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Restrictions in ATP diffusion within sarcomeres can provoke ATP-depleted zones impairing exercise capacity in chronic obstructive pulmonary disease

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ABSTRACT

Background: Chronic obstructive pulmonary disease (COPD) is characterized by the inability of patients to sustain a high level of ventilation resulting in perceived exertional discomfort and limited exercise capacity of leg muscles at average intracellular ATP levels sufficient to support contractility.
Methods: Myosin ATPase activity in biopsy samples from healthy and COPD individuals was implemented as a local nucleotide sensor to determine ATP diffusion coefficients within myofibrils. Ergometric parameters clinically measured during maximal exercise tests in both groups were used to define the rates of myosin ATPase reaction and aerobic ATP re-synthesis. The obtained parameters in combination with AK- and CK-catalyzed reactions were implemented to compute the kinetic and steady-state spatial ATP distributions within control and COPD sarcomeres.
Results: The developed reaction–diffusion model of two-dimensional sarcomeric space identified similar, yet extremely low nucleotide diffusion in normal and COPD myofibrils. The corresponding spatio-temporal ATP distributions, constructed during imposed exercise, predicted in COPD sarcomeres a depletion of ATP in the zones of overlap between actin and myosin filaments along the center axis at average cytosolic ATP levels similar to healthy muscles.
Conclusions: ATP-depleted zones can induce rigor tension foci impairing muscle contraction and increase a risk for sarcomere damages. Thus, intra-sarcomeric diffusion restrictions at limited aerobic ATP re-synthesis can be an additional risk factor contributing to the muscle contractile deficiency experienced by COPD patients.
General significance: This study demonstrates how restricted substrate mobility within a cellular organelle can provoke an energy imbalance state paradoxically occurring at abounding average metabolic resources.

Abbreviations: AK, adenylate kinase; *CK*, creatine kinase; *PK*, pyruvate kinase; *Cr*, creatine; *PCr*, phosphocreatine; FEV_1, forced expiratory volume in 1 s; *FVC*, forced vital capacity; D_{LCO}, diffusing capacity of the lung for carbone monoxide; PaO_2, arterial partial pressure of oxygen; $PaCO_2$, arterial partial pressure of carbone dioxide; sO_2, oxygen saturation of hemoglobin; VO_2, oxygen consumption rate; VO_{2max}, maximal oxygen consumption rate; *r*, coordinate along the radius, from a boundary to the central axis, of a sarcomere; *x*, coordinate along the longitudinal axis, from Z- to M-line, of a sarcomere; *D*, isotropic diffusion coefficient for metabolites; D_r, coefficient for metabolite diffusion along the radius of a sarcomere; D_x, coefficient for metabolite diffusion in the longitudinal direction of a sarcomere; K_m^*, Michaelis constant of ATPase reaction measured in permeabilized myocytes; V_{max}^*, maximal rate of ATPase reaction in permeabilized myocytes used for calculation of apparent diffusion coefficients *D*; K_m^{ATP}, michaelis constant of ATPase reaction in the reaction–diffusion model; $V_{O_2}^{ADP}$, maximal rate of ADP-induced mitochondria respiration; K_m^{ADP}, Michaelis constant for ADP reflecting the half-maximal rate of mitochondria respiration; $V_{ATPase}^{max}(x)$, maximal rate of ATPase reaction in a sarcomere; V_{ATPase}^0, maximal rate of ATPase reaction; $\langle V_{ATPase}\rangle$, average sarcomeric ATPase activity; $\langle V_{ATPase}^{exp}\rangle$, experimentally estimated myofibrillar ATPase (mATPase) activity; V_{syn}, rate of ATP re-synthesis normalized to a sarcomere; V_{syn}^{max}, maximal rate or capacity of ATP re-synthesis; V_{syn}^a, aerobic ATP re-synthesis during endurance cycle ergometry calculated based on VO_{2max}, VO_2 at rest, and muscle mass; $V_{AK}(r,x)$, AK reaction rate in a sarcomere; $V_{CK}(r,x)$, CK reaction rate in a sarcomere.

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1. Introduction

Impaired production of ATP, as well as its defective communication, utilization or sensing can lead to inefficient operation of skeletal or cardiac muscles, and when persistent may precipitate the development of myopathic processes and maladaptive remodeling [1–7]. However, even under significant mismatch between ATP availability/production and consumption, critical impairment of contractile functions occurs at average ATP levels that appear to be sufficient to maintain contractility. For instance, significant cyanide-induced metabolic poisoning, after glycogen depletion, impaired muscle operation at resulting average cytosolic ATP levels that are just 20% below normal concentrations, estimated to be 8–10 mM [8,9], accompanied by shortening of action potentials sensitive to ATP deficit [10–12]. The implied functional ATP deficiency at high average intracellular ATP levels could be rationalized by the heterogeneity of the intracellular milieu consisting of biomembranes and structural protein complexes. Theoretical and computational studies revealed that this intracellular compartmentation results in diffusion restrictions limiting metabolite mobility [13–15]. Specifically, the intracellular diffusion barriers impose strong intracellular ATP gradients, such that local rather than average cytosolic ATP levels would determine the rate of ATPase reactions, as could occur with actomyosin contractile complexes within sarcomeres [16].

By assessing ATP gradients between bulk space and particular ATP-consuming/ATP-sensing sites, numerous *in vivo* and *in vitro* studies have suggested the presence of strong diffusion barriers within intracellular microdomains where residing ATPases set local ATP concentrations below cytosolic levels [16–22]. Specifically, the apparent diffusion coefficient within isolated rat myofibrils has been estimated at 0.1 $\mu m^2/s$ [16], which is ~3000 times lower than the free ATP diffusion in the cytosol or in aqueous phase [23]. Within sarcomeres, in vivo ^{31}P magnetic resonance spectroscopy revealed extremely low nucleotide mobility [24], which based on the atomic model of myosin complexes [25] could be associated with electrostatic interaction of ATP molecules with charged groups of surrounding proteins and/or by the tightly packed actomyosin filaments that can impede ATP accessibility to ATPase sites (Fig. 1A). Hence, analysis of muscle energetics and intra-sarcomeric diffusion coefficients that in combination would define spatial distributions of adenine nucleotides within sarcomeres can shed new light on impaired muscle function in the presence of sufficient intracellular energy resources observed in a number of metabolic disorders.

Herein we hypothesized that the limited intra-sarcomeric ATP diffusion, at a mismatch between the rates of myosin ATPase and aerobic ATP re-syntheses, is an additional risk factor capable of contributing to the locomotor muscle contractile deficiency in chronic obstructive pulmonary disease (COPD). COPD patients, due to their inability to sustain a high level of ventilation, are prone to metabolic stress in skeletal muscles leading to maladaptive remodeling [26,27]. Under imposed workloads, COPD is characterized by the failure of aerobic ATP re-synthesis resulting in perceived exertional discomfort and overall limited exercise capacity of leg muscles [27–31]. However, in patients with moderate to

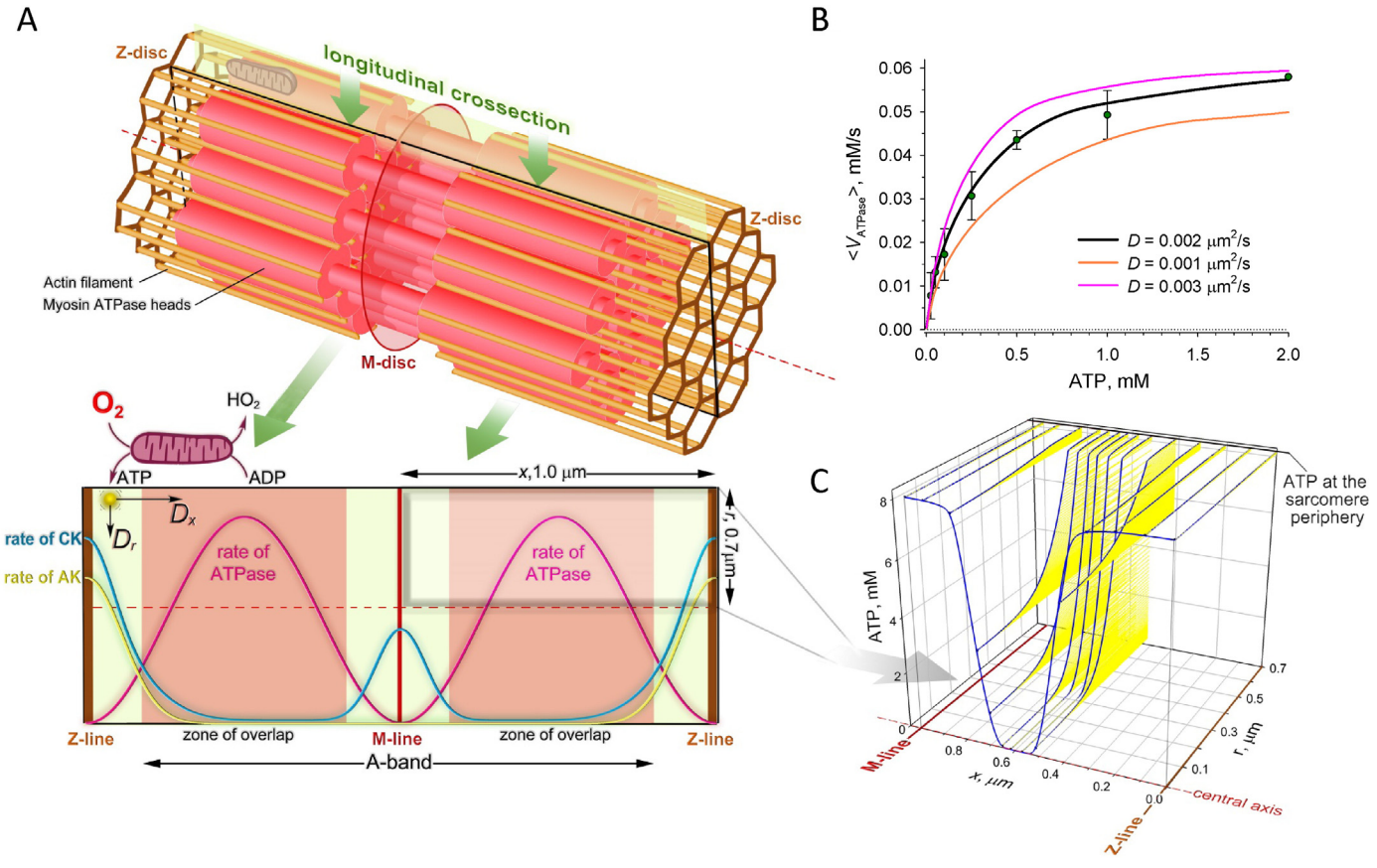

Fig. 1. Sarcomere structure and inner diffusion restrictions. A: Scheme illustrating the localization of ATPase, AK and CK activities within a sarcomere used for simulation by the reaction–diffusion model. The magnifying inset denotes a quarter of a sarcomere where spatial distributions of ATP were constructed. B: The experimentally measured rates of ATPase reaction in permeabilized control and COPD human myocytes were recalculated to the average sarcomeric ATPase activity $\langle V^{exp}_{ATPase} \rangle$ (see Materials and methods), averaged (data points are mean ± SD), and fitted using the reaction–diffusion model assuming isotropic diffusion of metabolites, i.e. $D = D_r = D_x$. The best fit at $D = 0.002$ $\mu m^2/s$ ($\chi^2 = 0.83$), as well as predicted ATP-dependent ATPase activities at different D values are shown for comparison. C: Spatial steady-state ATP distributions under isotropic substrate diffusion at $D = 0.002$ $\mu m^2/s$ are shown for a quarter of sarcomeres (inset in A:) from Z-line to M-line and from the periphery to the central axis, as indicated. ATP concentration outside of a sarcomere was assumed at 8.2 mM.

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