Published in final edited form as:

Heart Fail Rev. 2017 March ; 22(2): 167–178. doi:10.1007/s10741-016-9586-z.

Skeletal Muscle Bioenergetics in Aging and Heart Failure

Sophia Z. Liu¹ and David J. Marcinek^{1,2,3,*}

¹Departments of Radiology, University of Washington, Seattle WA 98109

²Department of Pathology, University of Washington, Seattle WA 98109

³Department of Bioengineering, University of Washington, Seattle WA 98109

Abstract

Changes in mitochondrial capacity and quality play a critical role in skeletal and cardiac muscle dysfunction. In vivo measurements of mitochondrial capacity provide a clear link between physical activity and mitochondrial function in aging and heart failure, although the cause and effect relationship remains unclear. Age-related decline in mitochondrial quality leads to mitochondrial defects that affect redox, calcium, and energy sensitive signaling by altering the cellular environment and lead to skeletal muscle dysfunction independent of reduced mitochondrial capacity. This reduced mitochondrial quality with age is also likely to sensitize skeletal muscle mitochondria to elevated angiotensin or beta-adrenergic signaling associated with heart failure. This synergy between aging and heart failure could further disrupt cell energy and redox homeostasis and contribute to exercise intolerance in this patient population. Therefore the interaction between aging and heart failure, particularly with respect to mitochondrial dysfunction, should be a consideration when developing strategies to improve quality of life in heart failure patients. Given the central role of the mitochondria in skeletal and cardiac muscle dysfunction, mitochondrial quality may provide a common link for targeted interventions in these populations.

Keywords

aging; heart failure; mitochondria; skeletal muscle

Introduction

The number of older adults (60 years or over) worldwide was 841 million in 2013 (four times higher than in 1950) and will almost triple by 2050, when it is expected to exceed two billion [1]. As people are living longer and the size of the elderly population increases so does the potential individual and societal burden of poor health and loss of independence. Because aging is the greatest risk factor for most chronic diseases, including heart failure, it is critical that we consider the potential interactions between primary disease and aging in research focused on mitigating the effects of chronic disease.

*Corresponding Author **Address for correspondence:** David J. Marcinek, Department of Radiology, Box 358050, University of Washington, Seattle, WA 98109, Phone: 206-221-6785, FAX: 206-616-7195, dmarc@uw.edu.
David Marcinek serves as a paid consultant to Stealth BioTherapeutics. Stealth is developing the compound SS-31 for clinical use. Sophia Liu has not conflict of interests to report.

A key factor contributing to loss of function in aging and chronic disease, especially heart failure, is the decrease of skeletal muscle function. Age-related loss of muscle mass and strength, defined as sarcopenia, is a key factor contributing to reduced exercise tolerance, increased all-cause morbidity, and loss of independence [2]. Aging individuals experience a ~10-20% per decade decline in skeletal muscle mass and 15% loss in leg muscle strength starting the fifth decade of life [3]. Compounding the health effects of age-related sarcopenia is the increased burden of chronic disease in the elderly, particularly cardiovascular disease. Age is the greatest risk factor for the development of heart failure, which has an incidence of less than 1% in 20-30s but more than 20% in the 80s [2]. As the leading cause of death in the United States heart failure costs the nation an estimated \$32 billion each year [4], and like aging, is associated with significant skeletal muscle dysfunction that contributes to reduced mobility and loss of independence. Thus improving quality of life in heart failure patients will depend not only on treating the cardiac dysfunction, but also the associated skeletal muscle deficits. Given the close relationship between aging and heart failure it is critical to consider the interaction between these conditions. This review will focus on the potential interactions between aging and heart failure on bioenergetics and mitochondrial function in the skeletal muscle.

Mitochondria in skeletal muscle (dys)function

It is becoming increasingly clear that mitochondria play a critical role in the loss of function associated with aging and chronic disease, particularly in high energy demand tissues like muscle, heart, and brain. This can be attributed to their place as a nexus between cell energy metabolism, redox biology, and cell signaling. ATP production by oxidative phosphorylation (OXPHOS) in the mitochondria is responsible for meeting the majority of ATP demand in skeletal muscle. ATP is produced through oxidation of metabolic fuel to provide reducing equivalents (NADH and FADH₂) that are used to generate a proton motive force across the inner mitochondrial membrane (IMM), which is then used to drive ATP synthesis through the F1FO ATPase in the IMM.

Mitochondria are also significant source of reactive oxygen species production in skeletal muscle. Superoxide (O₂⁻) is formed as a by-product of multiple electron transfer reactions of OXPHOS [5-7] and is then rapidly converted into other reactive oxygen (e.g. H₂O₂, OH⁻) and nitrogen species (ONOO⁻, NO₂, ...) by catalytic and small molecule antioxidants in the mitochondria and the cell. In addition, many mitochondrial enzymes such as nicotinamide nucleotide transhydrogenase (NNT), isocitrate dehydrogenase (IDH2), malic enzyme (ME), and glutamate dehydrogenase (GDH) play an essential role in regulating NAD(P)H/NAD(P)⁺ ratios and maintaining the cellular redox potential [8-10]. Thus, in addition to their role in maintaining energy homeostasis, the mitochondria are key contributors to redox homeostasis.

Many chronic diseases are associated with periods of sustained elevation of mitochondrial oxidant production that disrupts redox homeostasis and can ultimately lead to oxidative damage [11,12]. The interaction between mitochondrial energy metabolism and redox biology is also closely linked to their role in calcium buffering. The net negative charge across the inner mitochondrial membrane facilitates calcium uptake by the mitochondrial

matrix. This physiology plays an important role in calcium buffering in excitable cells like skeletal muscle [13]. However, excess calcium uptake leads to elevated mitochondrial oxidative stress, dissipation of the proton motive force, opening of the permeabilization transition pore, ultimately resulting in initiation of apoptosis or necrosis [13,14]. Under less extreme conditions the elevated oxidative stress can impair energy metabolism and lead to muscle dysfunction through oxidative damage or disruption of redox dependent signaling [15].

Meeting muscle ATP demand

The most obvious way in which mitochondrial dysfunction contributes to limitations of skeletal muscle performance is through the loss of mitochondrial capacity, defined as a reduced ability of the mitochondria to meet the ATP demand of the working muscle. Skeletal muscle ATP demand at rest is primarily driven by Na⁺/K⁺ pump and protein turnover [16,17]. Matching supply to resting ATP demand is essential for the maintenance of sarcolemma and T-tubular action ion gradients and to maintain cell homeostasis. During skeletal muscle contraction the ATP demand is primarily determined by mechanical output of cross bridge activity and calcium cycling [18,19]. Thus, tight coupling of ATP demand to ATP synthesis is important for muscle health and performance [20]. ATP demand during muscle contraction can be met using the phosphocreatine system, glycolysis, or oxidative phosphorylation. ATP demand during brief periods (seconds) of intense muscle activity can be met by the phosphocreatine/creatine kinase system and glycolytic ATP production. However, prolonged muscle contractions associated with sustained exercise and normal daily activities requires mitochondrial ATP production to meet the increased demand. In healthy skeletal muscle the maximum mitochondrial ATP production far exceeds the resting ATP demand. The theoretical maximum mitochondrial ATP production is estimated to be 40-fold and 20-fold greater than resting ATP production in humans [21] and mice [22,23], respectively. Thus under normal conditions there is sufficient reserve capacity to meet ATP demands and skeletal muscle mitochondria are seldom called upon to work at maximum capacity. However, the mitochondrial capacity sets an upper threshold that defines the ability of the cell to respond to transient increases in ATP demand due to increased muscle work. Therefore, as capacity declines the normal physiological conditions can exceed the ability of the cell to meet ATP demand and lead to muscle failure, fatigue, and exercise intolerance. Even in cases where the muscle is working below the energetic threshold a reduced mitochondrial capacity or efficiency requires the mitochondria to work at a larger fraction of their capacity to meet ATP demand leading to altered energy homeostasis [24]

Relationship between VO_{2max} and exercise tolerance and mitochondrial function

Clinically, maximum oxygen uptake, VO_{2max}, is a predictor of exercise tolerance and is a strong prognostic measurement for morbidity and mortality [25]. A VO_{2max} of ~17.5 ml/kg/min is the threshold for increased risk of morbidity and powerful predictor of mortality [25]. The decline in exercise tolerance and VO_{2max} with heart failure is well characterized and is discussed extensively in this issue. Maximal oxygen consumption also decreases with age at a rate of approximately 3-6% per decade in untrained population start from 20s and 30s. The rate of decline is accelerated under advanced age (>70) and disease conditions [26], which put heart failure patients closer to threshold for loss of independence.

The primary chronic symptom in HF is severe exercise intolerance with reduced $\text{VO}_{2\text{max}}$ /peak. $\text{VO}_{2\text{max}}$ is the product of cardiac output and Arterial-Venous oxygen difference ($\text{A-VO}_{2\text{diff}}$), which is associated with skeletal muscle oxygen diffusive capacity, O_2 transport, and fiber type composition[27]. Molina et al. [28] reported severely reduced $\text{VO}_{2\text{peak}}$ in heart failure patients with preserved left ventricular ejection fraction. Only about 50% of this decreased $\text{VO}_{2\text{peak}}$ is accounted for by reduced cardiac output suggesting a significant role for skeletal muscle dysfunction in exercise tolerance experienced by this population.

As activity level increases oxygen consumption by the skeletal muscle consumes a larger fraction of the total body oxygen uptake. Therefore maximum oxygen consumption provides an estimate of muscle oxygen consumption and thus mitochondrial function. Several studies have demonstrated a linear relationship between $\text{VO}_{2\text{max}}$ and muscle mitochondrial content and capacity across multiple species and humans [29], including healthy [30,31], aged [26], and heart failure [32,33] populations. Despite this relationship the capacity of skeletal muscle to consume oxygen is a complex phenotype that is affected by oxygen delivery and extraction, in addition to the oxidative capacity of the muscles. Measurement of $\text{VO}_{2\text{max}}$ has the advantage of being directly related to exercise tolerance making it both clinically and physiologically relevant. However, it does not provide an unequivocal measure of muscle mitochondrial function due to potential limitations to muscle oxygen delivery (reviewed by Wray et al in this issue). Thus, more direct measures of mitochondrial function are necessary to assess the effect of aging and heart failure on muscle mitochondria.

In vivo measurements of reduced mitochondrial capacity

Mitochondria are frequently viewed as isolated kidney-shaped structures in the cell that are responsible for oxidizing metabolic fuel to produce ATP in order to meet cell energy demands. As discussed above mitochondria are also dynamic organelles that interact with factors in their cell environment such as calcium concentrations and redox state. Thus, they can be viewed as important integrators of cell energy metabolism and cell signaling that are able to control diverse cellular pathways by performing diverse functions such as altering metabolite levels [34], redox environment [35,36], and calcium homeostasis [37-39]. For this reason direct measures of mitochondrial function *in vivo* are important tools for assessing mitochondrial dysfunction in skeletal muscle. One common direct measure of *in vivo* mitochondrial capacity is to use ^{31}P NMR spectroscopy to measure phosphocreatine dynamics. In this approach a brief period of ischemia or exercise is used to disrupt energy homeostasis and force the muscle to consume phosphocreatine (PCr) to meet ATP demand. The rate of return of PCr to resting levels is then used to calculate the theoretical maximum capacity for mitochondrial ATP production (ATP_{max}) (reviewed in [40,41]). In healthy muscles ATP_{max} correlates with mitochondrial content measured by citrate synthase activity and *ex vivo* respiratory capacity in human muscle [42], mitochondrial volume density by EM [43,44], and maximal mitochondrial respiratory capacity in permeabilized fibers [45].

Another recently developed approach assesses *in vivo* mitochondrial capacity using optical spectroscopy to measure the oxygen saturation state of hemoglobin and myoglobin to calculate the rate of oxygen consumption during recovery from brief periods of exercise or ischemia in muscle tissue [46-48]. This approach is based on the role of hemoglobin and

myoglobin in binding greater than 95% of the oxygen in the muscle. This novel development uses short (5-10s) periods of cuff inflation to block blood flow to the muscle throughout the recovery period [46] and correction for changes in blood volume during the recovery phase to allow the determination of the rate of oxygen consumption throughout the recovery phase [46]. In addition the short ischemia allows this protocol to separate muscle oxygen consumption from oxygen delivery, which confounded earlier attempts to use near-infrared spectroscopy (NIRS) during the recovery phase [49,50]. This NIRS approach represents a valuable tool for assessing *in vivo* mitochondrial capacity where use of NMR spectroscopy is limited by patient condition or access to instrumentation.

Several reports have demonstrated that *in vivo* skeletal muscle mitochondrial capacity declines in humans [51,52] and animal models [22,53] with both age and in heart failure [54]. Using ^{31}P NMR Conley et al [21] reported that both ATPmax and mitochondrial volume density decline with age in human quadriceps. The flux per mitochondrial volume also declines indicating that the loss of capacity in this muscle is due to both reduced content and quality of the mitochondria. A similar decline in *in vivo* ATPmax has been observed in aged mouse skeletal muscle [23,53]. In addition, Bhella et al. [55] also found reduced muscle oxidative metabolism by ^{31}P NMR analysis. In contrast to human muscle the lower ATPmax in mice was associated with elevated markers of mitochondrial content [23], suggesting greater mitochondrial defects (lower flux per unit mitochondria) in the mouse model. Southern et al. [54] used the NIRS approach described above to demonstrate a decline muscle oxidative capacity in forearm muscles of heart failure patients compared to healthy controls. These results are consistent with earlier observations that heart failure patients underwent greater energy stress (e.g. elevated Pi/PCr, and pH) following exercise than did healthy controls in the absence of differences in muscle oxygenation [56] and blood flow [57]. Further, under ischemic exercise heart failure patients had abnormal skeletal muscle metabolism compared to controls suggesting intrinsic muscle (peripheral) dysfunction rather than central (cardiovascular) limitation [58]. Interestingly, they did not find abnormalities of any intrinsic metabolic enzymes or correlation between biopsy and metabolic characteristics *in vivo*. The author suggested the importance of muscle mass loss could contribute to increased load for each fiber resulting in greater metabolic changes measured by the ratio of Pi/PCr *in vivo* [58]. Another potential mechanism for this disagreement between *in vivo* and *ex vivo* measures of mitochondrial function could be the influence of the cell environment on mitochondrial function. This is further discussed below.

There is ongoing debate whether the decline in skeletal muscle *in vivo* mitochondrial capacity with age is an intrinsic property of aging or dependent on reduced activity levels. In studies where activity level was carefully controlled through both questionnaires and monitoring no differences were observed for *in vivo* ATPmax in plantar flexion [59,60] and dorsiflexion [61] muscle groups between sedentary, activity-matched young and elderly groups. Comparing within age groups also demonstrates a clear interaction between *in vivo* mitochondrial function and activity level. Within a cohort of young adults sedentary lifestyle replicates the effects of age on mitochondrial function compared to a moderately active cohort [62]. This relationship also extends to elderly populations where ATPmax was correlated with preferred walking speed [45] and greater self-reported fatigability [63]. While it is not possible to determine causality from these association studies, they do

suggest that there is a close relationship between impaired mitochondrial function and exercise intolerance.

These *in vivo* measurements are key to understanding the interaction between aging, heart failure and mitochondrial function in skeletal muscle as they provide direct physiologically relevant measurements of peripheral energy metabolism. These studies clearly demonstrate that there is a functional limitation in aging skeletal muscle that reduces the reserve capacity for responding increasing metabolic demands. In this context, any added effect of heart failure will further reduce energetic reserves and close the gap between resting metabolism and maximal activity. Due to the limited ability to manipulate the system *in vivo*, combining *in vivo* analysis with *ex vivo* measurements provides an important strategy to better understand the mechanisms contributing to the functional declines in both aging and heart failure populations.

Reduced coupling of oxidative phosphorylation

In addition to loss of capacity there is a significant decline in the quality of mitochondria in aged skeletal muscle. Combining ^{31}P NMR with *in vivo* optical spectroscopy allows the measurement of resting ATP production and oxygen consumption by the skeletal muscle in humans and rodent models [24,64]. Using this approach we have found that the coupling of oxidative phosphorylation (P/O) declines with age in human [52] and mouse [23,24,53] skeletal muscles. This reduced coupling of OXPHOS was also observed in sedentary young adults compared to age matched controls supporting an important role for physical activity in regulating mitochondrial quality [62]. Altered redox stress provides a potential link between activity level, aging, and *in vivo* P/O. Increased redox stress in skeletal muscle with age is well-documented and is also elevated in sedentary individual [23,53,65-68]. Support for a role of redox stress in regulating *in vivo* P/O comes from mouse skeletal muscle where an acute, mild increase in redox stress associated with low dose paraquat treatment reduces P/O and ATPmax [22,23]. More importantly, acutely reversing mitochondrial redox stress with the mitochondrial targeted peptide elamipretide (SS-31) reverses age-related declines in P/O and ATPmax in mouse muscles [53]. These rapid responses to altered redox homeostasis, 24 hrs to paraquat and 1h to SS-31, suggest an important role for dynamic control of mitochondrial function by the cell environment through post-translational modification (PTM) to proteins [53,69]. Support for this hypothesis comes from studies identifying increased phosphorylation and acetylation of mitochondrial proteins as mechanism as mechanisms contributing to cardiac stress in heart failure [70,71].

The evidence indicates that elevated skeletal muscle oxidative stress whether associated with chronic conditions such as aging and heart failure or sustained inactivity can alter cell energetics. It is easy to see how chronic elevation of redox stress due to either aging or a sedentary lifestyle could result in bioenergetics deficits that would exacerbate those associated with heart failure and result in a downward spiral of increased inactivity, exercise intolerance, and muscle pathology.

Loss of mitochondrial quality

Pathways controlling skeletal muscle biogenesis and quality are disrupted in aging skeletal muscle. One of the key regulators of mitochondrial function is the peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1 α). PGC-1 α plays an important role in regulating mitochondrial biogenesis and antioxidant capacity [72-74] by interacting with multiple transcription factors to coordinate mitochondrial and antioxidant gene expression [75-79] including peroxisome proliferator-activated receptors (PPARs), nuclear respiratory factor family (NRF-1 and -2), estrogen-related receptor (ERR α), and mitochondrial transcription factor (TFAM). Many studies have demonstrated age-related declines in PGC-1 α expression in skeletal muscle in humans and rodent models [77,79,80,81] (Table 1) and skeletal muscle specific overexpression of PGC-1 α improves muscle mitochondrial function and performance in aged mice [78]. PGC-1 α is also regulated by AMPK and SIRT1 through manipulation of phosphorylation and acetylation PTMs. These PTMs determine localization of PGC-1 α between the cytoplasm and nucleus and thus regulate its activity [82,79]; phosphorylation of PGC-1 α facilitates its translocation into the nucleus, while deacetylation by SIRT1 traps PGC-1 α in the nucleus and increases its transcriptional co-activation [82]. The influence of AMPK and SIRT1 on PGC-1 α provides a critical link between the cellular environment and mitochondrial adaptation. Activation of AMPK by phosphorylation is facilitated by elevated AMP/ATP ratios in the cell, which is the result of energy stress [82]. AMPK is also activated in response to increased oxidative stress and elevated cytosolic calcium. Similarly, elevated NAD, also indicative of energy stress, enhances SIRT1 deacetylation of PGC-1 α and increased activity [83,84]

Disruption of this coordinated signaling between the mitochondria, cell environment and nucleus with age is likely to contribute to dysregulation of mitochondrial protein expression, lead to poor mitochondrial quality, and impair the ability of the muscle to respond to energy and redox stressors. Reduced cytoplasmic and nuclear NAD levels and elevated HIF-1 α signaling were associated with disruption of the coordinated expression between nuclear and mitochondrially-encoded proteins and poor stoichiometry of the electron transport system in gastrocnemius muscles [85], while elevation of NAD levels by supplementing the diet with NAD precursors improved mitochondrial stoichiometry. A similar loss of ETS stoichiometry was observed in the extensor digitorum longus (EDL) muscles of aged mice [86]. Analyses of the age-related changes in the mitochondrial proteome indicated that complex I proteins were preferentially elevated with age leading to inefficient respiratory function in the EDL, while the slow-twitch soleus muscle maintained ETS stoichiometry and mitochondrial efficiency with age [86]. These data support a disruption of coordinated signaling with age that will impair the ability of skeletal muscles to respond to physiological and pathological stress and would likely potentiate the effects of heart failure on skeletal muscle mitochondrial function.

In contrast to aging muscle there has been relatively little work examining molecular regulation of mitochondrial biogenesis and quality control in skeletal muscle myopathy associated with heart failure. When matched for activity level heart failure patients demonstrated similar levels mRNA expression for markers mitochondrial biogenesis (PGC-1 α and PGC-1 β , TFAM, NRF-1) and mitochondrial content in the vastus lateralis

compared to control subjects [87]. However, a recent study in mice supports a role for dysregulation of skeletal muscle mitochondria with heart failure. Zizola et al [88] demonstrated that stimulation of PPAR δ reversed the declines in fatty acid oxidation in skeletal muscle and exercise intolerance when initiated 8 weeks following experimentally-induced myocardial infarction (MI) in mice. In spite of the reduced fatty acid oxidation there was no change in citrate synthase or succinate dehydrogenase activities following MI in the skeletal muscles suggesting the deficit was not due to a general loss of mitochondria content in the skeletal muscle with heart failure, but rather loss of specific aspects of function. A similar decrement in fatty acid oxidation, that was also reversible by stimulation of the PPAR δ pathway, could be induced in C2C12 cells by treating with TNF- α , suggesting that circulating inflammatory cytokines induced by the myocardial infarction may contribute to this disruption of mitochondrial function with heart failure [32,88].

Other studies have observed decreased activities of mitochondrial marker enzymes in skeletal muscle homogenates from dogs with pacing induced heart failure [89-91] and human dilated cardiac myopathy [91,92]. However, this loss of mitochondrial enzyme activity, particularly in human studies, varies between laboratories and appears to be dependent on the model and mitochondrial marker used. Toth et al observed no significant effect of heart failure on citrate synthase and cytochrome oxidase activities or mitochondrial volume density in skeletal muscle of humans subjects matched for activity level, although they note a shift toward a larger size of mitochondria in the vastus lateralis of the heart failure patients [87]. Heart failure patients receiving optimum medical and device therapy also showed no changes in mitochondrial marker enzymes despite skeletal muscle abnormalities and diminished exercise capacity [93]. Therefore, reduced mitochondrial enzyme activities or even reduced content measured with EM is not a universal characteristic of skeletal muscle in heart failure, especially in human studies where controls and patients are matched for activity level.

The focus on *in vivo* oxidative capacity, *ex vivo* respiratory capacity, and mitochondrial enzyme activities may be missing more subtle underlying mitochondrial defects contributing to skeletal muscle dysfunction in both aging and heart failure. The intermyofibrillar (IFM) mitochondria from skeletal muscle of dogs with pacing induced heart failure demonstrated a specific defect in the phosphorylation system attributed to a shift in the isoform expression of the adenine nucleotide transporter. This suggests that this impairment was due to altered ability to exchange adenine nucleotides between the matrix and cytoplasm and was not associated with reduced specific activities of other complexes of the ETS in the IFM. However, the subsarcolemmal mitochondrial population demonstrated a defect in the ETS that was not relieved by bypassing the phosphorylation component of oxidative phosphorylation [91]. The breakdown in stoichiometry of the mitochondria can also be time-dependent. At 2 weeks following pressure overload induced heart failure mitochondria from rat gastrocnemius muscle had significantly elevated activities of ETS complexes I and II with unchanged complex III and IV activities [94] (Table 1). By 20 weeks this progressed to reduced activities for all four ETS complexes.

Disruption of ETS stoichiometry, particularly complex I, and inhibition of the phosphorylation system, in aging and heart failure are significant even in the absence of

reduced capacity because it results in elevated mitochondrial superoxide production and oxidative stress [86,94]. Recent work demonstrates that mitochondrial oxidative stress plays an important role in skeletal muscle dysfunction in both conditions. Skeletal muscle mitochondria from aged mice and those with heart failure have an increased capacity to produce H₂O₂ [53,95]. This increased mitochondrial oxidative stress can control mitochondrial function both *in vivo* [22,23] and *ex vivo* [96,97]. Inducing a mild oxidative stress in adult mice for 24 hours using low doses of paraquat recapitulates the reduced mitochondrial oxidative capacity (ATPmax), coupling efficiency (P/O) and depression of skeletal muscle metabolism observed *in vivo* in aged skeletal muscle [22,23]. The greater sensitivity of aged muscle to this stress is consistent with a decline in the ability of the aged skeletal muscle to buffer transient increases in oxidative stress [98-100].

Systemic effects linking heart failure to skeletal muscle mitochondrial deficits

Age-related mitochondrial deficits are the manifestation of underlying environmental, biochemical, genetic, and physiological conditions that accumulate over a lifetime. In contrast, skeletal muscle mitochondrial dysfunction in heart failure generally develops over a shorter period of time. One potential mechanism linking pathophysiology in the heart to mitochondrial dysfunction in the skeletal muscle is systemic beta-adrenergic stimulation. In the failing heart there is an increased release of norepinephrine and epinephrine by the cardiac nerves in an apparent attempt to increase heart rate and maintain cardiac output [101]. This results in a systemic beta-adrenergic stimulation, including the adrenergic receptors in skeletal muscle. In the cardiac muscle this elevated adrenergic stimulation leads to an increase in mitochondrial biogenesis, while in the skeletal muscle acute excessive adrenergic stimulation does not increase PGC1 α activation and mitochondrial biogenesis [102]. Furthermore, treatment of humans with clenbuterol, a beta2 agonist, led to reduced mitochondrial content and fatty acid oxidation in rat skeletal muscle [103]. One potential link between elevated adrenergic stimulation and skeletal muscle mitochondrial dysfunction is disruption of phospho-PTM of mitochondrial proteins through the cAMP-PKA pathway [104]. In this scenario stimulation of adrenergic receptors in the skeletal muscle leads to elevated cAMP in the mitochondria, particularly in the inner membrane space, which activates PKA dependent phosphorylation of mitochondrial proteins. Studies of the mitochondrial proteome indicate that over 60 proteins are phosphorylated, including subunits of each complex of the ETS [105,106]. Elevated phosphorylation of complex IV subunits can disrupt incorporation of complex IV into the supercomplex structure and reduce oxidative phosphorylation capacity [101,107]. This model is based on decreased formation of supercomplexes in the ETS resulting in impaired oxidative phosphorylation and elevated superoxide formation by the mitochondria. The increase in oxidative stress would then further disrupt mitochondrial function leading to a feedforward mechanism.

The renin-angiotensin system provides another potential mechanism linking cardiac dysfunction to mitochondrial deficits in the skeletal muscle. In response to low blood pressure the enzyme renin is secreted from the kidneys into the blood where it cleaves angiotensinogen to angiotensin I (ANG I), which is ultimately cleaved to angiotensin II (ANG II). Heart failure is associated with increased circulating ANG II [108,109]. Chronic infusion with ANG II leads to muscle atrophy through increase muscle protein degradation

and apoptosis [108], reduced exercise tolerance, and impaired mitochondrial function [110]. In a mouse model of high fat diet induced type 2 diabetes blocking ANG II receptors improved both mitochondrial function and exercise capacity, which supports a causal role for elevated ANG II in skeletal muscle mitochondrial dysfunction in heart disease [111].

The effect of elevated ANG II on mitochondrial deficits may be indirect. In the skeletal and cardiac muscle elevated ANG II leads to increased ROSs production by NADPH oxidases and increased oxidative stress [112-116]. Despite the primary stimulation of NADPH derived ROS, reducing mitochondrial ROS production, either by genetically expressing catalase in the mitochondria or treatment with the mitochondrial targeted peptide SS-31, prevents ANG II induced hypertrophy indicating that mitochondrial oxidative stress plays an important role in the ANG II induced mitochondrial dysfunction and pathology, at least in the heart [12,117,118] (Table 1). These data suggest that elevated ANG II increases ROS production from non-mitochondrial sources initiating a feed-forward cycle of mitochondrial dysfunction and further elevation of redox stress and mitochondrial deficits.

Altered calcium handling provides another potential pathway where either adrenergic stimulation through the cAMP-PKA system and/or the renin-angiotensin system could contribute to skeletal muscle mitochondrial deficits in heart failure. Excessive post-translational modification of the sarcoplasmic reticulum calcium release channel ryanodine receptor (RyR) through both PKA dependent phosphorylation [119,120] or thiol modification in response to redox stress [119,120] destabilizes the RyR and increases the open probability of the channel. This leads to increase calcium leak, elevated mitochondrial ROS production, and mitochondrial deficits that contribute to muscle dysfunction and atrophy [119]. In aged mouse muscle preventing calcium leak by stabilizing the interaction between the RyR and calstabin or reducing mitochondrial oxidative stress with mCAT both improved skeletal muscle function [121]. These data highlight how age-related changes in redox stress may synergize with systemic effects of cardiac dysfunction to magnify mitochondrial deficits and skeletal muscle dysfunction associated with heart disease.

Reduced mitochondrial quality control

One key point of interaction between aging and heart failure on mitochondrial dysfunction in skeletal muscle is the age-related decline in mitochondrial quality control. Autophagy is the primary pathway used to degrade organelles in the cell [122]. This process involves targeting damaged or dysfunctional cellular constituents to the lysosome through either microautophagy, macroautophagy, or chaperone mediated autophagy and is the main pathway by which dysfunctional mitochondria are cleared from the cell [122]. The efficiency of autophagic degradation of cellular constituents, including mitochondrial degradation, also known as mitophagy, is impaired with age [122-125]. The mitochondrial-lysosomal axis of aging hypothesizes that this decline in autophagy caused by the accumulation of incompletely digested oxidatively damaged molecules in the lysosome (lipofuscin) results in the accumulation of dysfunctional mitochondria with age [123,125-127]. In support of this hypothesis Kruse et al [86] recently reported an increase in the half-lives of mitochondrial proteins with age in both the extensor digitorum longus and the soleus muscles. It follows that an age-related decline in clearance of poorly functioning

mitochondria would contribute to the feedforward cycle of mitochondrial damage initiated by systemic effects of heart failure like those discussed above, while similar stressors in young muscle may be well tolerated due to more efficient clearance of dysfunctional mitochondrial and prevention of the feedforward cycle of mitochondrial dysfunction.

Direct targeting of mitochondrial oxidative stress

Further evidence for a causal role of mitochondrial derived oxidative stress comes from studies directly targeting mitochondrial oxidative stress to ameliorate skeletal muscle dysfunction in both aging and heart failure. Expressing catalase in the mitochondria (mCAT) is one of the most effective genetic interventions to reduce age-related dysfunction across multiple tissues, including heart and skeletal muscle [12,86,128,129]. Elevated mitochondrial oxidant production can impair other aspects of skeletal muscle function independent of the ability to generate sufficient ATP [15]. As discussed above oxidative post-translation modifications of the RyR in aging muscle can lead to destabilization of the RyR-calstabin complex and increased calcium leak resulting in loss of specific force, muscle atrophy, and decreased exercise tolerance [120,121]. This was prevented or delayed in mCAT mice, demonstrating the important role for mitochondrial oxidative stress in this process. Elevated basal calcium is likely lead to increased mitochondrial calcium uptake and further mitochondrial oxidative stress [121,130], thus creating a feedforward cycle and further skeletal muscle dysfunction. Interrupting this cycle by targeting mitochondrial oxidative stress can also improve skeletal muscle function in heart failure models [53] similarly to the results in aging muscle with SS-31 discussed earlier. Treating rats with mitoTEMPO, a mitochondrial targeted superoxide scavenger, for 10 weeks starting 8 weeks after induction of myocardial infarction improved mitochondrial dysfunction and increased force production in diaphragm [95]. These data support a causative role for mitochondrial oxidative stress in skeletal muscle dysfunction in both aging and heart failure. This common role for mitochondrial dysfunction creates a condition where the pathological effects of aging and heart failure could interact to potentiate the effects on skeletal muscle and exacerbate exercise intolerance.

Conclusion

Despite the fact that sarcopenia is a multifactorial condition, mitochondria provide a nexus linking cellular redox homeostasis, substrate metabolism, catabolic and anabolic signaling. In this review we attempted to highlight the potential for interaction between aging and heart failure to potentiate mitochondrial deficits in skeletal muscle. As we learn more about the complex interactions between the mitochondria and cell environment it is becoming increasingly clear that combining both *in vivo* and *ex vivo* approaches to assess skeletal muscle bioenergetics provides complementary information for not only identifying deficits, including changes in both mitochondrial capacity and quality, but also highlighting the underlying mechanisms. Similarities in bioenergetic deficits associated with aging, heart failure, and a sedentary lifestyle suggest it is important to consider the interaction between these factors when addressing exercise intolerance and skeletal muscle function in both clinical and research settings. We have attempted to emphasize that disruption of mitochondrial function by any one of these conditions can initiate a feedforward cycle that

sensitizes the muscle to further stressors and the acceleration of skeletal muscle pathology. While the ultimate initiating factor disrupting mitochondrial function remains controversial and may vary between conditions, evidence is accumulating for an important role for increased mitochondrial oxidative stress as a critical step in a feed-forward cycle leading to enhanced pathological outcomes in aging and heart failure. This provides a potential target for intervention to reduce both cardiac and skeletal muscle pathology and enhance quality of life of patients.

Acknowledgements

This publication was supported by awards from the National Institute on Aging of the National Institutes of Health AG001751, AG000057 and a Breakthrough in Gerontology Award from the American Federation for Aging Research.

Reference

1. <http://www.un.org/en/development/desa/population/publications/pdf/ageing/WorldPopulationAgeing2013.pdf>
2. Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De Simone G, Ferguson TB, Ford E, Furie K, Gillespie C, Go A, Greenlund K, Haase N, Hailpern S, Ho PM, Howard V, Kissela B, Kittner S, Lackland D, Lisabeth L, Marelli A, McDermott MM, Meigs J, Mozaffarian D, Mussolino M, Nichol G, Roger VL, Rosamond W, Sacco R, Sorlie P, Stafford R, Thom T, Wasserthiel-Smoller S, Wong ND, Wylie-Rosett J, American Heart Association Statistics C, Stroke Statistics S. Executive summary: heart disease and stroke statistics--2010 update: a report from the American Heart Association. *Circulation*. 2010; 121(7):948–954. [PubMed: 20177011]
3. Hughes VA, Frontera WR, Wood M, Evans WJ, Dallal GE, Roubenoff R, Fiatarone Singh MA. Longitudinal muscle strength changes in older adults: influence of muscle mass, physical activity, and health. *J Gerontol A Biol Sci Med Sci*. 2001; 56(5):B209–217. [PubMed: 11320101]
4. http://www.cdc.gov/dhdsp/data_statistics/fact_sheets/fs_heart_failure.htm
5. Brand MD. The sites and topology of mitochondrial superoxide production. *Exp Gerontol*. 2010; 45(7-8):466–472. [PubMed: 20064600]
6. Brand MD, Affouit C, Esteves TC, Green K, Lambert AJ, Miwa S, Pakay JL, Parker N. Mitochondrial superoxide: production, biological effects, and activation of uncoupling proteins. *Free Radic Biol Med*. 2004; 37(6):755–767. [PubMed: 15304252]
7. Fisher-Wellman KH, Neuffer PD. Linking mitochondrial bioenergetics to insulin resistance via redox biology. *Trends Endocrinol Metab*. 2012; 23(3):142–153. [PubMed: 22305519]
8. Jiang P, Du W, Mancuso A, Wellen KE, Yang X. Reciprocal regulation of p53 and malic enzymes modulates metabolism and senescence. *Nature*. 2013; 493(7434):689–693. [PubMed: 23334421]
9. Merritt TJ, Kuczynski C, Sezgin E, Zhu CT, Kumagai S, Eanes WF. Quantifying interactions within the NADP(H) enzyme network in *Drosophila melanogaster*. *Genetics*. 2009; 182(2):565–574. [PubMed: 19307608]
10. Gameiro PA, Laviolette LA, Kelleher JK, Iliopoulos O, Stephanopoulos G. Cofactor balance by nicotinamide nucleotide transhydrogenase (NNT) coordinates reductive carboxylation and glucose catabolism in the tricarboxylic acid (TCA) cycle. *J Biol Chem*. 2013; 288(18):12967–12977. [PubMed: 23504317]
11. Anderson EJ, Kypson AP, Rodriguez E, Anderson CA, Lehr EJ, Neuffer PD. Substrate-specific derangements in mitochondrial metabolism and redox balance in the atrium of the type 2 diabetic human heart. *J Am Coll Cardiol*. 2009; 54(20):1891–1898. [PubMed: 19892241]
12. Dai DF, Chiao YA, Marcinek DJ, Szeto HH, Rabinovitch PS. Mitochondrial oxidative stress in aging and healthspan. *Longev Healthspan*. 2014; 3:6. [PubMed: 24860647]
13. Feissner RF, Skalska J, Gaum WE, Sheu SS. Crosstalk signaling between mitochondrial Ca²⁺ and ROS. *Front Biosci (Landmark Ed)*. 2009; 14:1197–1218. [PubMed: 19273125]

14. Brookes PS, Levonen AL, Shiva S, Sarti P, Darley-USmar VM. Mitochondria: regulators of signal transduction by reactive oxygen and nitrogen species. *Free Radic Biol Med*. 2002; 33(6):755–764. [PubMed: 12208364]
15. Kramer PA, Duan J, Qian WJ, Marcinek DJ. The Measurement of Reversible Redox Dependent Post-translational Modifications and Their Regulation of Mitochondrial and Skeletal Muscle Function. *Front Physiol*. 2015; 6:347. [PubMed: 26635632]
16. Fitts RH. Cellular mechanisms of muscle fatigue. *Physiol Rev*. 1994; 74(1):49–94. [PubMed: 8295935]
17. Okamoto K, Wang W, Rounds J, Chambers EA, Jacobs DO. ATP from glycolysis is required for normal sodium homeostasis in resting fast-twitch rodent skeletal muscle. *Am J Physiol Endocrinol Metab*. 2001; 281(3):E479–488. [PubMed: 11500303]
18. Homsher E, Kean CJ. Skeletal muscle energetics and metabolism. *Annu Rev Physiol*. 1978; 40:93–131. [PubMed: 345955]
19. Rall JA. Energetic aspects of skeletal muscle contraction: implications of fiber types. *Exerc Sport Sci Rev*. 1985; 13:33–74. [PubMed: 3159582]
20. Blei ML, Conley KE, Kushmerick MJ. Separate measures of ATP utilization and recovery in human skeletal muscle. *J Physiol*. 1993; 465:203–222. [PubMed: 8024651]
21. Conley KE, Jubrias SA, Esselman PC. Oxidative capacity and ageing in human muscle. *J Physiol* 526 Pt. 2000; 1:203–210.
22. Siegel MP, Kruse SE, Knowels G, Salmon A, Beyer R, Xie H, Van Remmen H, Smith SR, Marcinek DJ. Reduced coupling of oxidative phosphorylation in vivo precedes electron transport chain defects due to mild oxidative stress in mice. *PLoS One*. 2011; 6(11):e26963. [PubMed: 22132085]
23. Siegel MP, Wilbur T, Mathis M, Shankland EG, Trieu A, Harper ME, Marcinek DJ. Impaired adaptability of in vivo mitochondrial energetics to acute oxidative insult in aged skeletal muscle. *Mech Ageing Dev*. 2012; 133(9-10):620–628. [PubMed: 22935551]
24. Marcinek DJ, Schenkman KA, Ciesielski WA, Lee D, Conley KE. Reduced mitochondrial coupling in vivo alters cellular energetics in aged mouse skeletal muscle. *J Physiol*. 2005; 569(Pt 2):467–473. [PubMed: 16254011]
25. Myers J, Prakash M, Froelicher V, Do D, Partington S, Atwood JE. Exercise capacity and mortality among men referred for exercise testing. *N Engl J Med*. 2002; 346(11):793–801. [PubMed: 11893790]
26. Fleg JL, Morrell CH, Bos AG, Brant LJ, Talbot LA, Wright JG, Lakatta EG. Accelerated longitudinal decline of aerobic capacity in healthy older adults. *Circulation*. 2005; 112(5):674–682. [PubMed: 16043637]
27. Upadhyay B, Haykowsky MJ, Eggebeen J, Kitzman DW. Exercise intolerance in heart failure with preserved ejection fraction: more than a heart problem. *J Geriatr Cardiol*. 2015; 12(3):294–304. [PubMed: 26089855]
28. Molina AJ, Bharadwaj MS, Van Horn C, Nicklas BJ, Lyles MF, Eggebeen J, Haykowsky MJ, Brubaker PH, Kitzman DW. Skeletal Muscle Mitochondrial Content, Oxidative Capacity, and Mfn2 Expression Are Reduced in Older Patients With Heart Failure and Preserved Ejection Fraction and Are Related to Exercise Intolerance. *JACC Heart Fail*. 2016; 4(8):636–645. [PubMed: 27179829]
29. Hoppeler H. The different relationship of VO₂max to muscle mitochondria in humans and quadrupedal animals. *Respir Physiol*. 1990; 80(2-3):137–145. [PubMed: 2218095]
30. Heath GW, Hagberg JM, Ehsani AA, Holloszy JO. A physiological comparison of young and older endurance athletes. *J Appl Physiol Respir Environ Exerc Physiol*. 1981; 51(3):634–640. [PubMed: 7327965]
31. Rogers MA, Hagberg JM, Martin WH 3rd, Ehsani AA, Holloszy JO. Decline in VO₂max with aging in master athletes and sedentary men. *J Appl Physiol* (1985). 1990; 68(5):2195–2199. [PubMed: 2361923]
32. Zizola C, Schulze PC. Metabolic and structural impairment of skeletal muscle in heart failure. *Heart Fail Rev*. 2013; 18(5):623–630. [PubMed: 23065040]

33. Mancini DM, Ferraro N, Tuchler M, Chance B, Wilson JR. Detection of abnormal calf muscle metabolism in patients with heart failure using phosphorus-31 nuclear magnetic resonance. *Am J Cardiol.* 1988; 62(17):1234–1240. [PubMed: 3195484]
34. Dudley GA, Tullson PC, Terjung RL. Influence of mitochondrial content on the sensitivity of respiratory control. *J Biol Chem.* 1987; 262(19):9109–9114. [PubMed: 3597408]
35. Cadenas E. Mitochondrial free radical production and cell signaling. *Mol Aspects Med.* 2004; 25(1-2):17–26. [PubMed: 15051313]
36. Garcia J, Han D, Sancheti H, Yap LP, Kaplowitz N, Cadenas E. Regulation of mitochondrial glutathione redox status and protein glutathionylation by respiratory substrates. *J Biol Chem.* 2010; 285(51):39646–39654. [PubMed: 20937819]
37. Pan X, Liu J, Nguyen T, Liu C, Sun J, Teng Y, Fergusson MM, Rovira, Allen M, Springer DA, Aponte AM, Gucek M, Balaban RS, Murphy E, Finkel T. The physiological role of mitochondrial calcium revealed by mice lacking the mitochondrial calcium uniporter. *Nat Cell Biol.* 2013; 15(12):1464–1472. [PubMed: 24212091]
38. Jouaville LS, Pinton P, Bastianutto C, Rutter GA, Rizzuto R. Regulation of mitochondrial ATP synthesis by calcium: evidence for a long-term metabolic priming. *Proc Natl Acad Sci U S A.* 1999; 96(24):13807–13812. [PubMed: 10570154]
39. Szalai G, Csordas G, Hantash BM, Thomas AP, Hajnoczky G. Calcium signal transmission between ryanodine receptors and mitochondria. *J Biol Chem.* 2000; 275(20):15305–15313. [PubMed: 10809765]
40. Kemp GJ, Ahmad RE, Nicolay K, Prompers JJ. Quantification of skeletal muscle mitochondrial function by ³¹P magnetic resonance spectroscopy techniques: a quantitative review. *Acta Physiol (Oxf).* 2015; 213(1):107–144. [PubMed: 24773619]
41. Campbell MD, Marcinek DJ. Evaluation of in vivo mitochondrial bioenergetics in skeletal muscle using NMR and optical methods. *Biochim Biophys Acta.* 2016; 1862(4):716–724. [PubMed: 26708941]
42. Lanza IR, Bhagra S, Nair KS, Port JD. Measurement of human skeletal muscle oxidative capacity by ³¹PMR spectroscopy: a cross-validation with in vitro measurements. *J Magn Reson Imaging.* 2011; 34(5):1143–1150. [PubMed: 22006551]
43. Percival JM, Siegel MP, Knowels G, Marcinek DJ. Defects in mitochondrial localization and ATP synthesis in the mdx mouse model of Duchenne muscular dystrophy are not alleviated by PDE5 inhibition. *Hum Mol Genet.* 2013; 22(1):153–167. [PubMed: 23049075]
44. Broskey NT, Boss A, Fares EJ, Greggio C, Gremion G, Schluter L, Hans D, Kreis R, Boesch C, Amati F. Exercise efficiency relates with mitochondrial content and function in older adults. *Physiol Rep.* 2015; 3(6)
45. Coen PM, Jubrias SA, Distefano G, Amati F, Mackey DC, Glynn NW, Manini TM, Wohlgemuth SE, Leeuwenburgh C, Cummings SR, Newman AB, Ferrucci L, Toledo FG, Shankland E, Conley KE, Goodpaster BH. Skeletal muscle mitochondrial energetics are associated with maximal aerobic capacity and walking speed in older adults. *J Gerontol A Biol Sci Med Sci.* 2013; 68(4): 447–455. [PubMed: 23051977]
46. Ryan TE, Erickson ML, Brizendine JT, Young HJ, McCully KK. Noninvasive evaluation of skeletal muscle mitochondrial capacity with near-infrared spectroscopy: correcting for blood volume changes. *J Appl Physiol (1985).* 2012; 113(2):175–183. [PubMed: 22582211]
47. Ryan TE, Southern WM, Brizendine JT, McCully KK. Activity-induced changes in skeletal muscle metabolism measured with optical spectroscopy. *Med Sci Sports Exerc.* 2013; 45(12):2346–2352. [PubMed: 23669881]
48. Ryan TE, Southern WM, Reynolds MA, McCully KK. A cross-validation of near-infrared spectroscopy measurements of skeletal muscle oxidative capacity with phosphorus magnetic resonance spectroscopy. *J Appl Physiol (1985).* 2013; 115(12):1757–1766. [PubMed: 24136110]
49. De Blasi RA, Almenrader N, Aurisicchio P, Ferrari M. Comparison of two methods of measuring forearm oxygen consumption (VO₂) by near infrared spectroscopy. *J Biomed Opt.* 1997; 2(2):171–175. [PubMed: 23014870]
50. Van Beekvelt MCP, Colier WNJM, Wevers RA, Van Engelen BGM. Performance of near-infrared spectroscopy in measuring local O₂ consumption and blood flow in skeletal muscle. 2001; 902

51. Larsen RG, Callahan DM, Foulis SA, Kent-Braun JA. Age-related changes in oxidative capacity differ between locomotory muscles and are associated with physical activity behavior. *Appl Physiol Nutr Metab*. 2012; 37(1):88–99. [PubMed: 22236246]
52. Amara CE, Shankland EG, Jubrias SA, Marcinek DJ, Kushmerick MJ, Conley KE. Mild mitochondrial uncoupling impacts cellular aging in human muscles in vivo. *Proc Natl Acad Sci U S A*. 2007; 104(3):1057–1062. [PubMed: 17215370]
53. Siegel MP, Kruse SE, Percival JM, Goh J, White CC, Hopkins HC, Kavanagh TJ, Szeto HH, Rabinovitch PS, Marcinek DJ. Mitochondrial-targeted peptide rapidly improves mitochondrial energetics and skeletal muscle performance in aged mice. *Aging Cell*. 2013; 12(5):763–771. [PubMed: 23692570]
54. Southern WM, Ryan TE, Kepple K, Murrow JR, Nilsson KR, McCully KK. Reduced skeletal muscle oxidative capacity and impaired training adaptations in heart failure. *Physiol Rep*. 2015; 3(4)
55. Bhella PS, Prasad A, Heinicke K, Hastings JL, Arbab-Zadeh A, Adams-Huet B, Pacini EL, Shibata S, Palmer MD, Newcomer BR, Levine BD. Abnormal haemodynamic response to exercise in heart failure with preserved ejection fraction. *Eur J Heart Fail*. 2011; 13(12):1296–1304. [PubMed: 21979991]
56. Mancini DM, Henson D, LaManca J, Levine S. Evidence of reduced respiratory muscle endurance in patients with heart failure. *J Am Coll Cardiol*. 1994; 24(4):972–981. [PubMed: 7930233]
57. Wiener DH, Fink LI, Maris J, Jones RA, Chance B, Wilson JR. Abnormal skeletal muscle bioenergetics during exercise in patients with heart failure: role of reduced muscle blood flow. *Circulation*. 1986; 73(6):1127–1136. [PubMed: 3698247]
58. Mancini DM, Coyle E, Coggan A, Beltz J, Ferraro N, Montain S, Wilson JR. Contribution of intrinsic skeletal muscle changes to 31P NMR skeletal muscle metabolic abnormalities in patients with chronic heart failure. *Circulation*. 1989; 80(5):1338–1346. [PubMed: 2805270]
59. Hart CR, Layec G, Trinity JD, Liu X, Kim SE, Groot HJ, Le Fur Y, Sorensen JR, Jeong EK, Richardson RS. Evidence of Preserved Oxidative Capacity and Oxygen Delivery in the Plantar Flexor Muscles With Age. *J Gerontol A Biol Sci Med Sci*. 2015; 70(9):1067–1076. [PubMed: 25165028]
60. Layec G, Trinity JD, Hart CR, Kim SE, Groot HJ, Le Fur Y, Sorensen JR, Jeong EK, Richardson RS. Impact of age on exercise-induced ATP supply during supramaximal plantar flexion in humans. *Am J Physiol Regul Integr Comp Physiol*. 2015; 309(4):R378–388. [PubMed: 26041112]
61. Lanza IR, Towse TF, Caldwell GE, Wigmore DM, Kent-Braun JA. Effects of age on human muscle torque, velocity, and power in two muscle groups. *J Appl Physiol* (1985). 2003; 95(6):2361–2369. [PubMed: 12923120]
62. Conley KE, Amara CE, Bajpeyi S, Costford SR, Murray K, Jubrias SA, Arakaki L, Marcinek DJ, Smith SR. Higher mitochondrial respiration and uncoupling with reduced electron transport chain content in vivo in muscle of sedentary versus active subjects. *J Clin Endocrinol Metab*. 2013; 98(1):129–136. [PubMed: 23150693]
63. Santanasto AJ, Glynn NW, Jubrias SA, Conley KE, Boudreau RM, Amati F, Mackey DC, Simonsick EM, Strotmeyer ES, Coen PM, Goodpaster BH, Newman AB. Skeletal Muscle Mitochondrial Function and Fatigability in Older Adults. *J Gerontol A Biol Sci Med Sci*. 2015; 70(11):1379–1385. [PubMed: 25167867]
64. Amara CE, Marcinek DJ, Shankland EG, Schenkman KA, Arakaki LS, Conley KE. Mitochondrial function in vivo: spectroscopy provides window on cellular energetics. *Methods*. 2008; 46(4):312–318. [PubMed: 18930151]
65. Nyberg M, Mortensen SP, Cabo H, Gomez-Cabrera MC, Vina J, Hellsten Y. Roles of sedentary aging and lifelong physical activity in exchange of glutathione across exercising human skeletal muscle. *Free Radic Biol Med*. 2014; 73:166–173. [PubMed: 24858720]
66. Hutter E, Skovbro M, Lener B, Prats C, Rabol R, Dela F, Jansen-Durr P. Oxidative stress and mitochondrial impairment can be separated from lipofuscin accumulation in aged human skeletal muscle. *Aging Cell*. 2007; 6(2):245–256. [PubMed: 17376148]
67. Jackson MJ. Interactions between reactive oxygen species generated by contractile activity and aging in skeletal muscle? *Antioxid Redox Signal*. 2013; 19(8):804–812. [PubMed: 23682926]

68. Jackson MJ. Monitoring of hydrogen peroxide and other reactive oxygen and nitrogen species generated by skeletal muscle. *Methods Enzymol.* 2013; 528:279–300. [PubMed: 23849872]
69. Marcinek DJ, Siegel MP. Targeting redox biology to reverse mitochondrial dysfunction. *Aging (Albany NY).* 2013; 5(8):588–589. [PubMed: 23945201]
70. Karamanlidis G, Lee CF, Garcia-Menendez L, Kolwicz SC Jr, Suthammarak W, Gong G, Sedensky MM, Morgan PG, Wang W, Tian R. Mitochondrial complex I deficiency increases protein acetylation and accelerates heart failure. *Cell Metab.* 2013; 18(2):239–250. [PubMed: 23931755]
71. O'Rourke B, Van Eyk JE, Foster DB. Mitochondrial protein phosphorylation as a regulatory modality: implications for mitochondrial dysfunction in heart failure. *Congest Heart Fail.* 2011; 17(6):269–282. [PubMed: 22103918]
72. Kelly DP, Scarpulla RC. Transcriptional regulatory circuits controlling mitochondrial biogenesis and function. *Genes Dev.* 2004; 18(4):357–368. [PubMed: 15004004]
73. Lehman JJ, Barger PM, Kovacs A, Saffitz JE, Medeiros DM, Kelly DP. Peroxisome proliferator-activated receptor gamma coactivator-1 promotes cardiac mitochondrial biogenesis. *J Clin Invest.* 2000; 106(7):847–856. [PubMed: 11018072]
74. St-Pierre J, Drori S, Uldry M, Silvaggi JM, Rhee J, Jager S, Handschin C, Zheng K, Lin J, Yang W, Simon DK, Bachoo R, Spiegelman BM. Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell.* 2006; 127(2):397–408. [PubMed: 17055439]
75. Lanza IR, Short DK, Short KR, Raghavakaimal S, Basu R, Joyner MJ, McConnell JP, Nair KS. Endurance exercise as a countermeasure for aging. *Diabetes.* 2008; 57(11):2933–2942. [PubMed: 18716044]
76. Knutti D, Kralli A. PGC-1, a versatile coactivator. *Trends Endocrinol Metab.* 2001; 12(8):360–365. [PubMed: 11551810]
77. Johnson ML, Robinson MM, Nair KS. Skeletal muscle aging and the mitochondrion. *Trends Endocrinol Metab.* 2013; 24(5):247–256. [PubMed: 23375520]
78. Wenz T, Rossi SG, Rotundo RL, Spiegelman BM, Moraes CT. Increased muscle PGC-1alpha expression protects from sarcopenia and metabolic disease during aging. *Proc Natl Acad Sci U S A.* 2009; 106(48):20405–20410. [PubMed: 19918075]
79. Ji LL, Kang C. Role of PGC-1alpha in sarcopenia: etiology and potential intervention - a mini-review. *Gerontology.* 2015; 61(2):139–148. [PubMed: 25502801]
80. Chabi B, Ljubicic V, Menzies KJ, Huang JH, Saleem A, Hood DA. Mitochondrial function and apoptotic susceptibility in aging skeletal muscle. *Aging Cell.* 2008; 7(1):2–12. [PubMed: 18028258]
81. Ghosh S, Lertwattanakarn R, Lefort N, Molina-Carrion M, Joya-Galeana J, Bowen BP, Garduno-Garcia Jde J, Abdul-Ghani M, Richardson A, DeFronzo RA, Mandarino L, Van Remmen H, Musi N. Reduction in reactive oxygen species production by mitochondria from elderly subjects with normal and impaired glucose tolerance. *Diabetes.* 2011; 60(8):2051–2060. [PubMed: 21677280]
82. Canto C, Gerhart-Hines Z, Feige JN, Lagouge M, Noriega L, Milne JC, Elliott PJ, Puigserver P, Auwerx J. AMPK regulates energy expenditure by modulating NAD⁺ metabolism and SIRT1 activity. *Nature.* 2009; 458(7241):1056–1060. [PubMed: 19262508]
83. Fernandez-Marcos PJ, Auwerx J. Regulation of PGC-1alpha, a nodal regulator of mitochondrial biogenesis. *Am J Clin Nutr.* 2011; 93(4):884S–890. [PubMed: 21289221]
84. Houtkooper RH, Canto C, Wanders RJ, Auwerx J. The secret life of NAD⁺: an old metabolite controlling new metabolic signaling pathways. *Endocr Rev.* 2010; 31(2):194–223. [PubMed: 20007326]
85. Gomes AP, Price NL, Ling AJ, Moslehi JJ, Montgomery MK, Rajman L, White JP, Teodoro JS, Wrann CD, Hubbard BP, Mercken EM, Palmeira CM, de Cabo R, Rolo AP, Turner N, Bell EL, Sinclair DA. Declining NAD⁽⁺⁾ induces a pseudohypoxic state disrupting nuclear-mitochondrial communication during aging. *Cell.* 2013; 155(7):1624–1638. [PubMed: 24360282]
86. Kruse SE, Karunadharma PP, Basisty N, Johnson R, Beyer RP, MacCoss MJ, Rabinovitch PS, Marcinek DJ. Age modifies respiratory complex I and protein homeostasis in a muscle type-specific manner. *Aging Cell.* 2016; 15(1):89–99. [PubMed: 26498839]

87. Toth MJ, Miller MS, Ward KA, Ades PA. Skeletal muscle mitochondrial density, gene expression, and enzyme activities in human heart failure: minimal effects of the disease and resistance training. *J Appl Physiol* (1985). 2012; 112(11):1864–1874. [PubMed: 22461439]
88. Zizola C, Kennel PJ, Akashi H, Ji R, Castellero E, George I, Homma S, Schulze PC. Activation of PPARdelta signaling improves skeletal muscle oxidative metabolism and endurance function in an animal model of ischemic left ventricular dysfunction. *Am J Physiol Heart Circ Physiol*. 2015; 308(9):H1078–1085. [PubMed: 25713305]
89. Moe GW, Marin-Garcia J, Konig A, Goldenthal M, Lu X, Feng Q. In vivo TNF-alpha inhibition ameliorates cardiac mitochondrial dysfunction, oxidative stress, and apoptosis in experimental heart failure. *Am J Physiol Heart Circ Physiol*. 2004; 287(4):H1813–1820. [PubMed: 15205165]
90. Marin-Garcia J, Goldenthal MJ, Moe GW. Abnormal cardiac and skeletal muscle mitochondrial function in pacing-induced cardiac failure. *Cardiovasc Res*. 2001; 52(1):103–110. [PubMed: 11557238]
91. Rosca MG, Hoppel CL. Mitochondria in heart failure. *Cardiovasc Res*. 2010; 88(1):40–50. [PubMed: 20668004]
92. Hoppel CL, Tandler B, Fujioka H, Riva A. Dynamic organization of mitochondria in human heart and in myocardial disease. *Int J Biochem Cell Biol*. 2009; 41(10):1949–1956. [PubMed: 19446651]
93. Middlekauff HR, Verity MA, Horwich TB, Fonarow GC, Hamilton MA, Shieh P. Intact skeletal muscle mitochondrial enzyme activity but diminished exercise capacity in advanced heart failure patients on optimal medical and device therapy. *Clin Res Cardiol*. 2013; 102(8):547–554. [PubMed: 23575739]
94. Schrepper A, Schwarzer M, Schope M, Amorim PA, Doenst T. Biphasic response of skeletal muscle mitochondria to chronic cardiac pressure overload - role of respiratory chain complex activity. *J Mol Cell Cardiol*. 2012; 52(1):125–135. [PubMed: 22100228]
95. Laitano O, Ahn B, Patel N, Coblenz PD, Smuder AJ, Yoo JK, Christou DD, Adhihetty PJ, Ferreira LF. Pharmacological targeting of mitochondrial reactive oxygen species counteracts diaphragm weakness in chronic heart failure. *J Appl Physiol* (1985). 2016; 120(7):733–742. [PubMed: 26846552]
96. Echtay KS, Esteves TC, Pakay JL, Jekabsons MB, Lambert AJ, Portero-Otin M, Pamplona R, Vidal-Puig AJ, Wang S, Roebuck SJ, Brand MD. A signalling role for 4-hydroxy-2-nonenal in regulation of mitochondrial uncoupling. *Embo J*. 2003; 22(16):4103–4110. [PubMed: 12912909]
97. Mailloux RJ, Adjeitey CN, Xuan JY, Harper ME. Crucial yet divergent roles of mitochondrial redox state in skeletal muscle vs. brown adipose tissue energetics. *FASEB J*. 2012; 26(1):363–375. [PubMed: 21940996]
98. Jackson MJ. Redox regulation of adaptive responses in skeletal muscle to contractile activity. *Free Radic Biol Med*. 2009; 47(9):1267–1275. [PubMed: 19748570]
99. Jackson MJ. Redox regulation of skeletal muscle. *IUBMB Life*. 2008; 60(8):497–501. [PubMed: 18629903]
100. Forman HJ, Ursini F, Maiorino M. An overview of mechanisms of redox signaling. *J Mol Cell Cardiol*. 2014; 73:2–9. [PubMed: 24512843]
101. Rosca MG, Hoppel CL. Mitochondrial dysfunction in heart failure. *Heart Fail Rev*. 2013; 18(5):607–622. [PubMed: 22948484]
102. Miura S, Kawanaka K, Kai Y, Tamura M, Goto M, Shiuchi T, Minokoshi Y, Ezaki O. An increase in murine skeletal muscle peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC-1alpha) mRNA in response to exercise is mediated by beta-adrenergic receptor activation. *Endocrinology*. 2007; 148(7):3441–3448. [PubMed: 17446185]
103. Benson DW, Foley-Nelson T, Chance WT, Zhang FS, James JH, Fischer JE. Decreased myofibrillar protein breakdown following treatment with clenbuterol. *J Surg Res*. 1991; 50(1):1–5. [PubMed: 1670961]
104. Rosca MG, Tandler B, Hoppel CL. Mitochondria in cardiac hypertrophy and heart failure. *J Mol Cell Cardiol*. 2013; 55:31–41. [PubMed: 22982369]
105. Rosca MG, Hoppel CL. New aspects of impaired mitochondrial function in heart failure. *J Bioenerg Biomembr*. 2009; 41(2):107–112. [PubMed: 19347572]

106. DiPilato LM, Cheng X, Zhang J. Fluorescent indicators of cAMP and Epac activation reveal differential dynamics of cAMP signaling within discrete subcellular compartments. *Proc Natl Acad Sci U S A*. 2004; 101(47):16513–16518. [PubMed: 15545605]
107. Rosca M, Minkler P, Hoppel CL. Cardiac mitochondria in heart failure: normal cardiolipin profile and increased threonine phosphorylation of complex IV. *Biochim Biophys Acta*. 2011; 1807(11):1373–1382. [PubMed: 21320465]
108. Brink M, Wellen J, Delafontaine P. Angiotensin II causes weight loss and decreases circulating insulin-like growth factor I in rats through a pressor-independent mechanism. *J Clin Invest*. 1996; 97(11):2509–2516. [PubMed: 8647943]
109. Wang Y, Seto SW, Golledge J. Angiotensin II, sympathetic nerve activity and chronic heart failure. *Heart Fail Rev*. 2014; 19(2):187–198. [PubMed: 23225134]
110. Kadoguchi T, Kinugawa S, Takada S, Fukushima A, Furihata T, Homma T, Masaki Y, Mizushima W, Nishikawa M, Takahashi M, Yokota T, Matsushima S, Okita K, Tsutsui H. Angiotensin II can directly induce mitochondrial dysfunction, decrease oxidative fibre number and induce atrophy in mouse hindlimb skeletal muscle. *Exp Physiol*. 2015; 100(3):312–322. [PubMed: 25580531]
111. Takada S, Kinugawa S, Hirabayashi K, Suga T, Yokota T, Takahashi M, Fukushima A, Homma T, Ono T, Sobirin MA, Masaki Y, Mizushima W, Kadoguchi T, Okita K, Tsutsui H. Angiotensin II receptor blocker improves the lowered exercise capacity and impaired mitochondrial function of the skeletal muscle in type 2 diabetic mice. *J Appl Physiol* (1985). 2013; 114(7):844–857. [PubMed: 23329824]
112. Dai DF, Johnson SC, Villarin JJ, Chin MT, Nieves-Cintrón M, Chen T, Marcinek DJ, Dorn GW 2nd, Kang YJ, Prolla TA, Santana LF, Rabinovitch PS. Mitochondrial oxidative stress mediates angiotensin II-induced cardiac hypertrophy and Galphaq overexpression-induced heart failure. *Circ Res*. 2011; 108(7):837–846. [PubMed: 21311045]
113. Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. *Circ Res*. 1994; 74(6):1141–1148. [PubMed: 8187280]
114. Mollnau H, Wendt M, Szocs K, Lassegue B, Schulz E, Oelze M, Li H, Bodenschatz M, August M, Kleschyov AL, Tsilimingas N, Walter U, Forstermann U, Meinertz T, Griendling K, Munzel T. Effects of angiotensin II infusion on the expression and function of NAD(P)H oxidase and components of nitric oxide/cGMP signaling. *Circ Res*. 2002; 90(4):E58–65. [PubMed: 11884382]
115. Semprun-Prieto LC, Sukhanov S, Yoshida T, Rezk BM, Gonzalez-Villalobos RA, Vaughn C, Michael Tabony A, Delafontaine P. Angiotensin II induced catabolic effect and muscle atrophy are redox dependent. *Biochem Biophys Res Commun*. 2011; 409(2):217–221. [PubMed: 21570954]
116. Wei Y, Sowers JR, Nistala R, Gong H, Uptergrove GM, Clark SE, Morris EM, Szary N, Manrique C, Stump CS. Angiotensin II-induced NADPH oxidase activation impairs insulin signaling in skeletal muscle cells. *J Biol Chem*. 2006; 281(46):35137–35146. [PubMed: 16982630]
117. Dai DF, Karunadharma PP, Chiao YA, Basisty N, Crispin D, Hsieh EJ, Chen T, Gu H, Djukovic D, Raftery D, Beyer RP, MacCoss MJ, Rabinovitch PS. Altered proteome turnover and remodeling by short-term caloric restriction or rapamycin rejuvenate the aging heart. *Aging Cell*. 2014; 13(3):529–539. [PubMed: 24612461]
118. Dai DF, Chen T, Szeto H, Nieves-Cintrón M, Kuttyavin V, Santana LF, Rabinovitch PS. Mitochondrial targeted antioxidant Peptide ameliorates hypertensive cardiomyopathy. *J Am Coll Cardiol*. 2011; 58(1):73–82. [PubMed: 21620606]
119. Andersson DC, Betzenhauser MJ, Reiken S, Meli AC, Umanskaya A, Xie W, Shiomi T, Zalk R, Lacampagne A, Marks AR. Ryanodine receptor oxidation causes intracellular calcium leak and muscle weakness in aging. *Cell Metab*. 2011; 14(2):196–207. [PubMed: 21803290]
120. Rullman E, Andersson DC, Melin M, Reiken S, Mancini DM, Marks AR, Lund LH, Gustafsson T. Modifications of skeletal muscle ryanodine receptor type 1 and exercise intolerance in heart failure. *J Heart Lung Transplant*. 2013; 32(9):925–929. [PubMed: 23953820]
121. Umanskaya A, Santulli G, Xie W, Andersson DC, Reiken SR, Marks AR. Genetically enhancing mitochondrial antioxidant activity improves muscle function in aging. *Proc Natl Acad Sci U S A*. 2014; 111(42):15250–15255. [PubMed: 25288763]

122. Cuervo AM, Bergamini E, Brunk UT, Droge W, French M, Terman A. Autophagy and aging: the importance of maintaining “clean” cells. *Autophagy*. 2005; 1(3):131–140. [PubMed: 16874025]
123. Terman A, Dalen H, Eaton JW, Neuzil J, Brunk UT. Mitochondrial recycling and aging of cardiac myocytes: the role of autophagocytosis. *Exp Gerontol*. 2003; 38(8):863–876. [PubMed: 12915208]
124. Coleman R, Silbermann M, Gershon D, Reznick AZ. Giant mitochondria in the myocardium of aging and endurance-trained mice. *Gerontology*. 1987; 33(1):34–39. [PubMed: 3596262]
125. Brunk UT, Terman A. The mitochondrial-lysosomal axis theory of aging: accumulation of damaged mitochondria as a result of imperfect autophagocytosis. *Eur J Biochem*. 2002; 269(8):1996–2002. [PubMed: 11985575]
126. Dayan D, Abrahams I, Buchner A, Gorsky M, Chimovitz N. Lipid pigment (lipofuscin) in human perioral muscles with aging. *Exp Gerontol*. 1988; 23(2):97–102. [PubMed: 2456941]
127. Dayan D, David R, Buchner A. Lipofuscin in human tongue muscle. *J Oral Pathol*. 1979; 8(2):121–125. [PubMed: 86606]
128. Dai DF, Santana LF, Vermulst M, Tomazela DM, Emond MJ, MacCoss MJ, Gollahon K, Martin GM, Loeb LA, Ladiges WC, Rabinovitch PS. Overexpression of catalase targeted to mitochondria attenuates murine cardiac aging. *Circulation*. 2009; 119(21):2789–2797. [PubMed: 19451351]
129. Dai DF, Chen T, Wanagat J, Laflamme M, Marcinek DJ, Emond MJ, Ngo CP, Prolla TA, Rabinovitch PS. Age-dependent cardiomyopathy in mitochondrial mutator mice is attenuated by overexpression of catalase targeted to mitochondria. *Aging Cell*. 2010; 9(4):536–544. [PubMed: 20456298]
130. Zalk R, Lehnart SE, Marks AR. Modulation of the ryanodine receptor and intracellular calcium. *Annu Rev Biochem*. 2007; 76:367–385. [PubMed: 17506640]
131. Kitzman DW, Nicklas B, Kraus WE, Lyles MF, Eggebeen J, Morgan TM, Haykowsky M. Skeletal muscle abnormalities and exercise intolerance in older patients with heart failure and preserved ejection fraction. *Am J Physiol Heart Circ Physiol*. 2014; 306(9):H1364–1370. [PubMed: 24658015]

Table 1

Skeletal muscle abnormalities in Heart Failure (HF).

Author, yr (#)	Population character	Key measurement	Outcome & Conclusion
Mancini et al., 1988 [33]	HF & age matched healthy control male (~54 yr)	HF:PeakVO ₂ 13.6±5ml/min/kg In vivo P31-NMR	Lower leg muscle mass loss in HF Calf metabolic abnormality part due to muscle atrophy During Exercise Pi/PCr: HF >>Control Recovery time post Ex: HF> control Impaired metabolic profile suggest intrinsic changes not only due to muscle loss
Southern et al., 2015 [54]	HF& age matched Control (~65 yr)	near-infrared spectroscopy	Oxidative capacity lower in HF & Exercise training improved oxidative capacity only in control, suggested reduced oxidative capacity and impaired training adaptation in HF
Mancini et al., 1994, [56]	HF & Control (~58yr)	Ventilatory capacity: progressive isocapnic hyperpnea respiratory muscle deoxygenation near-infrared spectroscopy	Reduced respiratory ventilator capacity without respiratory muscle deoxygenation in HF
Wiener et al., 1986 [57]	HF & age matched control (~60yr)	Forearm blood flow with plethysmography Metabolism: 31P-NMR	Forearm blood flow was similar but increased Pi/PCr upon exercise suggested abnormal metabolism in HF was not due to decreased muscle blood flow rather intrinsic changes in mitochondria or substrate utilization
Mancini et al., 1989 [58]	HF& age matched control (~57yr)	31P NMR: Pi/PCr and PH changes In vitro muscle biopsy: enzyme activity fiber type	HF fiber shift to increased fast, glycolytic type IIb fibers; decreased fatty acid oxidation enzyme activity; ³¹ P NMR also shown higher Pi/PCr; No correlation between Pi/PCr to VO ₂ and enzyme activity suggested intrinsic changes not due to metabolic response (Pi/PCr changes)
Toth et al., 2012 [87]	HF & Control, age and physical activity matched	18wks resistance training (RE) Muscle biopsy, in vitro analysis	Larger but fewer mitochondria per fiber without difference in content between HF and control; No difference in mitochondria enzyme activity and mRNA level of cytochrome oxidase RE increased mitochondrial transcription factor A and muscle strength for both HF and control, but did not alter mitochondrial size/content, enzyme or transcriptional regulator
Middlekauff et al., 2013[93]	HF & Control (~50 yr, peakVO ₂ 11.6ml/kg/min)	Muscle biopsy & in vitro biochemistry analysis: fiber typing & mitochondria enzyme activity	HF fibers shift to fast twitch; no difference in vascular index or many of mitochondrial enzyme activities; clonidine revealed no impact for HF suggested skeletal myopathy is not caused by neuroendocrine disuse
Zizola et al., 2015 [88]	Myocardial infarction induced mice	In vitro muscle biopsy: fiber typing & enzyme activity assay; ATP content	Muscle dysfunction associated with impaired PPARδ signaling; upon treating MI mice with PPARδ agonist corrected diminished oxidative capacity and FA metabolism (CPT1) and improved exercise capacity
Schrepper et al., 2012 [94]	Pressure overload hypertrophy in rat heart	In vitro assay form muscle biopsy: Isolation of fresh mitochondria mRNA and protein assay	Complex I & II early increase and the later decline (I, II & III) in respiratory capacity not due to differences in gene expression or supercomplex assembly.
Dai et al., 2011 [118]	AngII induced cardiomyopathy in mice treated with SS-31	Flow cytometric access MitoSOX and DCFDA signal	SS-31 reduced AngII induced ROS signal (mitochondrial superoxide and total cellular ROS)
Kitzman et al., 2014 [131]	HF with preserved ejection fraction (HFpEF)	Peak VO ₂ Fiber type capillary-to-fiber ratio	Reduced type I fiber & capillary to fiber ratio contribute to exercise intolerance among HF