Effect of pulmonary TNF-α overexpression on mouse isolated skeletal muscle function

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Submitted 11 March 2011; accepted in final form 19 June 2011

Zuo L, Nogueira L, Hogan MC. Effect of pulmonary TNF-α overexpression on mouse isolated skeletal muscle function. Am J Physiol Regul Integr Comp Physiol 301: R1025–R1031, 2011. First published June 22, 2011; doi:10.1152/ajpregu.00126.2011.—TNF-α is a proinflammatory cytokine that is involved in numerous pathological processes including chronic obstructive pulmonary disease (COPD). In the present study, we used a transgenic mouse model that overexpresses TNF-α in the lung (Tg9251) to test the hypothesis that chronic exposure to TNF-α (as seen in COPD) reduces skeletal muscle force production and fatigue resistance, particularly under low PO2 conditions. At 7–12 mo, body and muscle weight of both extensor digitorum longus (EDL) and soleus were significantly smaller in Tg9251 compared with littermate wild-type (WT) mice; however, the body-to-muscle weight ratio was not different between groups. EDL and soleus muscles were subjected to in vitro fatiguing contractile periods under high (~550 Torr) and low PO2 (~40 Torr). Although all muscles were less fatigue-resistant during low PO2 compared with high PO2, only the soleus fatigued more rapidly in Tg9251 mice (~12%) compared with WT at high PO2. The maximal tension of EDL was equally reduced in Tg9251 mice (28–34% decrease from WT under both PO2 conditions); but for soleus this parameter was smaller only under low PO2 in Tg9251 mice (~31%) decrease from WT). The peak rate of relaxation and the peak rate of contraction were both significantly reduced in Tg9251 EDL muscles compared with WT EDL under low PO2 conditions, but not in soleus. These results demonstrate that TNF-α upregulation in the lung impairs peripheral skeletal muscle function but affects fast- and slow-twitch muscles differentially at high and low PO2.

Peripheral muscle fatigue can often significantly limit exercise capacity (15), increased protein catabolism (17), or decreased muscle contractile properties affected by TNF-α overexpression in the lung.

MATERIALS AND METHODS

Animal care and whole muscle isolation. SP-C/TNF-α transgenic mice (Tg9251) were obtained as a kind gift from Dr. Charles G. Irvin (Vermont Lung Center, University of Vermont), crossed with C57BL/6 mice, and screened by PCR analysis (15). Male mice of 7–12 mo old were used in this study. All procedures were approved by the University of California San Diego institutional animal care and use committee. Before each experiment, animals were anesthetized by intraperitoneal administration of ketamine (70 mg/kg) and xylazine (10 mg/kg). Both extensor digitorum longus (EDL; mostly composed of fast-twitch fibers) and soleus (mostly composed of slow-twitch fibers) were carefully removed from both hindlimbs and mounted in experimental chambers (model 800MS; Danish Myo Technology, Aarhus, Denmark) in the presence of Tyrode’s solution (in mM: 121 NaCl, 5 KCl, 0.4 NaH2PO4, 1.8 CaCl2, 0.5 MgCl2, 24 NaHCO3, 5.5 glucose, 0.1 EGTA) bubbled continuously with 95% O2-5% CO2 (pH 7.4, room temperature). For each muscle, the tendon located on one end of the muscle was attached to a mobile lever arm that was fatigued; contractility; fiber-type

SKELETAL MUSCLE FUNCTION can be severely affected by heart and lung disease (4, 31). These disorders develop distinct clinical symptoms; however, they all result in skeletal muscle dysfunction [e.g., decrease in submaximal and maximal force (1)]. Chronic obstructive pulmonary disease (COPD), which is associated with reduced exercise capacity and quality of life, can result in significant muscle wasting and atrophy (28). Peripheral muscle fatigue can often significantly limit exercise tolerance in COPD patients (10). It has been speculated that the sustained pulmonary inflammation may have remote effects on peripheral tissues via chronic inflammatory mediators released into the circulation in COPD patients (3, 16). Among them is TNF-α, a polypeptide cytokine that controls antitumor and immune responses, which has been implicated as a key cytokine correlated with the progression of muscular dysfunction in COPD patients (25). In fact, previous studies have shown that mice that overexpress TNF-α in the lung develop chronic pulmonary inflammation (21) and skeletal muscle wasting (15).

The main postulated mechanisms of TNF-α action on skeletal muscle have been related to decreased muscle regenerative capacity (15), increased protein catabolism (17), or decreased myofibrillar function mediated by free radical production (11, 16). It is known that reactive oxygen species (ROS) are constantly produced in skeletal muscle and that a transition to hypoxia can lead to an increased production of ROS (42). During repetitive contractions (e.g., exercise), the intracellular oxygen tension measured in humans can reach values close to anoxia [i.e., 1–3 Torr; (33)]. Furthermore, it has been shown that COPD patients can become rapidly hypoxic during exercise due to poor lung function (e.g., pulmonary emphysema) (19, 27). While the impaired contractile and performance characteristics of muscles from COPD patients or models of COPD have been documented, the causes of muscle dysfunction are unclear.

To further evaluate the effects of COPD on skeletal muscle function, we used a transgenic mouse model in which TNF-α was overexpressed in the lung (Tg9251), thereby mimicking some of the pathophysiological conditions associated with COPD (9). We used this transgenic model to test the hypothesis that chronic exposure to TNF-α negatively affects skeletal muscle force production and fatigue resistance (i.e., muscle’s ability to resist fatigue) in isolated fast- and slow-twitch muscles, particularly during low PO2 conditions. An in vitro isolated muscle model was used to avoid many of the confounding factors related to in vivo contracting muscle (i.e., cardiovascular or endocrine system), thereby allowing precise investigation of muscle contractile properties affected by TNF-α overexpression in the lung.
RESULTS

Effects of chronic production of lung TNF-α on body and skeletal muscle weight. The transgenic animals used in this study are known to contain high amounts of TNF-α in the lung (~100 pg/ml in bronchoalveolar lavage fluid) and in blood (~9 pg/ml in serum) (15). These animals showed chronic pulmonary inflammation with reduced body and muscle weights (15). As shown in Table 1, ~17% reduction of the body weight and ~12% reduction of isolated muscle weight (Table 1) were observed in TG+ mice compared with their littermate wild-type (WT) mice. However, the muscle weight-to-body weight ratio was not changed (Table 1) in TG+ mice compared with WT mice.

Contractile performance of the isolated muscles. Muscle fatigue is defined as the muscle’s inability to maintain the initial maximal force development over time, which corresponds to an exercise-induced decrease in the muscle power. In our studies, muscles (EDL and soleus) were electrically stimulated in two consecutive repetitive contractile periods until they reached the fatigue point (for details, see MATERIALS AND METHODS). A longer time to reach the fatigue point represented an increased fatigue resistance. For EDL, the low PO2 condition significantly reduced the time to fatigue within the mouse groups (TG+ vs. WT, P < 0.05, Fig. 1, A and B). However, there were no changes observed in the isometric tension developed at different time points in the contractile period (Fig. 1A). The time to fatigue (Fig. 1B) was also not changed between the groups of mice (WT and TG+) at either high or low PO2 conditions in EDL muscles. Regarding the soleus (Fig. 1, C and D), similar to EDL, the decrease in the oxygen tension (low PO2) in the chamber significantly reduced the time to fatigue in both WT and TG+ (P < 0.05, Fig. 1, C and D), compared with high PO2. Compared with the repetitive contraction protocols at high PO2 between the groups, relative tension development was significantly reduced in soleus muscle from TG+ mice during the time period between 120 s and the time at fatigue point, compared with WT mice (P < 0.05, Fig. 1C). Time to fatigue was also significantly reduced in soleus muscle from TG+ mice compared with WT mice (252 ± 8 s vs. 222 ± 14 s for WT and TG+, respectively; P < 0.05, Fig. 1D). However, there was no difference in time to fatigue under low PO2 conditions (Fig. 1, C and D) in soleus between WT and TG+ mice.

Contractile parameters during each contractile bout. To investigate whether the contractile properties of the muscles from TG+ mice were changed from control, both absolute tension development and the tension development normalized by the muscle cross-sectional area were calculated.

Table 1. Whole body and muscle weights

<table>
<thead>
<tr>
<th>Animal</th>
<th>Body Weight, g</th>
<th>Muscle Weight, mg</th>
<th>Muscle Weight/Body Weight, mg/g</th>
<th>L0, mm</th>
<th>Cross-Sectional Area, mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EDL</td>
<td>Soleus</td>
<td>EDL</td>
<td>Soleus</td>
</tr>
<tr>
<td>WT</td>
<td>35.9 ± 1.3 (8)</td>
<td>10.7 ± 0.3 (9)</td>
<td>10.5 ± 0.2 (9)</td>
<td>0.30 ± 0.01</td>
<td>0.29 ± 0.01</td>
</tr>
<tr>
<td>TG+</td>
<td>28.5 ± 0.6* (7)</td>
<td>9.3 ± 0.3* (7)</td>
<td>9.3 ± 0.5* (7)</td>
<td>0.31 ± 0.01</td>
<td>0.32 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE; number in parenthesis are the number of animals analyzed (for body wt) or number of muscles analyzed (for muscle wt). L0, muscle optimal length; EDL, extensor digitorum longus; WT, wild type; TG+, transgenic mouse model that overexpresses TNF-α in the lung. *P < 0.05 vs. WT, Student’s t-test; †P < 0.05 vs. WT, one-way ANOVA.
work period. The results were summarized in Fig. 3 (EDL) and Fig. 4 (soleus). In both figures, the contraction period was analyzed using the time to reach 90% of the peak tension from the start (T90%; Figs. 3A and 4A) and the peak rate of contraction (+dP/dt, the maximal first derivative of the force development during the contraction, Figs. 3B and 4B), the relaxation period represented by one-half relaxation time (1/2RT), the 50% of the time between the peak of the developed force and the resting state, Fig. 3C and 4C, and the peak rate of relaxation (−dP/dt, the maximal first derivative of the force development during the relaxation, Figs. 3D and 4D).

When the contractile parameters of unfatigued contractions were compared between the WT and Tg⁺ groups, only −dP/dt was significantly smaller in Tg⁺ than in WT under both PO₂ conditions in EDL, (−3,615 ± 489 vs. −4,862 ± 496 mN-mm⁻²·s⁻¹ for high PO₂ and −3,415 ± 304 vs. −4,684 ± 474 mN-mm⁻²·s⁻¹ for low PO₂, P < 0.05; Fig. 3D). Interestingly, Tg⁺ soleus did not show any difference in contractile parameters compared with WT during the contraction and relaxation period of any unfatigued contractions (Fig. 4, A–D). This was quite consistent with the isometric tension development at high PO₂ in soleus (Fig. 2B).

However, when the contractile parameters were compared between high and low PO₂ during unfatigued contractions, the EDL demonstrated significant differences under low PO₂ compared with high PO₂ conditions. In the EDL muscle from WT mice, T₀⁹₀ was significantly reduced (90 ± 5 vs. 64 ± 5 ms for high vs. low PO₂, Fig. 3A, P < 0.05) and +dP/dt was significantly increased at low PO₂ (3,481 ± 433 vs. 4,355 ± 401 mN-mm⁻²·s⁻¹ for high vs. low PO₂, P < 0.05; Fig. 3B). No change in these parameters were detected in Tg⁺ mice.

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Fig. 3. Contractile parameters of the first and the last (fatigue point) contractions during the repetitive contractile period in EDL muscle from WT (open bars) and Tg⁺ (crossed bars) mice. Time to reach 90% of the peak tension (T90%) (A); peak contraction rate (+dP/dt) (B); half relaxation time (1/2RT) (C); peak relaxation rate (-dP/dt) (D). *P < 0.05, fatigue vs. the first contraction within the same group, paired t-test; †P < 0.05, Tg⁺ vs. WT within the same group, two-way ANOVA; ‡P < 0.05 low vs. high PO2 within the same panel, two-way repeated-measures ANOVA. Data were averaged from 9 muscles of 5 mice for WT, and 7 muscles of 5 mice for Tg⁺.

In contrast to the EDL, neither T90% nor +dP/dt was changed in the soleus from WT and Tg⁺ mice at low PO2. However, during the relaxation period, only in Tg⁺ mice -dP/dt from soleus muscle was significantly reduced under low PO2 (-1,909 ± 149 vs. -1,288 ± 296 mN·mm⁻²·s⁻¹ for high vs. low PO2, P < 0.05; Fig. 4D).

The contractile properties of the last contraction (fatigue point) showed the expected differences in the contraction and relaxation periods in both groups of mice. Specifically at the fatigue point, +dP/dt was significantly decreased in both EDL and soleus from WT and Tg⁺ mice (i.e., slowing of contraction, P < 0.05; Figs. 3B and 4B). The 1/2RT was increased, while -dP/dt was decreased, slowing down the relaxation (P < 0.05; Fig. 3, C and D for EDL and Fig. 4, C and D for soleus). Regarding contractile properties, no significant differences between WT and Tg⁺ mice (Figs. 3 and 4) were found at the fatigue point in both EDL and soleus muscles.

DISCUSSION

Our study showed that chronic production of TNF-α in the lung resulted in specific fiber-type-related muscle dysfunctions. Particularly, the maximal isometric tension development was decreased in muscle primarily composed of fast-twitch fibers (EDL), and fatigue resistance was reduced in muscle primarily composed of slow-twitch fibers (soleus).

Morphological effects. TNF-α upregulation in the lung reduced body and muscle weight (Table 1), which agreed with a previous report using the same mouse model (15). Although atrophy is characterized by a reduced muscle weight compared with overall body weight (40), there was no difference in the ratio of muscle to body weight between groups (Table 1), which suggests that the TNF-α effects were likely not related to the muscle atrophy per se. In other studies, the level of other cytokines including interleukin IL-6, interferon-γ, and TGF-β remained the same in some skeletal muscles (i.e., quadriceps) from patients with COPD compared with control (3), while in other skeletal muscles (i.e., intercostal muscles) from COPD patients, the expression of cytokines, including IL-6 and -1β, were significantly increased (5). However, the level of TNF-α seemed to be more responsive to COPD conditions and was significantly changed in both quadriceps and intercostal muscles from those patients (3, 5). Thus, although other inflammatory cytokines may be produced in Tg⁺ mice, the major factor that was changed in our transgenic mouse model was the

Fig. 4. Contractile parameters of the first and the last (fatigue point) contractions during the repetitive contractile period in soleus muscle from WT (open bars) and Tg⁺ (crossed bars) mice. Time to reach 90% of the peak tension (T90%) (A); peak contraction rate (+dP/dt) (B); half relaxation time (1/2RT) (C); peak relaxation rate (-dP/dt) (D). *P < 0.05, fatigue vs. the first contraction within the same group, paired t-test; †P < 0.05 low vs. high PO2 within the same panel, two-way repeated-measures ANOVA. Data were averaged from 9 muscles of 5 mice for WT, and 7 muscles of 5 mice for Tg⁺.
chronic elevation of TNF-α, so that the pathophysiological effects noted in the present study on skeletal muscle function were likely most dependent on the TNF-α elevation. Additionally, these animals had a life-long elevated TNF-α production, which could cause adaptations of other tissues as well. In fact, this lifelong TNF-α overexpression is different from normal COPD progression. However, the effect of this chronic transgenic overexpression of TNF-α on skeletal muscle function should be similar to the effect of elevated levels of TNF-α seen in many COPD patients. In our model, both body and muscle mass were reduced in lung-specific Tg+ mice (Table 1). In other similar models, research showed that there were no morphological differences between cardiac-specific Tg+ mice and WT littermates (16). However, these cardiac-specific Tg+ mice were 5 to 10 mo younger than the lung-specific Tg+ animals used in the present investigation. Therefore, the reduced mice size and function in the present model could be a result of a longer period of TNF-α exposure.

Skeletal muscle performance. Skeletal muscle contains fast-twitch (glycolytic) and slow-twitch (oxidative) fibers. The mouse EDL and soleus were representatives of fast- and slow-twitch muscles, respectively (6, 22, 29). Our data suggested that EDL from Tg+ had a similar fatigue resistance indicated by time to fatigue, compared with WT under both PO2 conditions (Fig. 1, A and B). However, the soleus from Tg+ was significantly less fatigue resistant (~12%) compared with WT, but only under high PO2 conditions (Fig. 1, C and D). This was likely because in slow-twitch fibers, the mitochondrial volume and density are fundamentally more important during repetitive contractions than in fast-twitch fibers (18). Furthermore, it has been shown that either mice or cultured skeletal muscle cells (i.e., C2C12) exposed chronically to TNF-α, have decreased mitochondrial protein content and biogenesis (32, 37). It is highly likely that the decreased fatigue resistance of Tg+ soleus under high PO2 in the present study was a result of a reduced aerobic capacity caused by chronic exposure to TNF-α present in the blood, which was not an important component of fatigue in a fast-twitch muscle (i.e., EDL) (6, 22, 29).

To mimic a hypoxic environment that would occur during exercise in COPD patients (34), the external PO2 in our setup was decreased to ~40 Torr prior to the contractile periods; however, the core of the muscle underwent much more hypoxic conditions during contractions (2). Thus, our data demonstrate that both EDL and soleus were less fatigue resistant under low PO2 compared with high PO2 conditions (Fig. 1). There was no difference in the time to fatigue between WT and Tg+ at low PO2 in both EDL and soleus (Fig. 1, B and D), since the mitochondria activity was likely highly restricted in such conditions (12, 36). These results strengthened the point that chronic TNF-α exposure affected fatigue in the slow-twitch muscle by decreasing the aerobic capacity.

Contractile differences. The effects of acute and chronic treatments of TNF-α on skeletal muscle function are contradictory in the literature (16, 31). While isolated EDL and soleus were not affected in mice that overexpressed TNF-α in cardiac muscle (16), acute exposure of exogenous TNF-α reduced the tension in single fast-twitch fibers (31), similar to the present investigation [i.e., 30% (Fig. 2, A and C) compared with 20% as reported (31)]. This acute effect resulted in a substantial decrease in either myosin ATPase activity or thin filament Ca2+ sensitivity (31). This could involve the generation of nitric oxide (NO) and ROS. Under our protocol, the 60-min rest period did not cause any substantial decline in tension over time, i.e., 60-min under 95% O2 bubbling at room temperature was sufficient for a full functional recovery in both EDL and soleus. In our model under both PO2 conditions, we observed a decreased tension in Tg+ EDL (Fig. 2, A and C) and a decreased −dP/dt (Fig. 3D), but neither T0% nor +dP/dt was changed (Fig. 3, A and B). This indicated that the rate of maximal tension development was not changed in Tg+ EDL under both PO2 conditions. The decreased −dP/dt under both PO2 conditions could relate to the reduced cross bridge dissociation rate or reduced Ca2+ uptake rate (26, 39). While both absolute and normalized maximal tensions were decreased in Tg+ EDL under both PO2 conditions (Fig. 2, A and C), it is likely that Ca2+ uptake rate could not be reduced (39). Thus, the reduced tension of Tg+ EDL was associated with a decreased cross bridge dissociation rate, which could be affected by either NO or ROS as shown in the literature (14, 24, 38, 39). These results suggested that chronic production of TNF-α likely affected the cross bridge dissociation rate of Tg+ EDL under both PO2 conditions, consistent with the hypothesis by Reid et al. (31) that TNF-α negatively affected either myosin ATPase activity or the thin-filament Ca2+ kinetics.

Furthermore, since the −dP/dt in Tg+ EDL was decreased by a similar level under both PO2 conditions compared with WT EDL, the present results suggested that fast-twitch muscles from Tg+ mice were not more susceptible to low PO2. Fast-twitch muscle preferentially utilizes glycolytic metabolism during a short period of contractions. The EDL maximal tension from Tg+ and WT was not changed at low PO2 for either WT or Tg+ mice (Fig. 2, A and C). These results agreed with a previous study on isolated dog muscles at different PO2 conditions (13). Interestingly, the rate of tension development was increased (i.e., decreased in T0% and increased +dP/dt; P < 0.05 in Fig. 3, A and B) at low PO2 compared with high PO2 in WT EDL. Previous research has shown that low PO2 increases Ca2+ release from the SR through ROS production (7, 8). It is possible that this increased rate of tension development could be a response of increased ROS level during hypoxia (42).

For soleus, unlike EDL, the maximal tension and the contractile parameters were not significantly different between WT and Tg+ mice at both high and low PO2 conditions (Figs. 2B and Fig. 4). These results suggested that TNF-α did not affect either excitation-contraction coupling or myofilament activation in a slow-twitch muscle. However, the normalized tension was reduced in Tg+ soleus compared with WT at low PO2 (Fig. 2D). The only contractile difference between high and low PO2 was the reduced −dP/dt. These data suggested that slow-twitch muscles from Tg+ mice had the similar contractile dysfunction to EDL, but this only occurred under hypoxia.

Perspectives and Significance

We used a transgenic model of pulmonary TNF-α overexpression (Tg+) mice, which mimics the conditions in some COPD patients (9), to study skeletal muscle contractile function and fatigue. These mice chronically produced TNF-α in their lungs, leading to a marked pulmonary inflammation (15).
The results of the present study demonstrated distinct effects of TNF-α overexpression on muscles of different fiber-type composition at both high and low PO₂. Muscle composed of primarily slow-twitch fibers were principally less fatigue resistant, while muscle composed primarily of fast-twitch fibers demonstrated more contractile dysfunction. These results may have important implications for understanding complications in peripheral skeletal muscles from patients with COPD.

ACKNOWLEDGMENTS

We acknowledge the assistance of Harrieth Wagner for conducting animal breeding and PCR analysis of the transgenic mice. We thank Drs. Peter Wagner and Ellen Breen for research support.

GRANTS

This research was supported by National Heart, Lung, and Blood Institute Grant PPG-HL-091830.

DISCLOSURES

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

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