ORIGINAL ARTICLES

Molecular and physiological events in respiratory muscles and blood of rats exposed to inspiratory threshold loading

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High-intensity exercise induces oxidative stress and inflammatory events in muscles. Tumor necrosis factor (TNF)-α may alter muscle protein metabolism or promote muscle regeneration. We hypothesized that a program of noninvasive chronic inspiratory loading of different intensities induces a differential pattern of physiological, molecular, and cellular events within rat diaphragms. Antioxidants and TNF-α blockade may influence those events. In the diaphragm, gastrocnemius, and blood of rats exposed to high-intensity inspiratory threshold loads (2 hour every 24 hours for 14 days), with and without treatment with N-acetyl cysteine or infliximab (anti-TNF-α antibody), inflammatory cells and cytokines, superoxide anion production, myogenesis markers, and muscle structure were explored. In all animals, maximum inspiratory pressure (MIP) and body weight were determined. High-intensity inspiratory loading for 2 weeks caused a decline in MIP and body weight, and in the diaphragm induced a reduction in fast-twitch fiber proportions and sizes, whereas inflammatory cells and cytokine levels, including TNF-α immunohistochemical expression, superoxide anion, internal nuclei counts, and markers of myogenesis were increased. Blockade of TNF-α improved respiratory muscle function and structure, and animal weight, and, in the diaphragm, reduced inflammatory cell numbers and superoxide anion production drastically while inducing larger increases in protein and messenger RNA levels and immunohistochemical expression of TNF-α, internal nuclei, and markers of muscle regeneration. Blunting of TNF-α also induced a reduction in blood inflammatory cytokines and superoxide anion production. We conclude that TNF-α synthesized by inflammatory cells or myofibers could have differential effects on muscle structure and function in response to chronic, noninvasive, high-intensity inspiratory threshold loading. (Translational Research 2014;163:478–493)

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Abbreviations: COPD = chronic obstructive pulmonary disease; ELISA = enzyme-linked immunosorbent assay; IL = interleukin; m-cadherin = muscle calcium-dependent cell adhesion; MIP = maximal inspiratory pressure; mRNA = messenger RNA; myf-6 = myogenic factor 6; MyHC = myosin heavy chain; NAC = N-acetylcysteine; TNF = Tumor necrosis factor

AT A GLANCE COMMENTARY

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Background

Chronic noninvasive inspiratory loading of different intensities induces a differential pattern of physiological, molecular, and cellular events within rat diaphragms, eventually influenced by antioxidants and tumor necrosis factor (TNF)-α blockade.

Translational Significance

Chronic, noninvasive, high-intensity inspiratory threshold loading reduced respiratory muscle function and body weight, altered muscle structure, and increased levels of inflammatory cells, cytokines, superoxide anions, internal nuclei, and myogenesis markers. TNF-α blockade improved respiratory muscle function and structure, and animal weight, and reduced inflammatory cell numbers and superoxide anions, while increasing TNF-α levels and muscle regeneration in diaphragms. These findings have therapeutic implications in exercise training of chronic respiratory patients with muscle dysfunction.

Skeletal muscle dysfunction, which is a major systemic manifestation in highly prevalent conditions such as chronic obstructive pulmonary disease (COPD), has relevant implications in patients' exercise capacity and quality of life.[1,2] General exercise and inspiratory muscle training have been shown to exert beneficial effects on clinical outcomes such as respiratory muscle performance and underlying structure among patients with severe COPD.[3-9] Nonetheless, other studies have also shown an increase in oxidative stress markers[10] and proinflammatory cytokines[11] in response to the administration of high-intensity inspiratory loads in animal models. In fact, strong muscle contractions leads to enhanced oxidant production,[12,13] which may result in the development of oxidative stress.[10] These are relevant findings, because patients with COPD already exhibit a greater production of oxidants in their diaphragms at rest.[14-16]

Inflammatory makers such as the pleiotropic cytokine tumor necrosis factor (TNF)-α, has long been considered to exert catabolic actions in muscles and to induce contractile dysfunction in chronic inflammatory conditions including cancer and COPD.[17] Furthermore, TNF-α was also shown to block protein synthesis, to enhance protein breakdown,[18] and to inhibit myogenesis through several mechanisms in *in vitro* studies.[19,20] Nevertheless, TNF-α is also involved in other processes such as muscle repair and regeneration,[21] growth,[22] and differentiation,[23] as well as in the delay of muscle proteolysis in dystrophic mice.[24] Other proinflammatory cytokines such as interleukin (IL)-6 and IL-1 seem to participate in muscle metabolism in contracting muscles. As such, muscle messenger RNA (mRNA) levels of IL-6 were shown to increase as early as 30 minutes after exercise and to reach their peak at the end of the exercise bout.[25] In another relevant investigation, high inspiratory loads were also shown to induce an increase in the synthesis of the cytokines TNF-α, IL-6, and IL-1 in the rat diaphragm.[11] More important, in humans, exercise of moderate to high intensity also enhanced the production of IL-6 and its release into the bloodstream.[26,27]

On the other hand, the contractile dysfunction induced by oxidants in the diaphragms of dogs exposed to several degrees of inspiratory resistive breathing[10] and in septic rats[28] was attenuated in response to antioxidant treatment with N-acetyl cysteine (NAC). Nevertheless, whether inspiratory threshold loading of high intensity may induce deleterious molecular events that could counteract the potential beneficial effects of chronic exercise on muscle structure and function should still be explored further. In addition, identification of whether cytokine release from the contracting muscles may be associated with muscle regeneration also needs to be elucidated. Moreover, the potential beneficial effects of blocking the actions of oxidants or proinflammatory cytokines (eg, TNF-α) within the contracting muscles also remain unidentified.

On this basis, we hypothesized that a program of chronic, noninvasive inspiratory threshold loading of different intensities would induce a differential pattern of physiological, molecular, and cellular events within the diaphragm myofibers of rats at the end of the study period. Furthermore, we also attempted to assess whether concomitant treatment of the animals with the antioxidant NAC or the anti-TNF-α antibody infliximab may interfere differentially with such responses. Accordingly, the study objectives were to determine (1) whole body weight and respiratory muscle function (force), (2) diaphragm levels of inflammatory and regeneration markers (cell proliferation and differentiation),