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Impact of aging on mitochondrial function in cardiac and skeletal muscle

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ABSTRACT

Both skeletal muscle and cardiac muscle are subject to marked structural and functional impairment with aging and these changes contribute to the reduced capacity for exercise as we age. Since mitochondria are involved in multiple aspects of cellular homeostasis including energetics, reactive oxygen species signaling, and regulation of intrinsic apoptotic pathways, defects in this organelle are frequently implicated in the deterioration of skeletal and cardiac muscle with aging. On this basis, the purpose of this review is to evaluate the evidence that aging causes dysfunction in mitochondria in striated muscle with a view towards drawing conclusions about the potential of these changes to contribute to the deterioration seen in striated muscle with aging. As will be shown, impairment in respiration and reactive oxygen species emission with aging are highly variable between studies and seem to be largely a consequence of physical inactivity. On the other hand, both skeletal and cardiac muscle mitochondria are more susceptible to permeability transition and this seems a likely cause of the increased recruitment of mitochondrial-mediated pathways of apoptosis seen in striated muscle. The review concludes by examining the role of degeneration of mitochondrial DNA versus impaired mitochondrial quality control mechanisms in the accumulation of mitochondria that are sensitized to permeability transition, whereby the latter mechanism is favored as the most likely cause.

1. Introduction

The heart and skeletal muscles undergo dramatic changes with aging that contribute to reduce exercise capacity as we age. In the context of the heart amongst the most important changes is a progressive cardiomyocyte loss and replacement fibrosis; factors that are key to the impaired diastolic function [1] and increased risk of heart failure [2] seen with aging. Similarly, in skeletal muscle there is also a progressive loss of myocytes, with heterogenous atrophy amongst the remaining muscle fibers, and contractile dysfunction [3]; changes that contribute to muscle weakness and increased fatigability [4] and which in advanced age also contribute to impaired mobility and loss of independence [5]. In view of the health consequences of these changes, understanding their mechanistic basis is important to improve health and quality of life in the elderly.

Mitochondria play a diversity of roles involved in the homeostatic maintenance of cells, ranging from energy production to reactive oxygen species (ROS) signaling to regulation of intrinsic cell death pathways [6]. It is thus logical to consider that dysfunction in these organelles likely plays a key role in the structural and functional deterioration of skeletal and cardiac muscle with aging [7]. Although there have been many studies examining age-related changes in mitochondrial function in skeletal and cardiac muscle, the literature is often contradictory for a variety of reasons, including differences in the type of preparation used to interrogate mitochondrial function (e.g., isolated organelles versus preparations where mitochondrial structure is intact) [8], wide variability in the ages of animals studied, and a relatively high reliance on inbred rodent strains that may be confounded by specific pathologies [9]. Notwithstanding these issues, accumulating evidence strongly implicates dysregulation of the mitochondrial permeability transition pore (mPTP) in both skeletal muscle [10,11] and heart [12,13] with aging, leading to an increase in apoptosis. Furthermore, aged mitochondria are more susceptible to dysfunction following ischemia-reperfusion [14,15], which may be important in explaining how mitochondria contribute to

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cardiomyocyte injury with advancing age. Amongst the putative causes of the accumulation of mitochondria that are sensitized to permeability transition and which evidence other features of impaired function with aging is impairment in mitochondrial quality control [16,17]. The evidence pertaining to these points will be reviewed below.

2. Mitochondrial respiratory capacity

Mitochondria generate adenosine triphosphate (ATP) through harnessing the potential energy created by the pumping of protons into the mitochondrial inter-membrane space. The pumping of these protons is coupled to the transfer of electrons from electron donors and electron acceptors down an electrochemical gradient within the electron transport chain, with the terminal electron acceptor being oxygen in a reaction occurring at complex IV of the mitochondria. For this reason, measurement of mitochondrial respiratory capacity (i.e., rate of oxygen consumption) can provide important insights into the capacity for aerobic ATP production by the mitochondria. A caveat to this is that the coupling of the mitochondrial proton gradient to ATP production varies under both physiological and pathological conditions, meaning that the amount of ATP produced per unit of oxygen consumed (i.e., the so-called P:O ratio) is also an important influence on aerobic ATP production. Due to their wide metabolic scope, both skeletal and cardiac muscle are highly reliant on mitochondria for aerobic synthesis of ATP and it may thus seem logical to think that declining mitochondrial respiratory capacity may contribute to impairments in contractile performance of heart and skeletal muscle with aging. However, evidence that the mitochondrial respiratory capacity is impaired in aging heart and skeletal muscle is equivocal and at least in part depends upon the physical activity status, as will be reviewed below.

2.1. Mitochondrial respiratory capacity in aging skeletal muscle

Although a reduction in skeletal muscle aerobic function with aging is well known and is seen even when aged muscles are provided with similar levels of oxygen delivery as young muscles [18], the role played by the mitochondrion in this decline is less clear. *In vivo* magnetic resonance spectroscopy measurements examining the dynamic rate of phosphocreatine (PCr) resynthesis (an indicator of mitochondrial respiration-induced generation of high energy phosphates) following muscle contractions generally find a reduction in aged muscle [19–22], consistent with an impaired respiratory capacity with aging, although this may not apply to physically active populations [23,24]. Note that, with the exception of one study finding a greater reduction in PCr synthesis rate than could be accounted for by changes in mitochondrial content quantified by electron microscopy [20], these *in vivo* measures do not account for muscle mitochondrial content. As such, impairment at the mitochondrial level, if present, could be obscured at the whole tissue level by a compensatory increase in mitochondrial content.

More direct measures of mitochondrial function in skeletal muscle include *ex vivo* preparations such as in saponin-permeabilized myofibers, a preparation where all mitochondria are represented and their structure preserved [25]. Using this method to infer organelle-specific changes in function requires normalization of respiration values to an index of mitochondrial content, such as biochemical activities (e.g., citrate synthase) or protein contents (e.g., voltage dependent anion channel or specific respiratory chain proteins). Alternatively, another approach facilitating in-depth study of mitochondrial function involves mechanically isolating the mitochondria. Although mitochondrial isolation may simplify issues relating to identifying an organelle-specific defect (assuming mitochondrial purity is similar between groups in the isolates; see [26] for discussion of this issue), there is evidence that the isolation process may not only exaggerate the impact of aging but also yield differences that are not seen in saponin-permeabilized myofibers [26]. This does not invalidate the use of isolated organelles, but certainly warrants caution in the interpretation of resulting data, as discussed elsewhere [8]. Both isolated mitochondria and saponin-permeabilized myofiber approaches have the advantage of permitting detailed study of mitochondrial function in response to the addition of various substrates and inhibitors; however, an important caveat is that the aging *milieu* is removed through the use of standardized incubation media. The potential significance of this will be addressed below.

Studies employing saponin-permeabilized myofibers find that whereas respiratory capacity of skeletal muscle is usually preserved with aging [10,11,27,28], except perhaps in rodent slow twitch muscle [10], after accounting for markers of mitochondrial content a reduction in intrinsic mitochondrial respiratory capacity may be seen in both rodent [10] and human skeletal muscle [11]. Measures of respiratory function in isolated mitochondria vary, with several studies identifying an organelle-specific deficit in respiratory capacity [29–33], but others no change [34,35], keeping in mind the aforementioned potential of the isolated mitochondrial preparation to exaggerate the aging impact [26]. Interestingly, recent work comparing mitochondrial respiratory function *in vivo* using magnetic resonance spectroscopic methods versus respirometry in saponin-permeabilized myofibers suggested that respiratory impairment of aged mitochondria was primarily a function of the aging *milieu* [36]. However, an important caveat here is that the method used to interrogate mitochondrial respiratory function in this study examined PCR resynthesis following cuff-occlusion of hindlimb blood flow [36] and it is possible that the *in vivo* results reflect in part a differential response of aged mitochondria to this cuff occlusion followed by reperfusion, as has been shown to occur in aging heart mitochondria (see sections addressing ischemia-reperfusion in heart, Sections 3.2 and 4.2, below). Thus, collectively, the available evidence suggests that there is a mild impairment in mitochondrial respiratory capacity with aging, but for the most part, aging muscle seems to adequately compensate for this problem since respiratory capacity per unit of muscle in *ex vivo* preparations appears to be largely preserved [10,11].

2.2. Mitochondrial respiratory capacity in aging heart

A reduced cardiomyocyte mitochondrial respiratory capacity has been suggested to contribute to reduce contractile function in the aging heart [37]. As with skeletal muscle, a variety of approaches have been taken to assess mitochondrial respiratory function in cardiac muscle with aging. *In vivo* spectroscopy methods are becoming increasingly popular to monitor cardiac metabolism in human patients. In this respect, there is evidence that with normal aging the ratio of PCr to adenosine triphosphate (ATP) (PCR:ATP) is reduced in the heart with aging [38,39], a finding that indicates a disproportionate depletion of high energy phosphates relative to their resynthesis by mitochondria, suggestive of respiratory impairment. Furthermore, this reduction in PCr:ATP ratio with aging is attenuated in physically active subjects [40], similar to what was reported above in skeletal muscle mitochondria [23,24], suggesting some of the decline may be avoided through physical activity. Since obtaining a cardiac biopsy from otherwise healthy human subjects is impractical, none of these prior studies can address whether the implied reductions in cardiac muscle respiratory capacity are secondary to reduced mitochondrial content and/or impaired organelle respiratory capacity.