Antianabolic Effects of Hypercapnia: No Country for Strong Men

Elevated levels of CO_2 (hypercapnia) are often observed in acute or chronic lung diseases, such as acute respiratory distress syndrome, chronic obstructive pulmonary disease (COPD), and cystic fibrosis, and reflect the effects of alveolar hypoventilation and altered gas exchange (1). In recent years, it has become increasingly evident that high CO_2 levels contribute to pulmonary disease states, and that hypercapnia is an independent risk factor and driver of poor outcomes in patients with the above-mentioned diseases (2–4). We now know that levels of CO_2 are sensed by various nonexcitable cells via an as-yet-unknown mechanism, leading to the activation of highly specific signaling cascades (5). Importantly, the primarily detrimental effects of hypercapnia are not limited to the lungs, where high CO_2 levels impair alveolar epithelial and bronchial airway function. In systemic tissues, high CO_2 levels also negatively affect cell proliferation, repair mechanisms, innate immunity, and skeletal muscle function (6–10). Loss of skeletal muscle mass and function, which is often observed in patients with COPD, correlates with increased morbidity and mortality in chronically ill patients (11). Thus, understanding the mechanisms by which CO_2 retention contributes to skeletal muscle wasting is not only interesting as a biological phenomenon, it is also important clinically, as interfering with these events may improve outcomes for hypercapnic patients with COPD.

In this issue of the *Journal*, Korponay and colleagues (pp. 74–86) convincingly demonstrate that elevated levels of CO_2 decrease protein anabolism in skeletal muscles by decreasing ribosomal biogenesis (12). By performing a pilot study in human quadriceps muscle biopsies from normo- and hypercapnic patients with a history of lung disease, the authors identified a marked reduction of ribosomal 45S pre-RNA in muscles from the hypercapnic patient group, suggesting decreased protein translation. In subsequent experiments, mice were exposed to 10% CO_2 (normoxic hypercapnia) or room air (normoxic normocapnia) for 60 days, and the *extensor digitorum longus* muscle was processed for an unbiased proteomic study. In line with the initial findings in the human cohort, the authors found a marked downregulation of several components of translation initiation in hypercapnic animals accompanied by a downregulation of "structural constituent of ribosome," as suggested by ontology enrichment analysis. Further *in vivo* and *in vitro* studies showed that protein synthesis in muscle fibers and cultured myotubes was significantly reduced during sustained hypercapnic exposure, as assessed by puromycin incorporation. Puromycin is an aminonucleoside antibiotic and a structural analog of aminoacyl-tRNA, and as such can be incorporated into elongating peptide chains via the formation of a peptide bond (13). Thus, the rate at which puromycin-labeled peptides are formed reflects the overall rate of protein synthesis.

Skeletal muscle wasting is a hallmark of various lung diseases and particularly of COPD; however, the nature of the mechanisms that drive muscle loss remains a topic of intense debate. Although it is evident that immobility associated with the disease leads to muscle wasting that is in part reversible with physical exercise, recent evidence shows that more specific mechanisms may cause a distinct COPD myopathy (11). Generally, muscle wasting could be a consequence of activated catabolic functions, such as proteasomal degradation and autophagy, or inhibited anabolic pathways. Korponay and colleagues demonstrate antianabolic effects of hypercapnia in skeletal muscle that are driven by AMP-activated protein kinase α2 (AMPKα2). This is of particular interest because AMPKα2 also promotes myotube degradation during elevated CO_2 by activating the ubiquitin proteasome system via the E3-ubiquitin ligase MuRF1 (muscle-specific Ring finger protein 1), which directly targets the myosin heavy chain (14). Interestingly, these catabolic effects of hypercapnia appear to be activated earlier than the antianabolic ones, as a previous study showed that ubiquitination-driven muscle degradation was evident after mice were exposed to 21 days of hypercapnia (14). However, the current study shows that this time course is not sufficient to downregulate protein synthesis that is evident after 60 days of hypercapnic exposure. Of note, it was recently shown that autophagy-driven degradation is also significantly enhanced in locomotor muscles of patients with COPD, which is mediated by AMPK as well (15). Thus, hypercapnia drives both antianabolic and catabolic skeletal muscle wasting by at least two (potentially three) distinct mechanisms. Further research is needed to confirm the involvement of autophagy in CO_2-induced muscle loss and to identify the relative contributions of these mechanisms to COPD-associated myopathy.

Although hypercapnia induces AMPKα2 in myotubes, it activates AMPKα1 in the lung epithelium, where the kinase markedly downregulates the Na,K-ATPase and thereby impairs alveolar epithelial function (9), further highlighting the specificity of the CO_2-induced pathways. It is well known that AMPK, a metabolic sensor, is rapidly activated upon cellular stress or starvation, leading to inhibition of energy-demanding (anabolic) pathways and upregulation of catabolic processes to generate ATP (16). Because muscle fibers require a considerable amount of energy and the Na,K-ATPase accounts for ∼40% of the energy needs of a resting cell, it seems logical that to survive, cells exposed to hypercapnia (mal)adapt by downregulating these energy-demanding processes. This may also explain why the hypercapnia-induced downregulation of protein translation was found to be muscle-fiber-type specific and more pronounced in type IIa and IIb/x fibers than in type I fibers

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in the current study. Skeletal muscles exhibit significant variability depending on their biochemical, mechanical, and metabolic demands. Although it may be an oversimplification, fast-twitch type II fibers generally contract more powerfully and rapidly than type I fibers, and thus require high energy on demand. Clearly, further studies are warranted to tease out the molecular mechanisms responsible for this specificity. To achieve that goal, it will be necessary to precisely characterize the downstream targets of AMPKα2 in the context of hypercapnia. Korponay and colleagues suggest that mTOR (mammalian target of rapamycin) is probably not involved in the hypercapnia-induced and AMPK-driven downregulation of protein synthesis. However, it is well documented that mTOR complex 1 is directly inhibited by AMPK, leading to inhibition of protein synthesis (17). mTOR is required for initiation of translation through its phosphorylation of substrates such as eukaryotic initiation factor (eIF) 4E binding protein 1 (4E-BP1) and p70 ribosomal protein S6 kinase. AMPK may also downregulate protein synthesis by inhibiting Raptor (regulatory-associated protein of mTOR), blocking ribosomal biogenesis through inhibition of TIF-1A (transcription intermediary factor 1 α) and activating eEF2K (eukaryotic elongation factor 2 kinase), thereby blocking the elongation process by phosphorylating eEF2. Thus, the potential involvement of mTOR in the above-mentioned mechanisms may require future research. Similarly, other AMPKα2-dependent but mTOR-independent regulators of protein synthesis will need to be investigated. Finally, as growing evidence suggests that the metabolic activity of cells impacts chromatin modifications and genome accessibility by inducing methylation, acetylation, phosphorylation, ubiquitination, or SUMOylation of histones (18), further research needs to focus on the potential effects of elevated CO_2 levels on these mechanisms in the context of skeletal muscle anabolism.

The findings of Korponay and colleagues are timely and important, and foster novel research that may ultimately lead to selective interventions against the maladaptive cellular responses to hypercapnia. The current study clearly shows that, as in the lung, hypercapnia is a nonpermissive environment for skeletal muscle—no country for strong men, indeed. ■

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