitro time to fatigue, oxidative enzyme activities, fiber composition and capillarity, and atrophy-related gene expression).

**Animals**

SP-C/TNF\( \alpha \)/+− mice on a C57BL/6 background were obtained from Dr. Y. Miyazaki (19, 41), and bred as heterozygous mice at the University of California, San Diego. In each litter, approximately half of the mice carried the SP-C/TNF\( \alpha \)+ gene, and the other half were negative for the transgene and used as the wild-type (WT), littermate control group. The University of California, San Diego, Institutional Animal Care and Use Committee (IACUC) approved the experimental procedures in this study.

**TNF\( \alpha \) ELISA**

TNF-\( \alpha \) was measured in serum and organs with an ELISA system that has a minimum detectable dose (MDD) of 0.36–7.21 pg/ml for mouse TNF\( \alpha \) (R & D Systems).

**Treadmill Running Tests**

Mice were exercised using an Omniscient Treadmill LC4 (Omnitech Electronics Columbus, OH) on a 10° incline in room air.

**Maximal running speed.** After a low-speed warm-up (15 cm/s for 5 min), mice were subjected to an incrementally increasing speed running test. Mice ran at 50, 55, 60, 65, 70, 75, and 80 cm/s for 20 s at each speed, respectively. The fastest speed that a mouse could run was recorded as its maximal running speed.

**Endurance.** Both WT and SP-C/TNF\( \alpha \)+ mice ran at 60% of the average maximal speed of SP-C/TNF\( \alpha \)+ mice (36 cm/s in this experiment) until exhaustion. In some mice, a clip was connected to the back of a shaved neck area and \( O_2 \) saturation was continuously monitored and recorded at 12-h intervals using a Comprehensive Lab Animal Monitoring System (CLAMS) from Columbus Instruments.

**Estimation of Air Space Enlargement by Mean Linear Intercept (MLI)**

A point-count morphometric technique was used to assess air space enlargement from paraffin-embedded, 7-μm sections. Lungs were inflated and fixed at an airway pressure of 20 cmH\(_2\)O as previously described (53). Lung volumes were estimated by fluid displacement.

**Cardiac Function Measurements**

**Transthoracic echocardiography.** Animals were anesthetized with 5% isoflurane for 1 min and then maintained at 1% isoflurane throughout the examination. The anterior chest wall was shaved and any remaining hair removed with Nair. Small needle electrodes for simultaneous electrocardiogram were inserted into one upper and two lower limbs. Images (M-mode, 2 dimensional, and Doppler) were acquired using the VisualSonics Vevo 2100, a division of SonoSite, ultrasound system with a linear transducer of 32–55 MHz. Measurements of heart rate (HR), left ventricular end-diastolic dimensions (LVEDD) and left ventricular end-systolic dimensions (LVESD), end-diastolic interventricular septal thickness (IVSd) and LV posterior wall thickness (LVPWd) were determined from the LV M-mode tracing. Percentage fractional shortening (%FS) was used as an indicator of systolic cardiac function. In addition, interrogation with pulsed wave Doppler (PWD) of the mitral inflow and tissue Doppler imaging (TDI) of the medial mitral annulus were used to quantify diastolic performance (52).

**Cardiac micromanometer catheterization.** Hemodynamic evaluation of right and left ventricular function was performed while mice were under general anesthesia [ketamine (100 mg/kg)/xylazine (10 mg/kg)]. After connection to a ventilator (Kent Scientific TOPO pressure ventilator), a 1.4-Fr (0.46 mm) micromanometer catheter (Millar Instruments, Houston, TX) was inserted via the right jugular vein into right atrium and advanced further into the right ventricle (RV). The instantaneous pressure at RV dP/dt\(_{\text{max}}\) was taken as corresponding to pulmonic valve opening and the instantaneous pressure at RV dP/dt\(_{\text{min}}\) as corresponding to pulmonic valve closure; during ejection, the RV pressure was considered equivalent to main pulmonary artery pressure. Following measurements in the RV, the Millar catheter was withdrawn from the RV and inserted via the right common carotid artery and into the LV where LV peak and end-diastolic pressures were measured. To assess contractile reserve, LV dP/dt\(_{\text{max}}\) was measured during an isotropic challenge with dobutamine at dosages of 0.75, 2, 4, 6, and 8 μg·kg\(^{-1}\)·min\(^{-1}\), with 3-min recordings at each dosage. All pressure recordings were continuously monitored using LabChart (ADInstruments, Colorado Springs, CO) systems (3).

**Skeletal Muscle Fatigue**

**Ex vivo fatigue index.** Isolated soleus and EDL fatigue was measured as previously described by Zuo et al. (60). Fatigue was measured at the optimal length (Lo). The soleus was stimulated with a 500-ms train duration, 0.5-ms pulses, 80 Hz at 0.15, 0.19, 0.25, 0.33, 0.43, 0.57, 0.75, 1.0, and 1.3 Hz trains per second, 1 min each. The EDL was stimulated with a 250-ms train duration, 0.5-ms pulse, 150 Hz at 0.15, 0.19, 0.25, 0.33, 0.43, 0.57, 0.75, and 1.0 Hz trains per second, 1 min each. The fatigue index was defined as the time to reach 30% of the initial tension.

**In situ fatigue.** Mice were anesthetized with isoflurane, and one sciatic nerve was exposed and connected to electrodes. The gastrocnemius complex was then separated from the bone and the Achilles tendon connected with a suture to a force transducer (Grass FT10). The gastrocnemius complex was set to Lo using single-twitch contractions and then stimulated to contract (with 10 V, 0.25 trains per second, 200-ms train duration) until the force generated fell to 50% of the initial force output and the time recorded. Mean arterial pressure was measured at the end of the contraction period and was on average 63 ± 8 mmHg.

**Analysis of Myosin Heavy Chain Protein (MHC)**

This method has been previously described (54). Briefly, frozen soleus samples were homogenized in a buffer containing 250 mM sucrose, 2 mM EDTA, 10 mM Tris, pH 7.4 at a volume of 50 μl/mg followed by sonication for 1 min. Homogenates were centrifuged at 10,000 \( g \) at 4°C for 10 min. The supernatant from this spin was used for citrate synthase and \( \beta \)-HAD assays. The remaining pellet was resuspended in 50 μl of ice-cold extraction buffer (100 mM Na\(_2\)O\(_2\)PO\(_4\), 10 mM EDTA, 1 mM DTT, pH 8.5), incubated for 30 min on ice, and centrifuged at 10,000 \( g \) at 4°C for 10 min. Supernatants from the second spin were electrophoresed on an SDS-PAGE gel and silver stained. ImageJ software (http://www.nih.gov) was used to measure the density of individual MHC bands in each lane. The data are expressed as percentage of MHC I, IIa, or IIb of the total density of MHCs detected per lane.

**Citrate Synthase (CS) and \( \beta \)-Hydroxylacyl CoA Dehydrogenase (\( \beta \)-HAD) Activity Assays**

Enzyme activity assays have been described previously (54). Briefly, the supernatant from the first spin of the above-mentioned
muscle sample preparation was assayed using the oxaloacetate method with a CS Assay Kit (Sigma, St. Louis, MO). The activity of β-HAD was measured using the NADH method.

NFκB p65 Binding Activity

The muscle samples were homogenized with passive lysis buffer (Promega, Madison WI). NFκB p65 DNA binding activity was measured using an ELISA-based kit (TransAM NF-κB p65, no. 40096 Active Motif, Carlsbad, CA).

Real-Time RT-PCR Assays

RNA isolation, reversed transcription, and PCR amplification were described previously (54). Changes in mRNA level were measured for the following genes: TNFα, TNFR-1, TNFR-2, VEGF, VEGFR-1, VEGFR-2, Ang-1, Ang-2, β-FGF, IL-6, IL-1β, INFγ, C-reactive protein, TGFβ, Atroglin-1, MuRF1, PGC-1α, PPARα, PPARγ1, and PPARδ. The PCR primers for the above-mentioned genes were designed with Vector NTI Deluxe (Tucows Toronto, Canada).

Statistics

Because it became evident prior to making any measurements that body weight in SP-C/TNFα+ mice was reduced in males but unaffected in females, all subsequent data from the male and female mice were analyzed separately. An ANOVA was used to compare body weights in WT and SP-C/TNFα+ at multiple time points and a Tukey post hoc test used to determine differences at each time point. A two-way ANOVA was used to compare type I and type II fiber sizes in soleus from SP-C/TNFα+ and WT male mice. Differences in physiological outcomes between WT and SP-C/TNFα+ groups within the same sex were analyzed with an unpaired Student’s t-test. P < 0.05 was considered significant.

RESULTS

TNFα Levels in Lung, Serum, Heart, and Skeletal Muscle of SP-C/TNFα+ Mice

TNFα protein levels were substantially increased in lungs, serum, and skeletal muscle but not in heart in SP-C/TNFα+ mice compared with WT mice of the same sex (Table 1). In the lungs, TNFα levels were higher in both SP-C/TNFα+ female and male mice compared with WT female and male mice (P < 0.01). Baseline TNFα levels in the lung of female mice were elevated compared with the WT male group (P < 0.05). In serum, TNFα levels were increased in both the female (P < 0.05) and male (P < 0.001) SP-C/TNFα+ groups compared with the respective sex WT group. TNFα+ levels in the serum of WT female mice were increased compared with WT male mice (P < 0.05). In soleus, TNFα was also elevated in both female (1.3-fold, P = 0.02) and male (1.6-fold, P = 0.001) SP-C/TNFα+ mice compared with WT. Similarly, TNFα levels in the gastrocnemius were elevated in both female (1.4-fold, P = 0.01) and male (1.7-fold, P < 0.001) SP-C/TNFα+ mice compared with WT. In the EDL, TNFα was also elevated in female (1.5-fold, P = 0.04) and male (1.7-fold, P = 0.003) SP-C/TNFα+ mice compared with WT.

Body Weight and Muscle Mass

Female WT and SP-C/TNFα+ mice did not differ in body weight over 10 mo (Fig. 1). Body weights of male SP-C/TNFα+ mice were detectably lower than the male WT group starting at 4 mo of age, and this difference progressively increased over the next months. At 12 mo of age, the male SP-C/TNFα+ mice weighed 23% less than the WT mice (P < 0.001). The weight of the soleus (WT, 9.22 ± 1.3 mg; SP-C/TNFα+, 7.68 ± 1.1 mg, P = 0.01), and both soleus type I and II fiber sizes (Fig. 2) were significantly reduced in male SP-C/TNFα+ mice but not in females. Gastrocnemius and EDL muscle mass and fiber size were not altered.

Impaired Exercise Capacity in Male SP-C/TNFα+ Mice

Maximal treadmill running speed was reduced by 11% in male SP-C/TNFα+ mice compared with WT (P = 0.03) (Fig. 3). Exercise endurance (time to exhaustion when run at a constant speed) was reduced by 24% only in male SP-C/TNFα+ mice (P = 0.04). Total activity in cage-confined mice was not different in male SP-C/TNFα+ mice compared with WT (Fig. 4A). However, there were small increases in the RER, V̇O₂, and V̇CO₂ as well as food and water intake at rest in male SP-C/TNFα+ mice compared with WT (Fig. 4B–F).

Pulmonary and Cardiac Function in SP-C/TNFα+ and WT Mice

Lung volumes quantified by fluid displacement at the same inflation pressure were increased by 2.0- and 1.6-fold, respectively, in female and male SP-C/TNFα+ mice compared with their sex-specific control group (P < 0.001). Evidence of emphysema was sought by evaluating the mean linear intercept, which was increased in both the female and male SP-C/TNFα+ mice by 2.36-fold (WT, 35.2 ± 1.2 μm; SP-C/TNFα+ 82.9 ± 4.5 μm, P < 0.001) and 2.07-fold (WT, 35.2 ± 1.2 μm; SP-C/TNFα+ 82.9 ± 4.5 μm, P < 0.01), respectively, compared with the sex-specific WT group. In the heart, increases in RV maximal pressure (WT 28.0 ± 6.5 mmHg; SP-C/TNFα+ 38.63 ± 7.5 mmHg, P < 0.05) and RV minimal dP/dt pressure (WT −1,689 ± 326 mmHg/s; SP-C/TNFα+ −2,460 ± 583 mmHg/s, P < 0.05) were found in male, but not female, SP-C/TNFα+ mice. Calculated peak

Table 1. TNFα levels in the lung, heart, skeletal muscle, and circulation

<table>
<thead>
<tr>
<th></th>
<th>Female</th>
<th>Male</th>
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<tbody>
<tr>
<td></td>
<td>Wild type (n = 8)</td>
<td>SP-C/TNFα+ (n = 8)</td>
</tr>
<tr>
<td>Lung, pg/mg</td>
<td>30.6 ± 6.8</td>
<td>895 ± 223*</td>
</tr>
<tr>
<td>Serum, pg/ml</td>
<td>6.8 ± 1.8</td>
<td>31.3 ± 10.4*</td>
</tr>
<tr>
<td>Heart, pg/mg</td>
<td>7.5 ± 1.9</td>
<td>7.8 ± 1.1</td>
</tr>
<tr>
<td>Gastrocnemius, pg/mg</td>
<td>2.8 ± 0.8</td>
<td>3.9 ± 0.9#</td>
</tr>
<tr>
<td>Soleus, pg/mg</td>
<td>3.6 ± 0.9</td>
<td>4.8 ± 0.9#</td>
</tr>
<tr>
<td>EDL, pg/mg</td>
<td>2.1 ± 0.5</td>
<td>3.1 ± 1.1#</td>
</tr>
</tbody>
</table>

Values represent means ± SD. EDL, extensor digitorum longus. Significant differences compared with the same-sex wild type (WT) are indicated (*P < 0.01, ‡P < 0.05) and between the male and female WT mice (‡P < 0.05).
higher in the male SP-C/TNFα/H9251/H11001
Locomotor Skeletal Muscle Function

four groups. both at rest and during exercise, was not different in any of the functional parameters were detected in female SP-C/TNFα/H9251/H11001 mice (data not shown). No significant differences in cardiac were also not different between male WT and SP-C/TNFα/H11021 (WT) (•) and SP-C/TNFα+ (○) mice. Values represent means ± SE. Differences between WT and SP-C/TNFα+ mice at each time point are indicated: *P < 0.05, #P < 0.01. WT-Female, n = 7–37; SP-C/TNFα+—Female, n = 6–40; WT-Male, n = 7–58; SP-C/TNFα+—Male, n = 5–36. The shaded gray area represents the 6- to 8-mo time interval in which mice were analyzed for changes in cardiac and skeletal muscle function.

(Fig. 2) Exercise capacity is selectively impaired in male but not female mice. Endurance times were not altered in female SP-C/TNFα+ mice. Values represent means ± SD; n = 6. *Difference between SP-C/TNFα+ and WT male mice, P < 0.05.

Skeletal Muscle Oxidative Phenotype

As shown in Fig. 6, A and B, in the soleus of female SP-C/TNFα+ mice, the percentage of MHC IIb was fourfold higher than in WT females (2.1 ± 0.8% vs. 0.5 ± 0.2%, P < 0.05). In the soleus of male SP-C/TNFα+ mice, the percentage of MHC IIb was 17-fold higher than in WT males (8.6 ± 3.5% vs. 0.5 ± 0.2%, P < 0.001). These changes were accompanied by 27% and 24% decreases in the activity level of the oxidative enzymes, CS (P < 0.02; Fig. 6C) and β-HAD (P < 0.045, Fig. 6E), respectively, in the soleus of SP-C/TNFα+ mice studied ex vivo, the time to fatigue (a time for force to fall to 30% of initial force during a repetitive contraction protocol) was decreased compared with WT soleus (P < 0.05). However, the fatigue response in the soleus was not different between WT and SP-C/TNFα+ females (Fig. 5A). EDL muscle in either sex also did not show a difference in the time to fatigue with TNFα overexpression (Fig. 5B). In situ gastrocnemius complex (soleus, plantaris, and gastrocnemius) time to fatigue (reflecting both oxygen availability and muscle contractile function) was reduced similarly in both the female (37%) and male (32%) SP-C/TNFα+ gastrocnemius complex compared with WT (P < 0.05) (Fig. 5C).

Locomotor Skeletal Muscle Function

In soleus from male SP-C/TNFα+ mice studied ex vivo, the time to fatigue (a time for force to fall to 30% of initial force

(27.7 ± 6.6 mmHg/s; SP-C/TNFα+ 37.5 ± 8.5 mmHg P < 0.05) and mean pulmonary artery pressures (WT 19.5 ± 5.5 mmHg; SP-C/TNFα+ 25.3 ± 6.2 mmHg P < 0.05) were higher in the male SP-C/TNFα+ mice. Cardiac output, stroke volume, LV end-diastolic pressure, LV maximal pressure and LV peak dP/dt were not altered by TNFα overexpression in either sex. Left ventricular pressures following dobutamine challenge (simulating cardiac output increases with exercise) were also not different between male WT and SP-C/TNFα+ mice (data not shown). No significant differences in cardiac functional parameters were detected in female SP-C/TNFα+ mice. Arterial oxygen saturation, measured by pulse oximeter, both at rest and during exercise, was not different in any of the four groups.

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TNFα+ male mice. No significant changes in citrate synthase activity and β-HAD activity levels were detected in the female soleus and EDL of either sex (Fig. 6, D and F). Interestingly, measuring total abundance of oxidative phosphorylation proteins by immunoblot in the soleus did not show any significant difference between groups (data not shown). Skeletal muscle capillary-to-fiber ratio, measured in the soleus and EDL, was not different between any of the groups (data not shown).

**DISCUSSION**

**Summary of Major Findings**

In this study of a lifelong pulmonary inflammation mouse model, circulating TNFα reached very high levels in the lung and circulation similarly in both male and female mice. However, exercise limitation was selectively impaired only in male SP-C/TNFα+ mice. Changes in the SP-C/TNFα mouse phenotype that could contribute to this exercise limitation and which were found to be selectively altered in male mice are a decrease in overall body weight, skeletal muscle atrophy, and an impaired muscle oxidative phenotype that was accompanied by increased TNFα, MuRF1, and atrogin1 and reduced PGC-1α expression.

**Reduced Body Weight and Loss of the Oxidative Muscle Phenotype**

A prominent change that occurred only in male SP-C/TNFα+ mice was an up to 23% lower body weight. Total...
activity in cage-confined mice was the same in both the SP-C/TNFα+ and WT male mice. A small increase in the RER, despite an increase in food and drink intake, suggests that the overall inflammatory state may contribute to a loss in body weight. In addition, the male soleus demonstrated a selective decrease in oxidative capacity (decreased percent of type I fibers and oxidative enzyme activity) and increased expression of two atrophy-related genes, atrogin and Murf1, even though all muscle types analyzed expressed elevated TNFα levels. This reduced oxidative capacity, detected from the reduced fatigue resistance of isolated muscles and reduced activity of oxidative enzymes, was not correlated with a reduction in the total abundance of mitochondrial proteins for oxidative phosphorylation. This finding would suggest that there might be an intrinsic impairment in the mitochondrial oxidative machinery. These data also suggest that loss in muscle mass as well as reduced muscle oxidative profile are not associated with physical inactivity, but related to the chronic pulmonary inflammatory state. Similar observations have been reported in some COPD patients that exhibit persistent weight loss despite an increase in dietary intake (10, 11).

There is evidence to support that both of these metabolic and atrophy pathways are aberrantly regulated in COPD patients and in mice exposed to cigarette smoke by the upstream metabolic regulator, PGC-1α (22, 43, 48, 54, 57). One unique function of PGC-1α is to stimulate muscle fiber remodeling. In PGC-1α-overexpressing mice, glycolytic fibers in the plantaris muscle have been reported to transition to oxidative fibers (39, 48). Inactivation of the PGC-1α gene in mice leads to the downregulation of genes involved in cellular respiration and results in decreased mitochondrial enzyme activity and ATP production in the heart (2). Selective expression of PGC-1α in oxidative but not glycolytic fibers may be one mechanism to restrict or target this muscle atrophy and/or loss of mitochondrial function to a subset of oxidative fiber types.

In contrast the canonical p65 NF-κB subunit, which is known to coordinate the regulation of inflammatory cytokines, was not upregulated in male SP-C/TNFα+ skeletal muscle. While there is evidence in muscle cell systems acutely treated with TNFα that the PGC-1α coactivator can repress NF-κB activation (16), in our study, total NF-κB DNA binding activity was increased in female SP-C/TNFα+ skeletal muscle and PGC-1α mRNA levels were unchanged. In vivo PGC-1α-NF-κB interactions were not measured. In contrast in male SP-C/TNFα+ skeletal muscle the level of PGC-1α mRNA was decreased and NF-κB DNA binding activity unaltered. Acute pulmonary inflammation, due to LPS instillation, and TNFα treatment have been reported to lead to muscle atrophy through an NF-κB mediated ubiquitin pathway. However, this does not appear to take place in response to chronic lung TNF-α expression (24, 32, 36, 37). An in vitro study performed in C2C12 cells suggests that coordinate expression of small heat shock proteins, such as αB crystalin, and NF-κB, may potentially protect myoblasts against TNF-α cytotoxicity by enhancing anti-apoptotic factors (1).

Soleus from male, but not female, TNFα overexpressing mice also demonstrate a more rapid fatigue response, as previously reported by Zuo et al. (60). However, when fatigue was assessed in situ, in the naturally perfused gastrocnemius complex, decreased fatigue resistance was observed in both female
and male SP-C/TNFα+ male mice. Analysis of the fatigue response in situ reflects not only the contractile ability of the muscle but oxygen available to skeletal muscle mitochondria. It should be noted that this experiment was performed in anesthetized mice. Thus anesthesia could attenuate diaphragm function in both male and female mice that already present an extensive degree of emphysema and elevation in pulmonary artery pressure. Another possibility is that the gastrocnemius is altered in both females and male TNFα+ mice. However, this larger muscle type cannot be accurately tested in an in vitro preparation. Experiments were limited to the smaller soleus and EDL muscles (4). Thus, under these experimental conditions, it is likely that reduced fatigue resistance in the mixed-fiber gastrocnemius complex of both sexes is potentially influenced by circulating cytokines, oxidative stress, or possible alterations in regional blood flow, noting that lung disease per se did not reduce arterial O2 saturation during exercise.

High Circulating TNF Levels and Normal Cardiac Function

Several cardiac-targeted TNFα transgenic mice have been engineered that exhibit a concentric hypertrophic cardiac phenotype or dilated cardiomyopathy depending on whether the membrane or soluble TNFα isoforms are overexpressed (6, 14, 15, 30, 38, 49). Cardiomyocyte TNFα overexpressing mice also have high circulating TNFα levels that lead to impaired skeletal muscle contractile function, particularly in the diaphragm (6, 15, 35). In contrast, in the present study TNFα expression in alveolar epithelial cells, under the control of the SP-C promoter, and the accompanying activation of inflammatory cells, resulted in an increase in serum TNFα levels (in the same ranges as the TNFα expressed by cardiomyocytes), but alterations in left ventricle structure and function at rest and in response to a dobutamine challenge were not detected (5, 6, 30, 35, 49). These observations suggest that the effect of TNFα on overall exercise capacity is not caused by cardiac dysfunction. The relatively mild increase in right ventricular pressure that was observed in male SP-C/TNFα+ mice likely reflects a response to the pathological vascular changes occurring in the lung. Thus these data suggest that high circulating TNFα levels alone are not necessarily a good indicator of imminent heart failure. Alternatively, the cellular source and presence or absence of additional protective factors may dictate whether cardiac dysfunction develops. Potential cardioprotective factors (i.e., TNFR2, Akt, TRAF2, STAT3, heat shock proteins) may also differ in their bioavailability depending on the sex and/or age of the mouse (7, 8, 25, 51). Male cardiomyocyte TNFα overexpression mice exhibit greater TNFR signaling and lower survival rates compared with female mice express-
ing the same TNFα transgene (28). However, in the lung-targeted TNFα overexpression model, female mice are protected from diminished growth and exercise capacity. One possibility is that the small elevation in wild-type female serum TNFα levels activates the TNFR2/JAK/STAT3 survival pathway (34). However, starting at 6 mo of age survival of female SP-C/TNFα+ mice rapidly declines (17), and this outcome suggests a decrease in an age-related, yet uniden-

Fig. 7. TNFα, angiopoietin-1, PGC-1α, atrogin-1, and MuRF1 mRNA level in the soleus and EDL of WT and SP-C/TNFα+ mice. Values represent means ± SE; n = 6. Difference between the WT and SP-C/TNFα+ group of the same sex are indicated: *P < 0.05.
ttified, female cardioprotective factor. Interestingly, STAT3 knockout mice and postpartum women with low serum STAT3 levels develop dilated cardiac myopathy shortly after the stress of giving birth due to high oxidative stress and premature regression of left ventricle capillaries (26). More studies will be required to understand the balance of TNFR1 and TNFR2 signaling and sex-specific protective factors that differentially regulate muscle function and overall survival in response to high TNFα expression in the lung.

Increased TNFα and Angiopoietin Expression Within Skeletal Muscle

Both female and male soleus and EDL muscles from pulmonary TNFα overexpressing mice have enhanced skeletal muscle TNFα and angiopietin expression. While a balance or interplay between VEGF, angiopoietin 1, and angiopoietin 2 are necessary to form mature vessels during development, angiopoietin 1 is not necessary to stabilize uninjured vessels in the adult organism (27). Consistent with this finding, the number of capillaries surrounding myofibers in the soleus and EDL of adult SP-C/TNFα+ mice in the present study was not altered. These data indicate that skeletal muscle in both male and female mice are responding to pulmonary TNFα overexpression. However, the activation of pathways that protect from or signal muscle atrophy and fatigue differ depending on sex.

Limitations of the SP-C/TNFα COPD Mouse Model

TNFα is often measured as one of a set of inflammatory biomarkers in serum from COPD patients. Disease symptoms often manifest in older individuals with COPD. However, TNFα overexpression occurs early on during the lifespan of the SP-C/TNFα+ transgenic mouse line and could allow compensatory mechanisms to be initiated during development. Nevertheless, TNFα expression in several locomotor skeletal muscles from SP-C/TNFα+ mice was elevated 1.7-fold compared with the control group, and this is in the range of the study reported by Remels et al. (43), which showed that a subset of COPD patients expressing 3.9-fold higher vastus lateralis muscle TNFα mRNA levels exhibited reduced body mass. Furthermore, while the levels of serum TNFα in healthy and COPD patients varies greatly among research studies, the level expressed by the SP-C/TNFα+ mouse greatly exceeds what has been reported in patients with COPD (13, 20, 42, 55, 59).

Potential Protective Role of Estrogen

Female SP-C/TNFα+ mice showed a much milder or even no response to the elevated circulatory TNFα levels in most of the physiological parameters measured. Thus it is possible that higher steroid levels in female mice have a protective effect. Estrogen and estrogen derivatives have been reported to attenuate the effects of multiple proinflammatory factors including TNFα (29) as well as protect against pulmonary hypertension (31, 45, 56), motoneuron apoptosis (12), and immobilization-induced atrophy (50). Furthermore, healthy men supplemented with estradiol were found to increase PGC-1α expression and augment β-oxidation of lipids (40). Conversely, mice engineered with an estrogen receptor (ERα) deficiency have an impaired oxidative metabolism and inflammation that is associated with increased skeletal muscle insulin resistance and increased Hsp72 (46). The more prominent pulmonary hypertension, weight loss, and attenuated oxidative metabolism in male SP-C/TNFα+ mice compared with female mice suggest that estrogen-related pathways may partially protect female mice from the robust expression of pulmonary TNFα.

Summary

In the present study, we investigated cardiac and skeletal muscle responses to lung overexpression of TNFα in male and female mice. SP-C/TNFα+ transgenic mice have a lifelong 30- to 80-fold elevation of circulatory TNFα and severe lung damage that could contribute to exercise limitation. Male (but not female) SP-C/TNFα+ mice showed significant decreases in exercise capacity and oxidative muscle fatigue resistance accompanied by a loss of whole body weight and skeletal muscle oxidative capacity. Cardiac function was, however, preserved. Although circulating TNFα levels were far higher in our mice than in patients with COPD, this phenotype reflects the situation in a subset of COPD patients (43). In female SP-C/TNFα+ mice, with similar circulating TNFα levels and lung damage, there was maintenance of body weight and little or no skeletal muscle dysfunction. These findings suggest that estrogen may have protective effects against the actions of TNFα.

GRANTS

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


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Fig. 8. NF-κB p65 subunit DNA binding activity is increased in female SP-C/TNFα+ skeletal muscle. DNA binding activity of the p65 NF-κB subunit was detected in gastrocnemius homogenates. Values represent means ± SE; n = 6. *P < 0.001 compared with all other groups.
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