Journal of the American Heart Association

CONTEMPORARY REVIEW

Skeletal Muscle Pathology in Pulmonary Arterial Hypertension and Its Contribution to Exercise Intolerance

Thaís C. F. Menezes, MD, PhD; Michael H. Lee, MD; Dara C. Fonseca Balladares, BSc; Kevin Nolan, BSc; Sankalp Sharma, BSc; Rahul Kumar, PhD; Eloara V. M. Ferreira, MD, PhD; Brian B. Graham, MD* Rudolf K. F. Oliveira, MD, PhD*

ABSTRACT: Pulmonary arterial hypertension is a disease of the pulmonary vasculature, resulting in elevated pressure in the pulmonary arteries and disrupting the physiological coordination between the right heart and the pulmonary circulation. Exercise intolerance is one of the primary symptons of pulmonary arterial hypertension, significantly impacting the quality of life. The pathophysiology of exercise intolerance in pulmonary arterial hypertension is complex and likely multifactorial. Although the significance of right ventricle impairment and perfusion/ventilation mismatch is widely acknowledged, recent studies suggest pathophysiology of the skeletal muscle contributes to reduced exercise capacity in pulmonary arterial hypertension, a concept explored herein.

Key Words: exercise intolerance ■ mitochondrial dysfunction ■ oxygen pathway ■ pulmonary hypertension ■ skeletal muscle dysfunction

Pulmonary arterial hypertension (PAH) is a hemodynamic condition resulting from pulmonary vascular dysfunction and increased pressure in the pulmonary arteries bed, leading to reduced physiological adaptation between the right heart and the pulmonary circulation.[1,2] The diagnosis of PAH requires clinical assessment including hemodynamic evaluation through right heart catheterization. PAH is characterized as precapillary disease, defined by a mean pulmonary artery pressure that exceeds 20 mm Hg, increased pulmonary vascular resistance >2 Wood units, and normal pulmonary artery wedge pressure.[1]

The cause of PAH is determined based on an extensive clinical and epidemiological evaluation.[1] The causes leading to PAH primarily involve the pulmonary vasculature, including schistosomiasis, HIV, connective tissues diseases (CTD), and drug and toxin-induced cases,[1] for example. Idiopathic PAH is a prototypical form that affects the pulmonary arteries. Exercise intolerance is generally the main symptom in those with PAH, significantly affecting quality of life.[2,3] Untreated, the median survival is ~2.5 years.[4]

The pathophysiology of exercise intolerance in PAH is complex and likely multifactorial. The role of central cardiac and pulmonary vascular components is well established, with decreased cardiac output especially during exercise.[2,5,6] An increase in right ventricular afterload resulting from pulmonary vascular remodeling leads to ventricle-arterial uncoupling, metabolic impairment, and progression to right heart dysfunction. Additionally, pulmonary vascular remodeling causes an imbalance between pulmonary ventilation and perfusion, leading to increased dead space. As a result, there is significant ventilation-perfusion heterogeneity, which contributes to an inefficient ventilatory response during exercise, along with increased central

Correspondence to: Rudolf K. F. Oliveira, MD, PhD, Division of Respiratory Diseases, Department of Medicine, Hospital São Paulo, Federal University of São Paulo, Rua Napoleão de Barros, 771, 3° andar, CEP 04024-002 São Paulo, SP, Brazil. Email: rudolf.oliveira@unifesp.br

*B. B. Graham and R. K. F. Oliveira contributed equally.

This article was sent to Sébastien Bonnet, PhD, Guest Editor, for review by expert referees, editorial decision, and final disposition.

For Sources of Funding and Disclosures, see page 9.

JAHA is available at: www.ahajournals.org/journal/jaha

Nonstandard Abbreviations and Acronyms

CTD	connective tissue disease
CTD-PAH	connective tissue disease-associated pulmonary arterial hypertension
LOXL2	lysyl oxidase homolog 2
MuRF	muscle RING Finger
PAH	pulmonary arterial hypertension
PAWP	pulmonary artery wedge pressure
SSc	systemic sclerosis
VO_2	oxygen uptake

chemoreflex activity, and respiratory muscle impairment.[5,6] Although the central cardiac and respiratory mechanisms are well established, recent evidence suggests that peripheral factors, particularly skeletal muscle abnormalities, likely also contribute significantly to exercise intolerance in PAH[3,6–10] (Figure 1).

This review aims to explore the multifactorial nature of exercise intolerance in PAH, with a particular emphasis on emerging evidence regarding the role of skeletal muscle dysfunction in exercise intolerance. To achieve this, we cover several key topics including normal exercise physiology; skeletal muscle dysfunction in PAH with a focus on vascular, morphological, and mitochondrial abnormalities; skeletal muscle dysfunction in connective tissue disease-associated PAH (CTD-PAH) specifically; and finally, conclude with summarizing the existing knowledge gaps and potential future directions in this area of research.

NORMAL EXERCISE PHYSIOLOGY

Exercise requires coordination across multiple organ systems and reflects the body's ability to maintain a balance between energy production and consumption. From daily activities to intense exertion, exercise demands ATP regenerated from ADP through either oxygen-dependent (oxidative phosphorylation) or oxygen-independent pathways.[7,11,12] Aerobic metabolism (oxygen-dependent) is more efficient and predominates during light to moderate intensity exercises.[7,13,14] Oxygen consumption increases during exercise.[11,13,15] When the oxygen supply is less

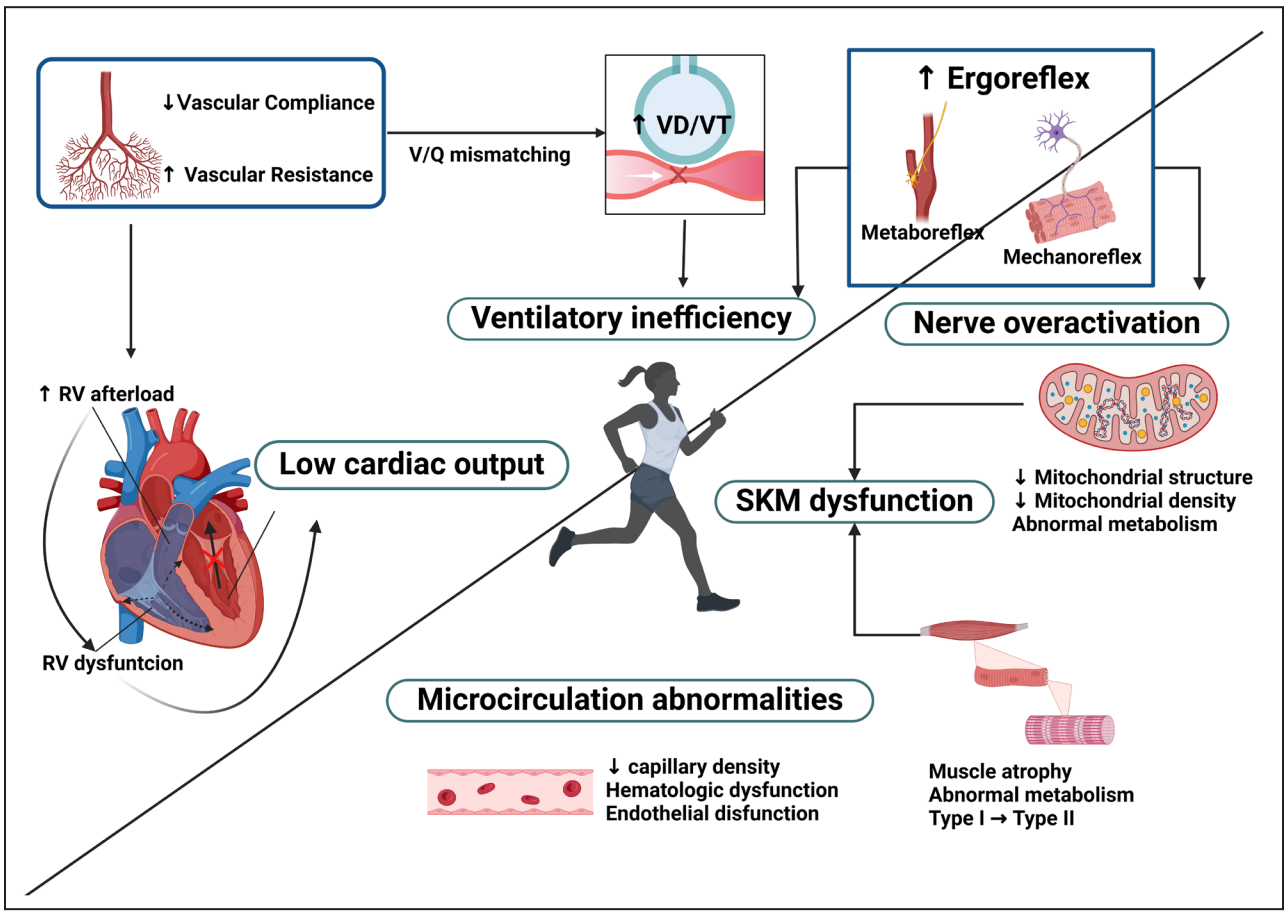

Figure 1. Exercise intolerance in patients with pulmonary arterial hypertension.
RV indicates right ventricle; SKM, skeletal muscle; V/Q, relation between ventilation and perfusion; and VD/VT, dead space ventilation ratio.

than the metabolic demand, due to high consumption or low supply, there is a relative increase in energy production through oxygen-independent pathways, also called anaerobic metabolism.[13]

Oxygen use for cellular metabolism requires transport from ambient air to tissue mitochondria, particularly in the skeletal muscle. This oxygen pathway involves several systems and can be didactically divided into 6 steps related to oxygen delivery: the convective oxygen transport components (the respiratory, cardiac, and hematologic systems) and oxygen extraction as related to diffusive oxygen transport components (the vascular, skeletal muscle, and nervous systems).[12,15] Figure 2 illustrates the oxygen pathway.

Inspired oxygen passes through airways to the alveoli, where gas exchange takes place. Alveoli are predominantly lined by type I pneumocytes, which are exceptionally thin (0.1 μm) to allow efficient gas diffusion. Surrounding the alveoli are capillaries of similar thickness that form a vascular network. This close proximity and thin structures facilitate the exchange of gases through simple diffusion.[13,16] Once oxygen diffuses into the capillaries it binds to hemoglobin molecules in red blood cells to form oxyhemoglobin.[17] The delivery of oxygen absorbed in the lungs depends on 2 important factors: cardiac preload and afterload. Preload refers to stretching of heart muscle fibers at the end of diastole, which is determined by the volume of venous blood return and elasticity of the cardiac tissue. Moderate stretching enhances the force of contraction (Frank–Starling mechanism) and increases stroke volume, but excessive preload overstretches myocardial fibers and reduces stroke volume.[18] Afterload, the resistance the right heart must overcome to eject blood, is primarily

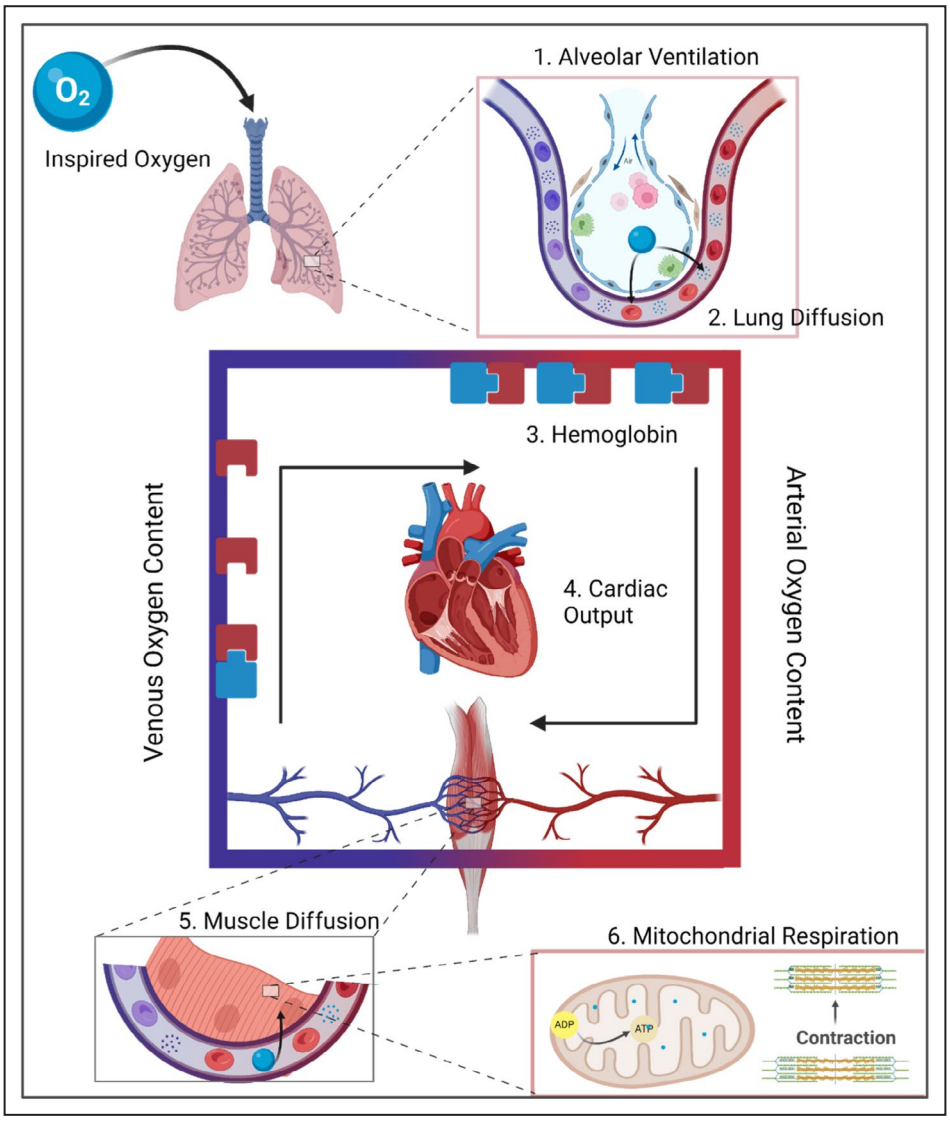

Figure 2. Illustration of the 6 steps of O_2 pathway from environment to mitochondria.
O_2 indicates oxygen.

determined by pulmonary vascular resistance and pulmonary arterial compliance, limited by pulmonary valve pathology. In PAH, high pulmonary vascular resistance and low pulmonary arterial compliance from distended and remodeled vessels increase afterload, decrease stroke volume, and reduce cardiac output.[1]

During exercise, increased blood flow to capillaries raises red blood cell velocity and local capillary hematocrit, enhancing oxygen perfusion to muscles through "longitudinal recruitment," which expands capillary surface area along its length.[15]

The regulation of blood flow in the skeletal muscle is regulated by the ergoreflex, composed of the mechanoreflex and metaboreflex. The mechanoreflex is activated by muscle contraction, and metabolite accumulation triggers the metaboreflex. Both signal to the central nervous system to increase ventilatory response and the blood flux to skeletal muscle, modifying vascular tone to optimize oxygen delivery and carbon dioxide removal.[19] In the skeletal muscle capillaries, oxygen leaves hemoglobin to move into tissues, following a decreasing O_2 pressure gradient.[15,20]

The skeletal muscle tissue is composed of fiber-shaped myocytes. The muscle fibers are composed of sarcolemma, sarcoplasm, and cell nuclei. Muscle contraction strength is directly related to the quality of sarcomere contractility. The release of calcium into the sarcoplasm facilitates the movement of actin and myosin filaments within the sarcomere, leading to the sliding of these filaments, which shortens the muscle cell and produces contraction.[3]

Myocytes are classified by the type of MyHC (myosin heavy chain) they express, which varies in mechanical, biochemical, and metabolic properties.[21] Generally, muscle fibers are categorized into 2 main groups: type I and type II. Type I fibers have higher oxidative metabolism, with greater capillary density and better endurance, while type II fibers have lower oxidative capacity, higher glycolytic capacity, and are suited for short-duration exercise.[22] Oxygen diffusibility into muscle fibers varies by cell type[23,24]: in type 1 fibers, muscle perfusion during rest and exercise remains elevated, enhancing aerobic metabolism.[25,26] In contrast, in type 2 fibers, muscle perfusion is generally lower, particularly during rest, which limits oxygen delivery and reduces the reliance on aerobic metabolism.[23] This difference is further accentuated by mitochondrial content: type 1 fibers are rich in mitochondria, enabling efficient oxidative phosphorylation, whereas type 2 fibers have fewer mitochondria, relying more heavily on anaerobic glycolysis for energy production.[27] Skeletal muscle is comprised of different fiber types, with each muscle containing varying proportions based on its function.

Muscle tissue requires a constant energy source to sustain contraction. Skeletal muscle stores energy as phosphocreatine, which bypasses the mitochondria and directly converts ADP into ATP. However, becausee this process is limited, mitochondrial metabolism must be optimized to meet higher energy demands. Through training with repeated high metabolic demand, biogenesis and mitochondrial fusion increase the ATP synthesis capacity of the mitochondrial network.[28–30] Concurrently, fission and mitophagy degrade dysfunctional organelles, promoting cellular hemostasis.[31]

Mitochondria structure is key to its energy production function, with a smooth and permeable outer membrane, an intermembrane space with an aqueous matrix, and a highly folded inner membrane forming cristae. These cristae house the electron transport chain complexes (Complexes I to IV) and ATP synthase (Complex V), which are essential for oxidative phosphorylation. The matrix contains citric acid cycle enzymes and mitochondrial DNA, which encodes many mitochondrial proteins. The mitochondrial complexes enable the electron transfer process, reducing molecular oxygen to water and pumping protons across the inner membrane, creating a proton gradient that powers ATP synthase to produce ATP.[30,32]

Traditionally, oxygen consumption by peripheral tissue, or oxygen uptake (VO_2), can be mathematically calculated by the Fick principle: VO_2 = cardiac output × $C(a\text{-}v)O_2$.[11] Cardiac output is derived by multiplying the stroke volume by heart rate. $C(a\text{-}v)O_2$ represents the difference between arterial and mixed-venous oxygen content. Oxygen content depends on hemoglobin oxygen carrying and oxygen solubility: $C(a/v)O_2 = (1.36 \times Hb(a/v) \times S(a/v)O_2) \times (0.003 \times P(a/v)O_2)$.[11,33–37] However, it is important to note that this equation mainly reflects the muscle perfusive component. As described previously, the muscle diffusive ability also plays an important role in oxygen consumption. Therefore, VO_2 can also be described as the relation between the ability of O_2 to diffuse and the pO_2 gradient between the capillary and the mitochondria ($VO_2 = D \times P(\text{capillary-mitochondria})O_2$), also known as Fick's law.[15,38] Figure 3 represents Fick's principle with the diffusive concept (ie, Fick's law), illustrating that maximum VO_2 is the point of intersection of oxygen transport and oxygen diffusibility in the muscle tissue.[38] Therefore, reductions in VO_2 can be related to impairment in the steps of oxygen delivery or oxygen extraction.[11,39]

SKELETAL MUSCLE DYSFUNCTION IN PAH

Beyond the PAH pathology in the pulmonary vasculature and right ventricle, skeletal muscle dysfunction in patients with PAH also significantly contributes to

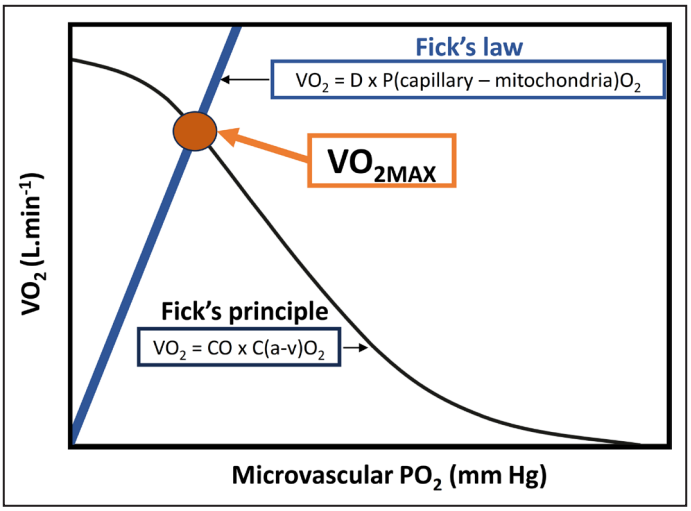

Figure 3. The relationship between uptake VO_2 and microvascular oxygen partial pressure.
Fick's law (blue line) describes the diffusion of oxygen from capillaries to mitochondria. Fick's principle (black line) shows VO_2 as the product of oxygen supply and muscle extraction capacity (difference in oxygen content between arterial and venous blood [$C(a\text{-}v)O_2$]). The point where the 2 curves intersect marks the maximum oxygen consumption (VO_{2MAX}), which is the maximum VO_2 during exercise. $C(a\text{-}v)O_2$ indicates difference in oxygen content between arterial and venous blood; CO, cardiac output; D, distance; O_2, oxygen; PO_2, oxygen partial pressure; and VO_{2MAX}, maximum oxygen consumption.

exercise intolerance and involves intrinsic vascular, morphological, and mitochondrial abnormalities.[6] Characterizing skeletal muscle pathophysiology in PAH is increasingly of interest among researchers, as it sheds light on the broader systemic effects of the disease and potential therapeutic targets. Due to its impact on quality of life and limited treatment options, both human and animal-model studies seek to understand the mechanism behind skeletal muscle abnormalities in PAH.[3,8,9]

Recent evidence highlights a dynamic interaction between skeletal muscle and pulmonary vascular remodeling, suggesting that skeletal muscle dysfunction may play a contributory role beyond a passive effect of systemic disease. A recent study proposed a novel endocrine signaling pathway for skeletal muscle modulation of pulmonary vascular remodeling through the SIRT3-CNPY2-LOXL2 (sirtuin-3-canopy fibroblast growth factor signaling regulator 2-lysyl oxidase homolog 2). Jheng et al compared findings from a skeletal muscle-specific SIRT3 knockout mouse model with left ventricle failure model rat and patients with pulmonary hypertension (PH) associated with preserved ejection fraction. The comparison revealed similar increased of LOXL2 and CNPY2 and similar alterations in pulmonary vascular remodeling and pressure. Mice with high-fat diet–induced PH-heart failure with preserved ejection fraction threated with recombinant LOXL2 protein developed increased pulmonary vascular proliferation, whereas skeletal muscle-specific deletion of LOXL2 improved pulmonary pressures.[40]

Skeletal Muscle Vascular Abnormalities

Microcirculation abnormalities in PAH encompass both the convective and diffusive components of oxygen transport and can be divided into 3 aspects: endothelial cell dysfunction, vascular dysfunction, and oxygen diffusion impairment.[6,41] Vascular stiffness and endothelial dysfunction inhibit effective diffusion of oxygen through the microvasculature to skeletal muscle tissues, ultimately resulting in decreased oxygen delivery to muscles.[42] Overactivation of the ergoreflex mechanism contributes to symptoms of dyspnea and exercise intolerance in patients with left heart failure.[19] Specific evidence of this occurring in PAH is still limited, but the loss of coupling between muscle signals and the autonomic nervous system, leading to hyperventilation and an imbalance in local blood flow, is present in PAH.[6]

Shulze et al compared rats with monocrotaline-PH with untreated controls and observed a difference in pO_2 in the interstitial muscle space at the start of exercise.[43] All the animals were treated with a vasodilator (sodium nitroprusside), resulting in an exaggerated decline in pO_2, which remained lower in monocrotaline-treated

animals compared with controls. These data support the concept that exercise intolerance in PH may be related to a diffusive oxygen defect, which could be associated with stiffening of skeletal muscle vessels leading to vascular dysfunction.[43–45]

Those findings are reinforced by recent studies that examined the convective and diffusive components of oxygen transport in PH. In another experiment, Schulze et al examined microcirculatory hemodynamics in monocrotaline-PH rats, revealing significant reductions in oxygen transport by both perfusive (capillary red blood cell flux and velocity) and diffusive (percentage of capillaries supporting continuous flow in skeletal muscles) mechanisms.[42] They suggest that this reduction in oxygen transport is likely linked to arteriolar regulation, resulting in impaired capillary hemodynamics.

In contrast, Zhang et al found evidence that reduced exercise capacity in PH occurs before intrinsic skeletal muscle dysfunction in rats with experimental PH induced by a combination of SU5416 and hypoxia, suggesting that the primary limitations in exercise tolerance are due to impaired blood flow rather than direct muscle pathology.[46] These data support the notion that early interventions targeting blood flow and oxygen delivery may help in PH-related exercise intolerance.

In human subjects, Potus et al observed a significant reduction in microRNA 126 (miR-126) expression within skeletal muscles, which correlated with decreased microvascular density quantified by loss of CD31+ endothelial cells, and worse exercise capacity.[47] They found that miR-126 dowregulation led to upregulation of SPRED-1 (sprouty related EVH1 domain containing 1) and, consequently, to a marked decrease in the downstream effectors of the VEGF (vascular endothelial growth factor) pathway including phospho-Raf and phospho-ERK (extracellular signal-regulated kinase) (Figure 4). Complementary experiments in healthy rats showed that targeted dowregulation of miR-126 in skeletal muscles recapitulated the reduction in microvascular density and exercise tolerance, whereas miR-126 upregulation in diseased rats partly restored their exercise tolerance. These results suggest that therapeutic strategies aimed at restoring miR-126 levels could potentially improve microcirculation and physical function in patients with PAH, offering a novel approach to addressing the systemic effects of this destabilization disease.[47]

Skeletal Muscle Morphological Abnormalities

Reduced muscle strength and endurance has been observed in patients with PAH.[3] These impairments are partially explained by a relative reduction in the number of oxidative type 1 cells and an increase in the number of glycolytic type II cells.[6] Muscle fiber differentiation is regulated by the FoxO1 pathway, with increased FoxO1 expression in type II fibers.[48] Kosmas et al demonstrated that FoxO1 inhibition in SU5416-hypoxia rats increased the ratio of type I to type II fibers,[49] similar to the phenotype of monocrotaline-treated rats reported by Xiang et al[50] Analysis of the type II fibers revealed more apoptosis leading to atrophy.[51]

Activation of autophagic/lysosomal proteolysis and the ubiquitin proteasome system leads to apoptosis. This process is a 3-enzyme ubiquitination cascade: E1

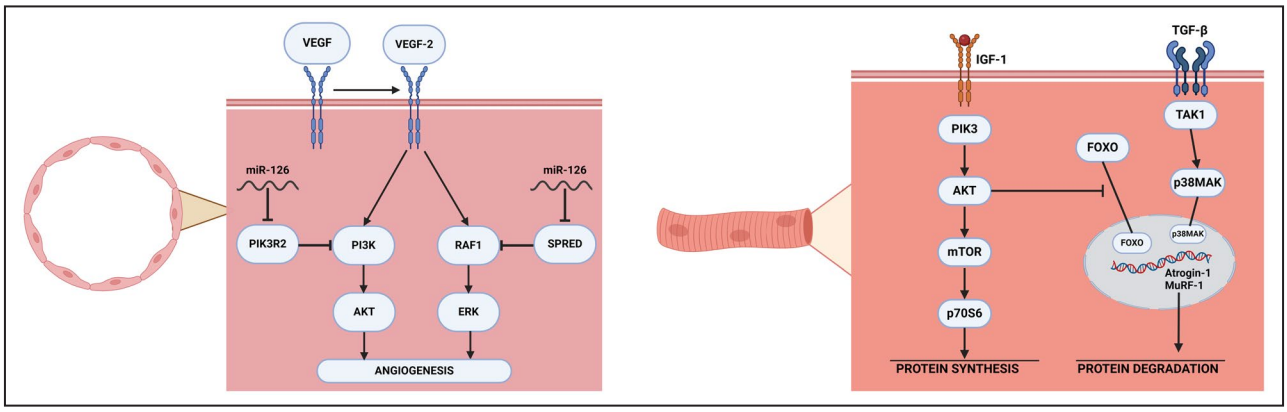

Figure 4. Vascular and skeletal muscle signaling pathways.
A, Skeletal muscle endothelial cells: miR-126 suppresses PIK3R2 and SPRED1, which negatively regulates VEGF signaling via the PI3 kinase and RAF1 kinase pathways, ultimately affecting angiogenesis. **B**, Summary schema of protein synthesis and degradation in muscle cells. AKT signaling can increase both the activity of protein synthesis pathways and inhibit protein degradation pathways. TAK1 signaling can increase protein degradation. AKT indicates protein kinase B; ERK, extracellular signal-regulated kinase; FOXO, Forkhead box O transcription factors; IGF-1, insulin-like growth factor 1; miR-126, microRNA 126; mTOR, mammalian target of rapamycin; MuRF-1, muscle RING-finger protein-1; p38MAPK, p38 mitogen-activated protein kinase; p70S6, ribosomal protein S6 kinase beta-1; PI3 kinase, phosphoinositide 3-kinase; PIK3R2, phosphoinositide-3-kinase regulatory subunit 2; RAF1, RAF proto-oncogene serine/threonine-protein kinase; SPRED1, sprouty-related EVH1 domain-containing protein 1; TAK1, transforming growth factor-beta-activated kinase 1; TGF-β, transforming growth factor-β; and VEGF, vascular endothelial growth factor.

(ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzyme), and E3 (ubiquitin ligase). In cardiac and skeletal muscle, MuRF (muscle RING finger) and atrogin-1 are E3 ligase enzymes that correlate with of skeletal muscle loss.[52,53] Knocking out these proteins is protective against myocardium and skeletal muscle atrophy.[54] Increased expression of MuRF has been found in diaphragm muscle fiber in rats with PH.[53–55]

Under normal conditions, proteolysis pathways are inhibited when protein synthesis pathways are activated.[56] When PI3 (phosphatidylinositide 3) kinase/AKT (protein kinase B) signaling pathway is activated, it induces FoxO transcription factor phosphorylation, inhibiting MurF1 and atrogin-1 expression.[56] These signaling pathways are diagrammed in Figure 4.

Further supporting these animal model data, Batt et al observed a significant shift toward more type II fibers, accompanied by muscle atrophy, in quadriceps femoris muscle in patients with PAH.[3] Increases of both atrogin-1 and MuRF1, lower levels of AKT, and increases of FoxO3 phosphorylation correlated to quadriceps muscle atrophy.[57] Decreased FoxO1 has also been described in lung and vascular smooth muscle cells in PAH.[58,59] Therefore, therapeutically targeting FoxO1 can potentially both improve PAH and skeletal muscle impairment.[57,58]

Muscle mass reduction does not occur exclusively among patients with PAH. Sarcopenia is present in different chronic diseases, such as chronic obstructive pulmonary disease and left heart failure, and is also inherent in the natural aging process.[60] The findings among different conditions, however, can be conflicting. In patients with chronic obstructive pulmonary disease, for example, researchers documented either no change or increased AKT and FoxO1 phosphorylation, perhaps representing a compensatory response to prevent further muscle atrophy.[61]

Use of a pulmonary artery banding model, which avoids hypoxia or toxins, suggests a significant impact of systemic circulation on the development of sarcopenia in heart failure, with more pronounced skeletal muscle impairment observed in left ventricle failure models compared with RV failure-models. In the left ventricle failure model there was a reduction in muscle weight and fiber diameter in the soleus and gastrocnemius muscles, accompanied by increased proteasome activity and expression of MuRF1 and atrogin-1 and the atrophy marker myostatin.[62]

Another mechanism that contributes to exercise intolerance is skeletal muscle weakness.[63] Previous studies have demonstrated that alterations in the regulatory pathways of calcium influx and efflux in the sarcoplasmic reticulum occurs in the skeletal muscle of mouse models of heart failure and human models with myopathies and chronic obstructive pulmonary disease.[3,64] This is also seen in respiratory muscles and in the peripheral muscles of patients with PAH.[3,64,65]

Skeletal Muscle Mitochondrial Abnormalities

Disruption in mitochondrial function can lead to significant metabolic disturbances.[30] A shift from oxidative to glycolytic metabolism has been described in pulmonary vascular and right ventricle cells, correlated to muscle dysfunction and exercise intolerance in PAH.[66,67] Levels of Mfn (mitofusin) positively correlate with increased mitochondrial fusion, enabling mitochondrial network development and redistribution of mitochondrial contents, preventing damaged mitochondria accumulation.[3] DecreasedMfn1 and 2 in PAH skeletal muscle cells is associated with muscle atrophy and mitochondrial metabolism inefficiency.[3]

An animal model comparing right and left ventricular failure identified mitochondrial dysfunction as a key finding, serving as an early marker of sarcopenia in left ventricle failure model. In left heart failure model, there was a significant decline in the activity of citrate synthase and complexes I and IV of the respiratory chain, both critical for energy production. Pyruvate-dependent state 3 respiration was significantly decreased, indicating impaired mitochondrial function, and the respiratory control index was notably lower, also reflecting reduced mitochondrial efficiency.[62]

In agreement, Malenfant et al analyzed skeletal muscle biopsies from patients with PAH and found decreased proteins related to mitochondrial oxidative phosphorylation, ATP production by the Krebs cycle, and fatty acid metabolism and increased proteins related to nonoxidative metabolism including pyruvate metabolism, gluconeogenesis, and lipid metabolism.[67] These findings are congruent with an observed increase in anaerobic glycolysis markers and decreased aerobic oxidative metabolism in the pulmonary vascular bed.[66]

SKELETAL MUSCLE DYSFUNCTION IN CTD-PAH

Among the causes of PAH, CTD-PAH has particularly poor clinical outcomes. PAH complicates a heterogeneous group of CTDs, including systemic sclerosis (SSc), systemic lupus erythematosus, rheumatoid arthritis, mixed connective tissue disease, antisynthetase syndrome, and dermato- and polymyositis. These CTDs collectively represent a systemic autoimmune response with an exacerbated inflammatory response.[1,68,69] Patients with CTD-PAH have worse morbidity and mortality, worse exercise capacity, and a lower therapeutic response rate to PAH-targeted therapy compared with other causes of PAH.[70,71] International PH registries show that the prevalence of CTD-PAH ranges from 13% to 50%, with SSc accounting for 50% to 70% of the cases.[68,69]

The systemic vascular and muscle involvement in CTD-PAH remains poorly understood. The prevalence of muscle disorders in patients with SSc is uncertain, with estimates spanning from 13% to 81%.[72] This variability largely stems from inconsistent criteria used to define muscle involvement, and the high prevalence of scleroderma-myositis syndrome.[73] Regardless of criteria, there is a consensus that skeletal muscle involvement in these patients is a negative prognostic factor.[72]

Two pathophysiological mechanisms have been proposed as potential causes of skeletal muscle impairment in SSc. The first involves peripheral microangiopathy leading to myopathy and the destruction of the vasculature and muscle tissue itself, often resulting in fibrosis.[74] Similar to other PAH causes, progressive damage leads to capillary rarefaction in skeletal muscle, evidenced by reduced CD31+ cells, increased endothelial cell apoptosis, and increased antiangiogenic hallmarks particularly in the VEGF pathway.[74,75] Of note, these vascular changes have also been observed in other connective diseases including dermatomyositis and polymyositis.[74,76]

Fibrosis also plays a crucial role in skeletal muscle impairment in SSc. Previous studies have shown a strong TGF-β (transforming growth factor-β) expression, a potent inducer of fibrillar collagen gene transcription and collagen secretion.[74,75,77] The presence of inflammatory cells in skeletal muscle in SSc has been variably reported in the literature. Whereas some studies demonstrate an increase in inflammatory cells in skeletal muscle (mostly T cells), others do not confirm this observation.[74,78] A humoral immune process leading to endothelial injury and intimal proliferation may be mediated by complement-fixing antibodies.[74] Myofiber atrophy associated with necrosis of the muscle fiber has also been reported, with histologic changes similar to other autoimmune diseases such as polymyositis, dermatomyositis, immune-mediated necrotizing myopathy, fibrosing myopathy, and nonspecific myositis.[79]

Muscle involvement may occur in other CTDs beyond SSc, including systemic lupus erythematosus, Sjogren's syndrome, and rheumatoid arthritis.[80] In systemic lupus erythematosus, true myositis is estimated to occur in only 4% to 16% of the cases,[80] with histopathological findings such as myositis, vasculitis, type II fiber atrophy, vascular wall thickening, and neurogenic muscle injury.[81,82] The low prevalence can be related to overlaps between disease symptoms and corticosteroid therapy effects.[80] Patients with rheumatoid arthritis experience a significant muscle strength reduction (25%–75%),[83] primarily due to structure muscle impairment, inflammation within the muscle, muscle tissue vasculitis, and neuropathy that leads to muscle weakness.[84,85] Other nonmuscle-related causes of weakness are global inactivity due to cachexia or diminished motion of joints (synovitis) and sarcopenia.[86]

Sjogren's syndrome also exhibits muscle involvement, as Lindvall et al demonstrated increased expression of MHC (major histocompatibility complex) class I, MHC class II, and membrane attack complex.[87] These findings are similar to those in dermatomyositis and suggest that the mechanism of myositis in Sjogren's syndrome is related to complement activation resulting in a microvasculopathy.[88,89]

In summary, muscle involvement in CTD-PAH encompasses multiple, complex, and heterogeneous mechanisms, which are poorly understood. Its occurrence is associated with a lower quality of life and higher mortality.[72] Understanding the histopathological features of CTD-related myopathy in CTD-PAH may provide valuable insights that could enhance the effectiveness of targeted therapies and improve clinical outcomes.

KNOWLEDGE GAPS AND FUTURE DIRECTIONS

Despite substantial progress in understanding many aspects of exercise limitation in PAH, there remains uncertainty regarding the pathology and clinical impact of skeletal muscle and metabolic impairment. Further evaluation of skeletal muscle vascular bed, muscle structure, and skeletal muscle mitochondrial metabolism is necessary. However, the considerable heterogeneity of PAH, with its diverse pathogenetic mechanisms, varying degrees of severity, and differing disease duration adds complexity to the task of investigating skeletal muscle dysfunction in PAH. Confounding factors in the analysis of skeletal muscle dysfunction in PAH include sedentary behavior and, consequently, physical deconditioning, as well as the natural aging process, which leads to changes in mitochondrial function.[90] Aging is also associated with muscle anabolism and a reduction in muscle sensitivity to AKT signaling.[90] In contrast to that observed in chronic diseases, however, the aging process appears to be accompanied by a reduction in type II fibers.[90]

Due to heterogeneity and limitations in studying human disease, investigations using animal models substantially contribute to understanding cellular mechanisms. One limitation with studies using PH animal models induced by monocrotaline or SU5416-hypoxia is that the muscle pathology is likely confounded by direct effects of the PH inciting stimulus (monocrotaline, SU5416, or hypoxia) on skeletal muscle. Therefore, the use of alterative PH animal models to study the skeletal muscle, such as the schistosomiasis-PH mouse model or PA banding, which restricts the stimulus to the pulmonary vasculature,[91,92] might be of particular utility.[1,62]

Studying PH animal models will enable determining the relative muscle weakness and exercise intolerance

in the different models, the time course, and how the pathology compares with that observed in human biospecimens. For example, comparing hypoxia-induced PH with PA banding can help clarify if global hypoxia directly affects the peripheral muscle phenotype. Similarly, comparing inflammatory PH causes such as schistosomiasis to noninflammatory causes such as PA banding will clarify if inflammation in the lungs contributes to the PH skeletal muscle pathology. For example, the relative mitochondrial density (fission or fusion) or changes in mitochondrial metabolism (density of mitochondrial complexes) can be compared across animal models. The animal models can then serve as a preclinical testing ground for developing future clinical interventions targeting skeletal muscle pathology and metabolism in PAH.

In human samples, precise morphometric and stereologic studies of the peripheral musculature including distinguishing type I and type II fibers, identifying the vascular pattern and density, and detecting the presence of fibrosis will rigorously characterize the skeletal muscle pathology in humans. There may be atrophy or hypertrophy of specific fiber types and important differences between PH causes, such as those caused by CTDs. Inflammatory infiltrates can be more precisely characterized, vis à vis those in the lungs. These descriptive but important studies will lay the groundwork for interrogating specific mechanisms of skeletal muscle disease pathophysiology.

Large data omics approaches can also be considered in future studies evaluating skeletal muscle dysfunction in PAH. For example, single-cell RNA sequencing can identify specific cell populations and the RNA phenotype of these cells, which can suggest pathologic differences between diseased and control specimens. Single-cell RNA sequencing can be integrated with proteomics to observe the effect on transcribed proteins and metabolomics to validate that the proteins ultimately affect metabolite uptake and use.

These data can directly lead to meaningful clinical interventions to aid patients with PAH. Skeletal muscle pathology likely can be pharmacologically targeted, potentially in combination with exercise training.[93,94] Changing the diet or introducing specific vitamins and minerals in the diet could provide critical enzymatic cofactors to promote successful metabolic and skeletal muscle rehabilitation in patients with PAH once the mechanisms of skeletal muscle dysfunction are better understood.

CONCLUSIONS

The pathophysiology of exercise limitation in patients with PAH is multifactorial. In addition to pulmonary and cardiac pathology, skeletal muscle impairment also contributes to exercise intolerance and poor quality of life in PAH. In this context, skeletal muscle myopathy, mitochondrial dysfunction with reduced oxidative capacity, and systemic vascular changes likely contribute to exercise impairment. Understanding the connective and diffusive components of the oxygen pathway, which ultimately lead to reduced oxygen supply or the ability of muscle to absorb and metabolize oxygen, is important to optimizing the current therapeutic interventions in PAH. By investigating the pathophysiology of the skeletal muscle in PAH, new therapeutic targets can be identified that will improve exercise capacity.

ARTICLE INFORMATION

Affiliations

Division of Respiratory Diseases, Department of Medicine, Hospital São Paulo, Federal University of São Paulo (UNIFESP), São Paulo, Brazil (T.C.M., E.V.F., R.K.O.); Division of Pulmonary and Critical Care Medicine, Department of Medicine, University of California, San Francisco, San Francisco, CA (M.H.L., D.C.F.B., K.N., R.K., B.B.G.); Department of Orthopaedic Surgery, University of California, San Francisco, CA (S.S.); and San Francisco Veteran Affairs Health Care System, San Francisco, CA (S.S.).

Sources of Funding

This work was supported by the Coordination for the Improvement of Higher Education Personnel, Brazil, to Thaís C. F. Menezes; by the United States Department of Defense (W81XWH2210457), by the Fulbright Scholarship Program (12398-BR), and by the National Institutes of Health (K08HL168310), USA, to Michael H. Lee; by the National Institutes of Health (R01HL135872 and P01HL152961), USA, to Brian B. Graham; and by the National Council for Scientific and Technological Development (313284/2021-0 and 409180/2022-0), Brazil, to Rudolf K. F. Oliveira.

Disclosures

None.

REFERENCES

1. Humbert M, Kovacs G, Hoeper MM, Badagliacca R, Berger RMF, Brida M, Carlsen J, Coats AJS, Escribano-Subias P, Ferrari P, et al. 2022 ESC/ERS guidelines for the diagnosis and treatment of pulmonary hypertension. *Eur Heart J*. 2022;43:3618–3731. doi: 10.1093/eurheartj/ehac237
2. Waxman AB. Exercise physiology and pulmonary arterial hypertension. *Prog Cardiovasc Dis*. 2012;55:172–179. doi: 10.1016/j.pcad.2012.07.003
3. Batt J, Ahmed SS, Correa J, Bain A, Granton J. Skeletal muscle dysfunction in idiopathic pulmonary arterial hypertension. *Am J Respir Cell Mol Biol*. 2014;50:74–86. doi: 10.1165/rcmb.2012-0506OC
4. McLaughlin VV, Shillington A, Rich S. Survival in primary pulmonary hypertension. *Circulation*. 2002;106:1477–1482.
5. Sun X-G, Hansen JE, Oudiz RJ, Wasserman K. Exercise pathophysiology in patients with primary pulmonary hypertension. *Circulation*. 2001;104:429–435. doi: 10.1161/hc2901.093198
6. Malenfant S, Lebret M, Breton-Gagnon É, Potus F, Paulin R, Bonnet S, Provencher S. Exercise intolerance in pulmonary arterial hypertension: insight into central and peripheral pathophysiological mechanisms. *Eur Respir Rev*. 2021;30:200284. doi: 10.1183/16000617.0284-2020
7. Dhakal BP, Malhotra R, Murphy RM, Pappagianopoulos PP, Baggish AL, Weiner RB, Houstis NE, Eisman AS, Hough SS, Lewis GD. Mechanisms of exercise intolerance in heart failure with preserved ejection fraction. *Circ Heart Fail*. 2015;8:286–294. doi: 10.1161/CIRCHEARTFAILURE.114.001825
8. Howden EJ, Ruiz-Carmona S, Claeys M, De Bosscher R, Willems R, Meyns B, Verbelen T, Maleux G, Godinas L, Belge C, et al. Oxygen pathway limitations in patients with chronic thromboembolic pulmonary hypertension. *Circulation*. 2021;143:2061–2073. doi: 10.1161/CIRCULATIONAHA.120.052899

9. Bauer R, Dehnert C, Schoene P, Filusch A, Bärtsch P, Borst MM, Katus HA, Meyer FJ. Skeletal muscle dysfunction in patients with idiopathic pulmonary arterial hypertension. *Respir Med*. 2007;101:2366–2369.
10. Malenfant S, Potus F, Mainguy V, Leblanc E, Malenfant M, Ribeiro F, Saey D, Maltais F, Bonnet S, Provencher S. Impaired skeletal muscle oxygenation and exercise tolerance in pulmonary hypertension. *Med Sci Sports Exerc*. 2015;47:2273–2282. doi: 10.1249/MSS.0000000000000696
11. Kisaka T, Stringer WW, Koike A, Agostoni P, Wasserman K. Mechanisms that modulate peripheral oxygen delivery during exercise in heart failure. *Ann Am Thorac Soc*. 2017;14:S40–S47. doi: 10.1513/AnnalsATS.201611-889FR
12. Calbet JAL, Losa-Reyna J, Torres-Peralta R, Rasmussen P, Ponce-González JG, Sheel AW, de la Calle-Herrero J, Guadalupe-Grau A, Morales-Alamo D, Fuentes T, et al. Limitations to oxygen transport and utilization during sprint exercise in humans: evidence for a functional reserve in muscle O_2 diffusing capacity. *J Physiol*. 2015;593:4649–4664. doi: 10.1113/JP270408
13. Houstis NE, Eisman AS, Pappagianopoulos PP, Wooster L, Bailey CS, Wagner PD, Lewis GD. Exercise intolerance in heart failure with preserved ejection fraction. *Circulation*. 2018;137:148–161. doi: 10.1161/CIRCULATIONAHA.117.029058
14. Melamed KH, Santos M, Oliveira RKF, Urbina MF, Felsenstein D, Opotowsky AR, Waxman AB, Systrom DM. Unexplained exertional intolerance associated with impaired systemic oxygen extraction. *Eur J Appl Physiol*. 2019;119:2375–2389. doi: 10.1007/s00421-019-04222-6
15. Poole DC, Musch TI. Capillary-mitochondrial oxygen transport in muscle: paradigm shifts. *Function (Oxf)*. 2023;4:zqad013. doi: 10.1093/function/zqad013
16. Weibel ER. Lung morphometry: the link between structure and function. *Cell Tissue Res*. 2017;367:413–426. doi: 10.1007/s00441-016-2541-4
17. Brunori M, Miele AE. Modulation of allosteric control and evolution of hemoglobin. *Biomol Ther*. 2023;13:572. doi: 10.3390/biom13030572
18. Konstam MA, Kiernan MS, Bernstein D, Bozkurt B, Jacob M, Kapur NK, Kociol RD, Lewis EF, Mehra MR, Pagani FD, et al. Evaluation and Management of Right-Sided Heart Failure: a scientific statement from the American Heart Association. *Circulation*. 2018;137:e578–e622. doi: 10.1161/CIR.0000000000000560
19. Aimo A, Saccaro LF, Borrelli C, Fabiani I, Gentile F, Passino C, Emdin M, Piepoli MF, Coats AJS, Giannoni A. The ergoreflex: how the skeletal muscle modulates ventilation and cardiovascular function in health and disease. *Eur J Heart Fail*. 2021;23:1458–1467. doi: 10.1002/ejhf.2298
20. Poole DC, Musch TI, Colburn TD. Oxygen flux from capillary to mitochondria: integration of contemporary discoveries. *Eur J Appl Physiol*. 2022;122:7–28. doi: 10.1007/s00421-021-04854-7
21. Larsson L, Moss RL. Maximum velocity of shortening in relation to myosin isoform composition in single fibres from human skeletal muscles. *J Physiol*. 1993;472:595–614. doi: 10.1113/jphysiol.1993.sp019964
22. Qaisar R, Bhaskaran S, Van Remmen H. Muscle fiber type diversification during exercise and regeneration. *Free Radic Biol Med*. 2016;98:56–67. doi: 10.1016/j.freeradbiomed.2016.03.025
23. McDonough P, Behnke BJ, Padilla DJ, Musch TI, Poole DC. Control of microvascular oxygen pressures in rat muscles comprised of different fibre types. *J Physiol*. 2005;563:903–913. doi: 10.1113/jphysiol.2004.079533
24. Behnke BJ, McDonough P, Padilla DJ, Musch TI, Poole DC. Oxygen exchange profile in rat muscles of contrasting fibre types. *J Physiol*. 2003;549:597–605. doi: 10.1113/jphysiol.2002.035915
25. Richardson RS, Noyszewski EA, Leigh JS, Wagner PD. Lactate efflux from exercising human skeletal muscle: role of intracellular po2. *J Appl Physiol (1985)*. 1998;85:627–634.
26. Arthur PG, Hogan MC, Bebout DE, Wagner PD, Hochachka PW. Modeling the effects of hypoxia on ATP turnover in exercising muscle. *J Appl Physiol (1985)*. 1992;73:737–742.
27. Conde Alonso S, Gajanand T, Ramos JS, Antonietti J-P, Borrani F. The metabolic profiles of different fiber type populations under the emergence of the slow component of oxygen uptake. *J Physiol Sci*. 2020;70:27. doi: 10.1186/s12576-020-00754-1
28. Colburn TD, Hirai DM, Craig JC, Ferguson SK, Weber RE, Schulze KM, Behnke BJ, Musch TI, Poole DC. Transcapillary PO 2 gradients in contracting muscles across the fibre type and oxidative continuum. *J Physiol*. 2020;598:3187–3202.
29. Hepple RT. Skeletal muscle: microcirculatory adaptation to metabolic demand. *Med Sci Sports Exerc*. 2000;32:117–123. doi: 10.1097/00005768-200001000-00018
30. Spinelli JB, Haigis MC. The multifaceted contributions of mitochondria to cellular metabolism. *Nat Cell Biol*. 2018;20:745–754. doi: 10.1038/s41556-018-0124-1
31. Hood DA, Memme JM, Oliveira AN, Triolo M. Maintenance of skeletal muscle mitochondria in health, exercise, and aging. *Annu Rev Physiol*. 2019;81:19–41. doi: 10.1146/annurev-physiol-020518-114310
32. Protasoni M, Zeviani M. Mitochondrial structure and bioenergetics in normal and disease conditions. *Int J Mol Sci*. 2021;22:586. doi: 10.3390/ijms22020586
33. Joseph P, Oliveira RKF, Eslam RB, Agarwal M, Waxman AB, Systrom DM. Fick principle and exercise pulmonary hemodynamic determinants of the six-minute walk distance in pulmonary hypertension. *Pulm Circ*. 2020;10:204589402095757.
34. Grafton G, Cascino TM, Perry D, Ashur C, Koelling TM. Resting oxygen consumption and heart failure: importance of measurement for determination of cardiac output with the use of the Fick principle. *J Card Fail*. 2020;26:664–672. doi: 10.1016/j.cardfail.2019.02.004
35. Koch B, Schäper C, Ittermann T, Spielhagen T, Dörr M, Völzke H, Opitz CF, Ewert R, Gläser S. Reference values for cardiopulmonary exercise testing in healthy volunteers: the SHIP study. *Eur Respir J*. 2009;33:389–397. doi: 10.1183/09031936.00074208
36. Ho JE, Zern EK, Lau ES, Wooster L, Bailey CS, Cunningham T, Eisman AS, Hardin KM, Farrell R, Sbarbaro JA, et al. Exercise pulmonary hypertension predicts clinical outcomes in patients with dyspnea on effort. *J Am Coll Cardiol*. 2020;75:17–26. doi: 10.1016/j.jacc.2019.10.048
37. Myers J, Kaminsky LA, Lima R, Christle JW, Ashley E, Arena R. A reference equation for Normal standards for VO_2 max: analysis from the fitness registry and the importance of exercise National Database (FRIEND registry). *Prog Cardiovasc Dis*. 2017;60:21–29. doi: 10.1016/j.pcad.2017.03.002
38. Wagner PD. Determinants of maximal oxygen transport and utilization. *Annu Rev Physiol*. 1996;58:21–50. doi: 10.1146/annurev.ph.58.030196.000321
39. Katz SD, Maskin C, Jondeau G, Cocke T, Berkowitz R, LeJemtel T. Near-maximal fractional oxygen extraction by active skeletal muscle in patients with chronic heart failure. *J Appl Physiol (1985)*. 2000;88:2138–2142.
40. Jheng J-R, Bai Y, Noda K, Huot JR, Cook T, Fisher A, Chen Y-Y, Goncharov DA, Goncharova EA, Simon MA, et al. Skeletal muscle SIRT3 deficiency contributes to pulmonary vascular remodeling in pulmonary hypertension due to heart failure with preserved ejection fraction. *Circulation*. 2024;150:867–883. doi: 10.1161/CIRCULATIONAHA.124.068624
41. Long GM, Troutman AD, Gray DA, Fisher AJ, Lahm T, Coggan AR, Brown MB. Skeletal muscle blood flow during exercise is reduced in a rat model of pulmonary hypertension. *Am J Physiol Regul Integr Comp Physiol*. 2022;323:R561–R570. doi: 10.1152/ajpregu.00327.2021
42. Schulze KM, Weber RE, Horn AG, Colburn TD, Ade CJ, Poole DC, Musch TI. Effects of pulmonary hypertension on microcirculatory hemodynamics in rat skeletal muscle. *Microvasc Res*. 2022;141:104334. doi: 10.1016/j.mvr.2022.104334
43. Schulze KM, Weber RE, Colburn TD, Horn AG, Ade CJ, Hsu W-W, Poole DC, Musch TI. The effects of pulmonary hypertension on skeletal muscle oxygen pressures in contracting rat spinotrapezius muscle. *Exp Physiol*. 2021;106:2070–2082. doi: 10.1113/EP089631
44. Chamorro N, Pozo RD, García-Lucio J, Torralba Y, Blanco I, López-Meseguer M, Ribas J, Rodríguez D, Barberà JA. Peripheral arterial stiffness and endothelial dysfunction in pulmonary arterial hypertension. *Eur Respir J*. 2015;46(suppl 59):PA2452. doi: 10.1183/13993003.congress-2015.PA2452
45. Singh I, Oliveira RKF, Naeije R, Oldham WM, Faria-Urbina M, Waxman AB, Systrom DM. Systemic vascular distensibility relates to exercise capacity in connective tissue disease. *Rheumatology*. 2021;60:1429–1434. doi: 10.1093/rheumatology/keaa510
46. Zhang P, Da Silva Goncalves Bos D, Vang A, Feord J, McCullough DJ, Zimmer A, D'Silva N, Clements RT, Choudhary G. Reduced exercise capacity occurs before intrinsic skeletal muscle dysfunction in experimental rat models of pulmonary hypertension. *Pulm Circ*. 2024;14:e12358.
47. Potus F, Malenfant S, Graydon C, Mainguy V, Tremblay È, Breuils-Bonnet S, Ribeiro F, Porlier A, Maltais F, Bonnet S, et al. Impaired angiogenesis and peripheral muscle microcirculation loss contribute to exercise intolerance in pulmonary arterial hypertension. *Am J Respir Crit Care Med*. 2014;190:318–328. doi: 10.1164/rccm.201402-0383OC
48. Xu M, Chen X, Chen D, Yu B, Huang Z. FoxO1: a novel insight into its molecular mechanisms in the regulation of skeletal muscle differentiation and fiber type specification. *Oncotarget*. 2016;8:10662–10674.

49. Kosmas K, Michael Z, Papathanasiou AE, Spyropoulos F, Adib E, Jasuja R, Christou H. Skeletal muscle dysfunction in experimental pulmonary hypertension. *Int J Mol Sci*. 2022;23:10912. doi: 10.3390/ijms231810912
50. Xiang G, Ying K, Jiang P, Jia M, Sun Y, Li S, Wu X, Hao S. Growth differentiation factor 11 induces skeletal muscle atrophy via a STAT3-dependent mechanism in pulmonary arterial hypertension. *Skelet Muscle*. 2022;12:10. doi: 10.1186/s13395-022-00292-x
51. dalla Libera L, Zennaro R, Sandri M, Ambrosio GB, Vescovo G. Apoptosis and atrophy in rat slow skeletal muscles in chronic heart failure. *Am J Physiol Cell Physiol*. 1999;277:C982–C986.
52. Campen MJ, Paffett ML, Colombo ES, Lucas SN, Anderson T, Nysus M, Norenberg JP, Gershman B, Hesterman J, Hoppin J, et al. Muscle RING Finger-1 promotes a maladaptive phenotype in chronic hypoxia-induced right ventricular remodeling. *PLoS One*. 2014;9:e97084. doi: 10.1371/journal.pone.0097084
53. Nguyen T, Bowen TS, Augstein A, Schauer A, Gasch A, Linke A, Labeit S, Adams V. Expression of MuRF1 or MuRF2 is essential for the induction of skeletal muscle atrophy and dysfunction in a murine pulmonary hypertension model. *Skelet Muscle*. 2020;10:12. doi: 10.1186/s13395-020-00229-2
54. Bodine SC, Latres E, Baumhueter S, Lai VK-M, Nunez L, Clarke BA, Poueymirou WT, Panaro FJ, Na E, Dharmarajan K, et al. Identification of ubiquitin ligases required for skeletal muscle atrophy. *Science*. 2001;294:1704–1708. doi: 10.1126/science.1065874
55. de Man FS, van Hees HWH, Handoko ML, Niessen HW, Schalij I, Humbert M, Dorfmüller P, Mercier O, Bogaard H-J, Postmus PE, et al. Diaphragm muscle fiber weakness in pulmonary hypertension. *Am J Respir Crit Care Med*. 2011;183:1411–1418. doi: 10.1164/rccm.201003-0354OC
56. Sandri M. Signaling in muscle atrophy and hypertrophy. *Physiology (Bethesda)*. 2008;23:160–170. doi: 10.1152/physiol.00041.2007
57. Beharry AW, Sandesara PB, Roberts BM, Ferreira LF, Senf SM, Judge AR. HDAC1 activates FoxO and is both sufficient and required for skeletal muscle atrophy. *J Cell Sci*. 2014;127:1441–1453. doi: 10.1242/jcs.136390
58. Savai R, Al-Tamari HM, Sedding D, Kojonazarov B, Muecke C, Teske R, Capecchi MR, Weissmann N, Grimminger F, Seeger W, et al. Pro-proliferative and inflammatory signaling converge on FoxO1 transcription factor in pulmonary hypertension. *Nat Med*. 2014;20:1289–1300. doi: 10.1038/nm.3695
59. Stenmark KR, Hu C-J, Pullamsetti SS. How many FOXs are there on the road to pulmonary hypertension? *Am J Respir Crit Care Med*. 2018;198:704–707. doi: 10.1164/rccm.201804-0702ED
60. Barreiro E, Jaitovich A. Muscle atrophy in chronic obstructive pulmonary disease: molecular basis and potential therapeutic targets. *J Thorac Dis*. 2018;10:S1415–S1424. doi: 10.21037/jtd.2018.04.168
61. Doucet M, Russell AP, Léger B, Debigaré R, Joanisse DR, Caron M-A, LeBlanc P, Maltais F. Muscle atrophy and hypertrophy signaling in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2007;176:261–269. doi: 10.1164/rccm.200605-704OC
62. Knapp F, Niemann B, Li L, Molenda N, Kracht M, Schulz R, Rohrbach S. Differential effects of right and left heart failure on skeletal muscle in rats. *J Cachexia Sarcopenia Muscle*. 2020;11:1830–1849. doi: 10.1002/jcsm.12612
63. Manders E, Ruiter G, Bogaard H-J, Stienen GJM, Vonk-Noordegraaf A, de Man FS, Ottenheijm CAC. Quadriceps muscle fibre dysfunction in patients with pulmonary arterial hypertension. *Eur Respir J*. 2015;45:1737–1740.
64. Green HJ, Burnett M, Duhamel TA, D'Arsigny C, O'Donnell DE, Webb KA, Ouyang J. Abnormal sarcoplasmic reticulum Ca2+−sequestering properties in skeletal muscle in chronic obstructive pulmonary disease. *Am J Physiol Cell Physiol*. 2008;295:C350–C357. doi: 10.1152/ajpcell.00224.2008
65. Middlekauff HR. Making the case for skeletal myopathy as the major limitation of exercise capacity in heart failure. *Circ Heart Fail*. 2010;3:537–546. doi: 10.1161/CIRCHEARTFAILURE.109.903773
66. Lee MH, Menezes TCF, Reisz JA, Ferreira EVM, Graham BB, Oliveira RKF. Exercise metabolomics in pulmonary arterial hypertension: where pulmonary vascular metabolism meets exercise physiology. *Front Physiol*. 2022;13:963881:1–11. doi: 10.3389/fphys.2022.963881
67. Malenfant S, Potus F, Fournier F, Breuils-Bonnet S, Pflieger A, Bourassa S, Tremblay È, Nehmé B, Droit A, Bonnet S, et al. Skeletal muscle proteomic signature and metabolic impairment in pulmonary hypertension. *J Mol Med (Berl)*. 2015;93:573–584. doi: 10.1007/s00109-014-1244-0
68. Humbert M, Sitbon O, Yaïci A, Montani D, O'Callaghan DS, Jaïs X, Parent F, Savale L, Natali D, Günther S, et al. Survival in incident and prevalent cohorts of patients with pulmonary arterial hypertension. *Eur Respir J*. 2010;36:549–555.
69. McGoon MD, Miller DP. Reveal: a contemporary US pulmonary arterial hypertension registry. *Eur Respir Rev*. 2012;21:8–18.
70. Chung L, Liu J, Parsons L, Hassoun PM, McGoon M, Badesch DB, Miller DP, Nicolls MR, Zamanian RT. Characterization of connective tissue disease-associated pulmonary arterial hypertension from REVEAL. *Chest*. 2010;138:1383–1394. doi: 10.1378/chest.10-0260
71. Galiè N, Olschewski H, Oudiz RJ, Torres F, Frost A, Ghofrani HA, Badesch DB, McGoon MD, McLaughlin VV, Roecker EB, et al. Ambrisentan for the treatment of pulmonary arterial hypertension. *Circulation*. 2008;117:3010–3019.
72. Paik JJ, Wigley FM, Mejia AF, Hummers LK. Independent Association of Severity of muscle weakness with disability as measured by the Health Assessment Questionnaire disability index in scleroderma. *Arthritis Care Res*. 2016;68:1695–1703. doi: 10.1002/acr.22870
73. Lóránd V, Czirják L, Minier T. Musculoskeletal involvement in systemic sclerosis. *Presse Med*. 2014;43:e315–e328. doi: 10.1016/j.lpm.2014.03.027
74. Corallo C, Cutolo M, Volpi N, Franci D, Aglianò M, Montella A, Chirico C, Gonnelli S, Nuti R, Giordano N. Histopathological findings in systemic sclerosis-related myopathy: fibrosis and microangiopathy with lack of cellular inflammation. *Ther Adv Musculoskelet Dis*. 2017;9:3–10. doi: 10.1177/1759720X16671928
75. Varga J, Trojanowska M, Kuwana M. Pathogenesis of systemic sclerosis: recent insights of molecular and cellular mechanisms and therapeutic opportunities. *J Scleroderma Relat Disord*. 2017;2:137–152. doi: 10.5301/jsrd.5000249
76. Volpi N, Pecorelli A, Lorenzoni P, Di Lazzaro F, Belmonte G, Aglianò M, Cantarini L, Giannini F, Grasso G, Valacchi G. Antiangiogenic VEGF isoform in inflammatory myopathies. *Mediat Inflamm*. 2013;2013:219313. doi: 10.1155/2013/219313
77. Nikitorowicz-Buniak J, Denton CP, Abraham D, Stratton R. Partially evoked epithelial-mesenchymal transition (EMT) is associated with increased TGFβ signaling within lesional scleroderma skin. *PLoS One*. 2015;10:e0134092. doi: 10.1371/journal.pone.0134092
78. Manetti M, Guiducci S, Romano E, Bellando-Randone S, Lepri G, Bruni C, Conforti ML, Ibba-Manneschi L, Matucci-Cerinic M. Increased plasma levels of the VEGF165b splice variant are associated with the severity of nailfold capillary loss in systemic sclerosis. *Ann Rheum Dis*. 2013;72:1425–1427. doi: 10.1136/annrheumdis-2012-203183
79. Bratoiu I, Burlui AM, Cardoneanu A, Macovei LA, Richter P, Rusu-Zota G, Rezus C, Badescu MC, Szalontay A, Rezus E. The involvement of smooth muscle, striated muscle, and the myocardium in scleroderma: a review. *Int J Mol Sci*. 2022;23:12011. doi: 10.3390/ijms231912011
80. Mogyoróssy S, Nagy-Vincze M, Griger Z, Dankó K, Szabó NA, Szekanecz Z, Szűcs G, Szántó A, Bodoki L. Novel aspects of muscle involvement in immune-mediated inflammatory arthropathies and connective tissue diseases. *Autoimmun Rev*. 2023;22:103311. doi: 10.1016/j.autrev.2023.103311
81. Lim KL, Abdul-Wahab R, Lowe J, Powell RJ. Muscle biopsy abnormalities in systemic lupus erythematosus: correlation with clinical and laboratory parameters. *Ann Rheum Dis*. 1994;53:178–182. doi: 10.1136/ard.53.3.178
82. Jakati S, Rajasekhar L, Uppin M, Challa S. Sle myopathy: a clinicopathological study. *Int J Rheum Dis*. 2015;18:886–891.
83. Stenström CH, Minor MA. Evidence for the benefit of aerobic and strengthening exercise in rheumatoid arthritis. *Arthritis Rheum*. 2003;49:428–434. doi: 10.1002/art.11051
84. Ali Z, Ranganathan P, Perry A, Gelbart M. Localized striated muscle vasculitis in rheumatoid arthritis. *J Clin Rheumatol*. 2007;13:35.
85. Ancu C, Pomîrleanu DC, Anton C-R, Moraru E, Anton E, Chirieac RM, Ancu E. Rheumatoid myositis, myth or reality? A clinical, imaging and histological study. *Romanian J Morphol Embryol*. 2014;55:781–785.
86. Santo RCE, Fernandes KZ, Lora PS, Filippin LI, Xavier RM. Prevalence of rheumatoid cachexia in rheumatoid arthritis: a systematic review and meta-analysis. *J Cachexia Sarcopenia Muscle*. 2018;9:816–825. doi: 10.1002/jcsm.12320
87. Lindvall B, Bengtsson A, Ernerudh J, Eriksson P. Subclinical myositis is common in primary Sjögren's syndrome and is not related to muscle pain. *J Rheumatol*. 2002;29:717–725.

88. Kissel JT, Halterman RK, Rammohan KW, Mendell JR. The relationship of complement-mediated microvasculopathy to the histologic features and clinical duration of disease in dermatomyositis. *Arch Neurol*. 1991;48:26–30. doi: 10.1001/archneur.1991.00530130034016
89. Emslie-Smith AM, Engel AG. Microvascular changes in early and advanced dermatomyositis: a quantitative study. *Ann Neurol*. 1990;27:343–356. doi: 10.1002/ana.410270402
90. Dao T, Green AE, Kim YA, Bae S-J, Ha K-T, Gariani K, Lee M-R, Menzies KJ, Ryu D. Sarcopenia and muscle aging: a brief overview. *Endocrinol Metab (Seoul)*. 2020;35:716–732. doi: 10.3803/EnM.2020.405
91. Kumar R, Lee MH, Kassa B, Fonseca Balladares DC, Mickael C, Sanders L, Andruska A, Kumar M, Spiekerkoetter E, Bandeira A, et al. Repetitive schistosoma exposure causes perivascular lung fibrosis and persistent pulmonary hypertension. *Clin Sci (Lond)*. 2023;137:617–631. doi: 10.1042/CS20220642
92. Mamazhakypov A, Veith C, Schermuly RT, Sydykov A. Surgical protocol for pulmonary artery banding in mice to generate a model of pressure-overload-induced right ventricular failure. *STAR Protoc*. 2023;4:102660. doi: 10.1016/j.xpro.2023.102660
93. Nici L. Pulmonary rehabilitation: mechanisms of functional loss and benefits of exercise. *Respir Care*. 2024;69:640–650. doi: 10.4187/respcare.11705
94. Mainguy V, Maltais F, Saey D, Gagnon P, Martel S, Simon M, Provencher S. Effects of a rehabilitation program on skeletal muscle function in idiopathic pulmonary arterial hypertension. *J Cardiopulm Rehabil Prev*. 2010;30:319–323. doi: 10.1097/HCR.0b013e3181d6f962