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Regulation of Skeletal Muscle Mitochondrial Function: Genes to Proteins

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Abstract

The impact of aging on mitochondrial function and the deterministic role of mitochondria on scenescence continue to be topics of vigorous debate. Many studies report that skeletal muscle mitochondrial content and function are reduced with aging and metabolic diseases associated with insulin resistance. However, an accumulating body of literature suggests that physical inactivity typical of aging may be a more important determinant of mitochondrial function than chronological age, *per se*. Reports of age-related declines in mitochondrial function have spawned a vast body of literature devoted to understanding the underlying mechanisms. These mechanisms include decreased abundance of mtDNA, reduced mRNA levels, as well as decreased synthesis and expression of mitochondrial proteins, ultimately resulting in decreased function of the whole organelle. Effective therapies to prevent reverse, or delay the onset of the aformentioned mitochondrial changes, regardless of their inevitability or precise underlying causes, require an intimate understanding of the processes that regulate mitochondrial biogenesis, which necessitates the coordinated regulation of nuclear and mitochondrial genomes. Herein we review the current thinking on regulation of mitochondrial biogenesis by transcription factors and transcriptional co-activators and the role of hormones and exercise in initiating this process. We review how exercise may help preserve mitochondrial content and functionality across the lifespan, and how physical inactivity is emerging as a major determinant of many age-associated changes at the level of the mitochondrion. We also review evidence that some mitochondrial changes with aging are independent of exercise or physical activity and appear to be inevitable consequences of old age.

INTRODUCTION

There are many features of skeletal muscle that have intrigued inquisitive minds for centuries. Among all cell types, skeletal muscle possesses the unique ability to increase metabolic rate nearly 100-fold during the transition from a basal resting state to near maximal contractile activity. Though perhaps underappreciated by many, this contractile activity of skeletal muscle, combined with the mechanics of tendons and bones, enables us to navigate our environment. The luxury of locomotion comes at a cost. Energy currency in the form of high energy phosphates (ATP, PCr) is necessary to ensure the proper execution of a coordinated series of events, namely 1) the maintenance of the sarcolemmal membrane potential, 2) interaction of the contractile proteins, and 3) calcium handling by the sarcoplasmic reticulum. It is remarkable that myocytes are able to maintain metabolic homeostasis in the face of such a dramatic increase in energetic demand (Dawson *et al.*

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CONFLICT OF INTEREST
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1977). Indeed, depletion of cellular ATP is seldom observed, even under extreme conditions such as sustained high-intensity muscle activity during ischemia (Lanza *et al.* 2007). Thus, skeletal muscle is quite well-adapted for prolonged periods of energetically costly contractile activity.

Skeletal muscle owes its metabolic prowess to a defining evolutionary event that occurred approximately 1.5 billion years ago when a eukaryotic cell symbiosed with a Protobacterial ancestor (Balaban *et al.* 2005,Gray *et al.* 1999). In what has become known as the serial endosymbiosis theory, the eubacterium evolved into the modern day mitochondrion; an organelle that has been dubbed the “powerhouse of the cell” because of its role in generating a readily usable form of chemical energy; ATP. Mitochondria generate ATP by oxidizing carbon substrates in the tricarboxylic acid cycle, creating a transmembrane proton gradient by the action of the electron transport system, and harnessing this potential energy to phopshorylate ADP (Figure 1).

Although the precise evolutionary origin of the organelle remains uncertain, the assumption of a protobacterial ancestry stems largely from the fact that mitochondria contain their own genome that is physically and functionally distinct from the nuclear genome. Human mitochondrial DNA is circular and encodes 13 respiratory chain proteins as well as 22 transfer RNAs and 2 ribosomal RNAs essential for the transcription and translation of the mitochondrial genome (Anderson *et al.* 1981). However, proteins encoded by the mitochondrial genome represent but a small fraction of the thousand or so proteins that make up the mitochondrion, the remainder of which are encoded by nuclear DNA and are imported into the mitochondrion after synthesis in the cytosol. Thus, it seems that over a very long period of time, the nucleus has assumed the majority of the control over mitochondrial biogenesis, though mitochondria retain exclusive governance of a small number of essential proteins, without which the organelle could not function.

Mitochondria have an emergent role in regulating lifespan. From a standpoint of basic survival, highly functional mitochondria in skeletal muscle promote survival by enabling organisms to generate sufficient energy to catch prey or avoid becoming prey. Although physical activity is no longer required to obtain food in most Westernized cultures, mitochondria still appear to play a critical role in the overall health and survival of our species. Mitochondria regulate cellular function in ways that go well beyond their role in energy homeostasis. For example, mitochondria are involved in the process of cell death through the release of apoptogenic factors such as apoptosis inducing factor, endonuclease G, and cytochrome c, which respectively induce DNA condensation, DNA degradation, and apoptosome formation (van Gurp *et al.* 2003). Mitochondria are also a major source of cellular reactive oxygen species (ROS), originating as superoxide at various sites along the electron transport machinery (Balaban *et al.* 2005) (figure 1). ROS are generally regarded as being detrimental to cellular function by modifying the structure and function of proteins, lipids, and nucleic acids. However, ROS are also believed to play an important physiological role in cell signaling through pathways that are sensitive to the cellular redox state (Stowe *et al.* 2006). Thus, although mitochondria were once uninvited guests in the eukaryotic cell, they have since made themselves indispensable by assuming functions that are critical to proper cellular function. Herein we will begin by examining mitochondrial function in the context of aging, followed by discussion of factors that are purported to regulate mitochondrial biogenesis.

AGING MITOCHONDRIA

One of the most profound physical features of human aging is the decline in skeletal muscle mass (sarcopenia), muscle fiber quantity, and preferential loss of type 2 motor units (Lexell

1995). The functional consequences of this loss of muscle mass are profound, including decreased muscle strength, power, and ability to safely perform activities of daily living (Grimby 1995,Wolfson *et al.* 1995). The decline in muscle strength with aging often exceeds the decline in muscle mass, reflected by reduced force per cross sectional area (i.e., specific strength) (Davies *et al.* 1986), but not when noncontractile tissue is accounted for (Kent-Braun & Ng 1999). A mechanism underlying the age-related declines in muscle mass is likely to involve decreases in protein synthesis, which have been demonstrated at the level of the whole body (Nair 1995), mixed muscle (Rooyackers *et al.* 1996,Yarasheski *et al.* 1993), and myosin heavy chain (Balagopal *et al.* 2001). The effect of aging on muscle endurance or fatigue, another key determinant of physical function, is more ambiguous (Kent-Braun 2009,Larsson & Karlsson 1978,Lennmarken *et al.* 1985). Metabolic disturbances such as a progressive decline in whole body peak oxygen uptake have been observed, even after correcting for lean mass (Grimby & Saltin 1966,Rogers *et al.* 1990,Short *et al.* 2005). At the cellular level, there has been a strong movement to understand how the mitochondrion changes with age because of its importance in energy metabolism and physical performance. To date, many reports favor the notion that mitochondrial function declines with age, although this remains a topic for vigorous debate. As reviewed below, although there is a great body of evidence to suggest that mitochondrial function is impaired with aging, it is becoming increasingly clear that much of this association is driven by the commensurate changes in physical activity, disease, nutrition, or other environmental factors rather than age *per se*.

Mitochondrial content

Skeletal muscle mitochondria were once thought to be small, spherical or bean shaped organelles based on their appearance in electron micrographs, but are now known to exist as extensively branched reticula with two distinct sub-populations (Kirkwood *et al.* 1986). Subsarcolemmal mitochondria are localized within a few microns of the plasma membrane, and intermyofibrillar mitochondria are distributed among the contractile proteins. The ability to sustain a steady-state level of muscular work is a function of the number of mitochondria or, more accurately, the volume density of mitochondria (Chance *et al.* 1985). Various methods have been used to demonstrate that mitochondrial content decreases with aging, including electron microscopy (Conley *et al.* 2000), mitochondrial DNA copy number (Barazzoni *et al.* 2000,Menshikova *et al.* 2006,Short *et al.* 2005,Welle *et al.* 2003), cardiolipin content (Menshikova *et al.* 2006), and citrate synthase activity (Houmard *et al.* 1998,Rooyackers *et al.* 1996,Short *et al.* 2005,Welle *et al.* 2000). There is little doubt that reduced mitochondrial content in peripheral tissue such as skeletal muscle, combined with impaired central hemodynamics, is partly to blame for age-related declines in whole-body peak oxygen uptake (Robinson *et al.* 1973,Rogers *et al.* 1990).

Mitochondrial function ex vivo

When viewed from a standpoint of bioenergetics, the function of mitochondria is to generate ATP. Thus, the most direct way to assess mitochondrial function in this context is to measure ATP synthesis rates. Recombinant firefly luciferase can be used to measure the rates of ATP production by monitoring light emission as a substrate luciferin is oxidized in an ATP-dependent manner (DeLuca & McElroy 1974). By adding ADP in the presence of specific combinations of substrates, it is possible to measure the rates of ATP synthesis as a result of electron flow through distinct complexes along the cytochrome chain. Using this method, we measured mitochondrial ATP production rates in mitochondria isolated from muscle tissue isolated from humans across a wide age range (Short *et al.* 2005). Substrates glutamate and malate were used to isolate electron flow exclusively into respiratory chain complex I (NADH dehydrogenase). The combination of succinate and the complex I inhibitor rotenone were used to isolate electron flow exclusively to complex II (succinate

dehydrogenase). As shown in figure 2, mitochondrial ATP synthesis rates declined with increasing age at a rate of 8% per decade for respiratory chain complexes I and II. The fact that these ATP production rates are expressed per gram of tissue is of critical importance to the interpretation of the data. When expressed in this way, one must use caution in the use of the term “mitochondrial dysfunction”, which implies that there is an intrinsic defect in the organelle that limits ATP synthesis. As noted above, the decline in mitochondrial content with aging is likely to be a major determinant of the age-related decline in mitochondrial ATP synthesis rates, although impaired function at the level of the organelle may also be a factor. Moreover, the age-related decline in ATP synthesis rates occurs in parallel with decreasing mtDNA copy number (Short *et al.* 2005). To address whether there is an intrinsic mitochondrial defect with aging, we expressed ATP synthesis rates per milligram of mitochondrial protein (figure 2**),** which removes the contribution of age-related differences in mitochondrial content. Although normalizing to mitochondrial protein content slightly lessens the age-related decline in ATP synthesis rates, there remains a significant reduction (~5% per decade) that cannot be explained simply by a decline in mitochondrial content. These changes appear to be independent of physical activity as the study included only individuals who exercised less than 30 minutes per day, twice per week for the 9 months preceding the study. Although activity questionnaires revealed no differences in habitual physical activity levels across the age range, it is possible that the results could be impacted by more subtle changes in physical activity patterns that would be undetectable by questionnaire. More sensitive, objective assessments of habitual physical activity such as accelerometry have become essential components of study which aim to assess the independent effects of aging and physical activity patterns on mitochondrial function (Kent-Braun & Ng 2000,Lanza *et al.* 2005,Lanza *et al.* 2007). Thus, aging is associated with an intrinsic impairment in mitochondrial function that goes beyond a simple reduction in mitochondrial content. This apparent gradual decline in mitochondrial function across the lifespan is mirrored by a similar rate of decline in whole-body VO_2 peak (Short *et al.* 2005). Although age-related changes to central hemodynamics (e.g., cardiac output) undoubtedly play a major role in the age-related decline in VO_2 peak, we find that muscle mitochondrial capacity and VO_2 peak are well-correlated (Short *et al.* 2005), suggesting that peripheral changes at the level of the mitochondria should not be discounted.

As an alternative to measuring ATP synthesis rates, it is common to assess mitochondrial function by measuring the rates of oxygen consumption. Oxygen, acting as the final electron acceptor at the level of complex IV (cytochrome c oxidase), is consumed at a rate that is generally proportional to the rate of ATP synthesis, assuming that the two processes are well-coupled. For more than half a century, researchers have been using polarographic oxygen electrodes to study mitochondrial physiology (Chance & WILLIAMS 1955). More recently, several groups have applied this methodology to study the effects of aging on human skeletal muscle mitochondrial function. In general, the results support the notion that mitochondrial respiratory activity declines with age (Boffoli *et al.* 1994), even after expressing respiration rates relative to mitochondrial protein (Trounce *et al.* 1989). These observations are in-line with the notion that, irrespective of age-related declines in mitochondrial content, there appears to be impairment in mitochondrial function with aging. It is, however, worth highlighting the recent work of Rasmussen and colleagues, who report more modest changes in mitochondrial function with aging (Rasmussen *et al.* 2003a). A unique aspect of this work was the meticulous attention to the isolation procedures to maximize the integrity, yield, and purity of the isolated mitochondria. Mitochondrial function was assessed from respiration rates and activities of thirteen different enzymes that were normalized to mitochondrial protein content or citrate synthase activity. Although fatty acid oxidation and respiration rates using complex I substrates declined with age, the activities of several key mitochondrial enzymes (e.g., citrate synthase, cytochrome c oxidase, succinate dehydrogenase) were unaltered. The authors propose that age-related

mitochondrial dysfunction is less profound than suggested by many investigators, possibly due to suboptimal isolation procedures and collagen contamination in the mitochondrial fraction. A similar study by the same research group found that mitochondria isolated from skeletal muscle of older humans (70+ years) exhibited some evidence of altered mitochondrial function when compared with young humans (20+ years), but that mitochondrial capacity was related to physical activity and not chronological age (Rasmussen *et al.* 2003b). More recently, Hutter et al. found no evidence of age-related changes in respiration rates of human permeabilized muscle fibers (Hutter *et al.* 2007). Furthermore, these investigators also found that the level of reactive oxygen species was higher in muscle tissue from young compared to older humans; findings that are in stark contrast with the free radical theory of aging (Hutter *et al.* 2007). The extent to which aging *per se* impacts mitochondrial function continues to be a topic of debate, particularly when consideration is given to physical activity, nutrition, and other lifestyle changes that are likely to influence mitochondrial function independently of chronological age.

Mitochondrial function in vivo

Techniques such as phosphorous magnetic resonance spectroscopy (^{31}P-MRS) allow mitochondrial function to be assessed *in vivo*. Although *in vitro* methods are advantageous in probing the intrinsic function of the organelle at distinct stages of oxidative phosphorylation, *in vivo* methods permit mitochondrial assessment under physiological conditions where all circulatory and regulatory systems are intact. Many, but not all, *in vivo* ^{31}P-MRS experiments reveal age effects that are consistent with those observed by *in vitro* measurements from muscle biopsy tissue (table 1). Years after Britton Chance pioneered the use of ^{31}P-MRS to investigate human muscle energetics (Chance *et al.* 1980), several groups applied this method to show that oxidative capacity decreases with age (Conley *et al.* 2000,McCully *et al.* 1991,McCully *et al.* 1993) or remains unchanged (Chilibeck *et al.* 1998,Kent-Braun & Ng 2000,Schunk *et al.* 1999,Taylor *et al.* 1997). Several years later, Petersen et al. measured steady-state basal oxidative phosphorylation flux using a unique ^{31}P-MRS approach and observed a nearly 50% reduction in the basal rate of mitochondrial ATP synthesis in older compared to young participants (Petersen *et al.* 2003). This reduction in basal ATP flux was also observed in aging mice (Marcinek *et al.* 2005). Furthermore, these investigators paired near-infrared spectroscopy and ^{31}P-MRS to demonstrate that mitochondrial coupling was reduced in skeletal muscle of senescent mice. Reduced phosphorylation activity in the basal state is often assumed to reflect mitochondrial dysfunction, but it is unlikely that a decline in resting oxidative phosphorylation flux is consequence of decreased mitochondrial capacity since basal flux represents a small fraction of the overall mitochondrial oxidative capacity. It is important to consider that the findings could also simply reflect reduced mitochondrial content, lower metabolic demand in the resting state (i.e., basal metabolic rate), or reduced oxygen or substrate delivery due to vascular factors. Other groups have measured muscle oxidative capacity by ^{31}P-MRS using a kinetic analysis of phosphocreatine (PCr) recovery following a brief bout of exercise. Oxidative capacity in the vastus lateralis muscle was found to be significantly reduced with age, as evidenced by slower PCr recovery following a bout of femoral nerve stimulation (Conley *et al.* 2000). Mitochondrial volume density was lower in older participants, which accounted for only a small portion of the age-related decline in oxidative capacity. There remained a significant effect of age after normalizing oxidative capacity for mitochondrial content, indicative of some inherent dysfunction at the level of the organelle. As shown in table 1, not all *in vivo* studies have reached this conclusion. A series of studies published by the Kent-Braun group have consistently found that oxidative capacity in the human tibialis anterior muscle is well-preserved with age (Kent-Braun & Ng 2000,Lanza *et al.* 2005,Lanza *et al.* 2007,Larsen *et al.* 2009). A unifying strength of these studies is the careful measurement and matching of daily physical activity levels between age groups. As

reviewed elsewhere (Russ & Kent-Braun 2004), much of the decline in mitochondrial function observed with old age may be secondary to the sedentary lifestyle that often accompanies aging. The idea that certain muscle groups are more susceptible to the detriments of aging has long been discussed, but only recently has oxidative capacity been measured *in vivo* in morphologically and functionally distinct muscle groups in well-matched young and older adults (Larsen *et al.* 2009). The above report indicated that oxidative capacity was reduced with age in the vastus lateralis but not the tibalis anterior muscle (Larsen *et al.* 2009). In sum, the disparity in the literature concerning the effects of aging on muscle mitochondrial function is likely explained by methodological differences, muscle group specificity of age-related changes, and subject characteristics such as age, health, and physical activity levels.

Mechanisms of mitochondrial changes with aging

There is uncertainty concerning the degree to which lifestyle factors versus aging *per se* contribute to declining mitochondrial content and function with old age. The underlying mechanisms are beginning to emerge as investigators probe various factors between the expression of genes and the assembly of the functional organelle. At the level of gene expression, numerous mRNA transcripts for genes encoding mitochondrial proteins are known to be significantly reduced with aging (Barazzoni *et al.* 2000,Calleja *et al.* 1993,Short *et al.* 2005,Welle *et al.* 2000). This decline in mRNA template availability may be the result of reduced gene transcription or mRNA instability with aging. Transcripts corresponding to the mitochondrial genome are undoubtedly influenced by declining mtDNA copy number with age (Barazzoni *et al.* 2000,Lanza *et al.* 2008,Menshikova *et al.* 2006,Short *et al.* 2005). Consequently, the expression of the proteins encoded by these genes would logically be expected to be influenced by the availability of the mRNA template. We recently measured the expression of numerous skeletal muscle proteins using isotopic tags and mass spectrometry and found that mitochondrial proteins encoded by both nuclear and mitochondrial genomes were significantly decreased with aging (Lanza *et al.* 2008,Short *et al.* 2005) (figure 5a). Protein expression, reflecting the balance between protein synthesis and degradation, is also influenced by the translational rate of gene transcripts. Using stable isotopes of amino acids, we found that the *in vivo* synthesis rates of mitochondrial proteins decreased with aging (Rooyackers *et al.* 1996). It is uncertain whether aging affects the synthesis rates of mitochondrial proteins encoded by both genomes in the same way. Recent advances in measuring the synthesis rates of individual mitochondrial proteins (Jaleel *et al.* 2008) will allow us to address this question in the future. Although specific rates of mitochondrial protein breakdown have yet to be measured *in vivo*, it is likely that mitochondrial protein turnover is reduced with aging based on the observations that Lon protease expression, a key enzyme for mitochondrial proteolysis, is reduced in older mice (Bota *et al.* 2002). In support of this notion we recently reported that whole body proteolysis decreases with aging (Henderson *et al.* 2009). A potential mismatch between rates of protein synthesis and breakdown may lead to an overall decrease in protein expression, but an important consequence of decreased protein turnover is the accumulation of oxidatively damaged dysfunctional proteins. Indeed, aging is associated with increased protein carbonylation (Hepple *et al.* 2008)and nitrotyrosine-modified proteins (Fugere *et al.* 2006). In addition to proteins, nucleic acids also demonstrate increased levels of oxidative damage with aging (Michikawa *et al.* 1999,Short *et al.* 2005), particularly mtDNA, whose susceptibility is increased by its proximity to the source of damaging reactive oxygen species and the lack of protection by histones. Mitochondrial fusion and fission appear to be critical for the maintenance of mtDNA (Herlan *et al.* 2003). At this point, the link between mitochondrial fusion/fission, aging, and mtDNA damage remains in its nascent stages (Lee *et al.* 2007). Altogether there are multiple factors at the levels of gene expression, protein

synthesis, protein quality, and mitochondrial dynamics that can account for reductions in mitochondrial number and function with aging.

Insulin sensitivity, aging, and the role of mitochondria

Insulin stimulates glucose uptake in peripheral tissues such as skeletal muscle and suppresses hepatic glucose production. The term “insulin sensitivity” is used to qualify the vigorousness of the response of target tissues to changing insulin levels. Insulin sensitivity is generally believed to decline with old age (Defronzo 1979,Houmard *et al.* 1995,Petersen *et al.* 2003,Short *et al.* 2003) but in much the same way as the aging mitochondrion, the reported effects of age vary considerably depending on the sample demographics. Studies in relatively lean individuals often show that insulin sensitivity is well maintained across the lifespan (Broughton *et al.* 1991,Lanza *et al.* 2008,Seals *et al.* 1984). Accumulating evidence supports the notion that physical inactivity and adiposity, not chronological age, are strong predictors of the decline in insulin sensitivity with age (Basu *et al.* 2003,Kohrt *et al.* 1993,Szoke *et al.* 2008).

Mitochondria have been implicated in the age-related decline in insulin sensitivity. This notion is largely driven by correlative evidence, specifically in obese (Simoneau & Kelley 1997), type 2 diabetic (Kelley *et al.* 2002), and elderly individuals (Petersen *et al.* 2003,Short *et al.* 2005). A reasonable hypothesis has been proposed whereby impaired mitochondrial function leads to decreased insulin signaling as a result of lipid metabolite accumulation (Shulman 2000). Although championed by many, this relationship has become clouded by numerous instances where mitochondrial function and insulin sensitivity are clearly dissociated (Boushel *et al.* 2007,Lanza *et al.* 2008,Pospisilik *et al.* 2007,Short *et al.* 2003,Toledo *et al.* 2008). A recent observation from our lab demonstrates that insulin sensitivity is similar in young and older men in spite of a significant age-related reduction in mitochondrial oxidative capacity (Lanza *et al.* 2008). Although mitochondria are frequently bestowed a causative role in the pathogenesis of insulin resistance, this remains a controversial topic that merits further investigation. As we will discuss below, there is some intriguing evidence that mitochondrial abnormalities observed in certain insulin resistant populations may be a consequence of impaired insulin signaling given that mitochondrial biogenesis has been shown to be regulated, in part, by insulin.

EFFECTS OF EXERCISE ON MITOCHONDRIAL FUNCTION

Numerous physiological stimuli (e.g., temperature, stress, hormones, hypoxia, caloric intake) are known to affect mitochondrial physiology, but endurance exercise remains one of the most robust, simple, cost-effective, and well-studied stimuli for mitochondrial biogenesis. Holloszy, having noted that the muscles of wild animals exhibited higher mitochondrial content than their domesticated counterparts, followed up on these observations by showing that prolonged treadmill running in rats increased mitochondrial oxidative capacity (Holloszy 1967), thus opening the floodgates for decades of research into mitochondrial adaptations to exercise. Countless studies have documented the beneficial effects of endurance exercise in a wide variety of species. Specifically, endurance training increased mitochondrial content, mitochondrial enzyme activities, and muscle oxidative capacity (Baldwin *et al.* 1972,Bizeau *et al.* 1998,Chow *et al.* 2007,Davies *et al.* 1981,Demirel *et al.* 1999,Dudley *et al.* 1982,Gollnick & King 1969,Holloszy 1967,Howald *et al.* 1985,Kent-Braun *et al.* 1990,Short *et al.* 2003,Zamora *et al.* 1995). We recently conducted a study in mice in an effort to better define the precise molecular and cellular mechanisms underlying the effects of exercise on mitochondrial content and function (Chow *et al.* 2007). Mice performed treadmill running for 5 days per week for 8 weeks at 80% of peak O_2 consumption. As expected, endurance training improved mitochondrial function in skeletal muscle, as evidenced by increased whole body peak O_2 consumption, mitochondrial

enzyme activities (citrate synthase, B-hydroxyacyl Coenzyme A dehydrogenase, cytochrome c oxidase), and maximal mitochondrial ATP synthesis rates. Microarray analyses revealed that these training adaptations were accompanied by increased gene transcripts corresponding to mitochondrial proteins encoded by both genomes. These exercise-induced changes in mitochondrial gene transcripts occurred in conjunction with increases in mtDNA copy number and the expression of peroxisome proliferative-activated receptor-γ coactivator-1α (PGC-1α), a transcriptional co-activator that is known to be a key regulator of mitochondrial biogenesis. This and other cellular signals which are believed to be responsible for mitochondrial biogenesis with exercise will be discussed next.

Signaling pathways linking endurance exercise to mitochondrial biogenesis

What is the cellular signal that links endurance exercise with proliferation of mitochondria? Several comprehensive review articles address this question in detail (Holloszy 2008,Lin *et al.* 2005,Scarpulla 2002), so the forthcoming paragraphs will focus primarily on the acute and chronic signals that mediate mitochondrial biogenesis in response to endurance exercise. As mentioned earlier, the vast majority of mitochondrial proteins are encoded by the nuclear genome, but there are a handful of proteins encoded by the mitochondrial genome that are essential to the proper function of the organelle. Thus, successful proliferation of mitochondria requires coordinated control of the expression of 2 distinct genomes. Significant advances in understanding this complex regulation have been made over the past 20 years with the discovery of several transcription factors which regulate the transcription of mitochondrial proteins. Mitochondrial transcription factor A (TFAM) regulates the transcription and expression of the mitochondrial genome (Fisher *et al.* 1987), while nuclear respiratory factors (NRF-1, NRF-2) modulate the transcription of nuclear-encoded mitochondrial proteins (Evans & Scarpulla 1989,Scarpulla 2002,Virbasius *et al.* 1993). TFAM is a product of the nuclear genome, whose promoter includes binding sites for NRF-1 and NRF-2 (Virbasius & Scarpulla 1994). Thus, by demonstrating that NRF-1 and NRF-2 activate the TFAM promoter, Scarpulla's group established a link between the expression of the two genomes. However, the binding of transcription factors to gene promoters is generally not sufficient to activate gene transcription, which often requires enzymatic activity of coactivator proteins (Spiegelman & Heinrich 2004). This role is fulfilled by the protein coactivator PGC-1α, which activates NRF-1, NRF-2, and several additional transcription factors involved with mitochondrial biogenesis, fatty acid oxidation, and gluconeogenesis (Lin *et al.* 2005,Puigserver *et al.* 1998). Thus, as a result of the pioneering work of Scarpulla's and Spiegelman's groups we now have a clearer picture of the coordinated transcriptional control of mitochondrial biogenesis and an upstream inducible coactivator which has become widely recognized as an important regulator of mitochondrial biogenesis.

Endurance exercise initiates mitochondrial biogenesis through PGC-1α in two distinct ways: a rapid increase in PGC-1α *activity* followed by a more chronic increase in PGC-1α *expression*. The initial rapid phase of adaptation was first revealed from 3 key observations by Wright and colleagues (Wright *et al.* 2007). First, gene transcripts and expression of certain mitochondrial proteins increased at similar or faster rates compared to PGC-1α expression in response to exercise. Second, NRF-1 and NRF-2 were bound to their respective promoters in advance of any increase in PGC-1α expression. Third, most PGC-1α is found within the cytosol of resting skeletal muscle but moves into the nuclei in response to exercise. The authors propose that in advance of any changes in PGC-1α expression, exercise rapidly initiates the process of mitochondrial biogenesis by activating PGC-1α (Wright *et al.* 2007).

There are currently two protein kinases that are believed to activate (i.e., phosphorylate) PGC-1α in response to exercise, namely p38 mitogen activated protein kinase (p38 MAPK)

and AMP-activated protein kinase (AMPK) (Jager *et al.* 2007,Knutti *et al.* 2001,Puigserver *et al.* 2001). During muscle activity, the neural excitation of the sarcolemmal t-tubules and contraction of the sarcomeres are coupled by the release of calcium from the sarcoplasmic reticulum. Increased cytosolic Ca^{2+} leads to the activation of calcium/calmodulin-dependent protein kinase (CAMKII) (Rose & Hargreaves 2003). CAMKII appears to be an upstream activator of p38MAPK (Wright *et al.* 2007), providing a link between muscle activity and phosphorylation of PGC-1α. Another important intracellular signal is the increase in AMP concentration that occurs as ATP and ADP are hydrolyzed during contractile activity. AMPK, which is activated in response to elevated cellular AMP, induces mitochondrial biogenesis (Winder *et al.* 2000) and phosphorylates PGC-1α (Jager *et al.* 2007). The aforementioned work represents a sampling of the body of literature that has led to an emerging pathway by which exercise acutely induces mitochondrial biogenesis in advance of changes in PGC-1α expression (figure 3).

In addition to the acute changes in PGC-1α activity, exercise also induces a delayed but sustained signal for mitochondrial biogenesis through increased PGC-1α mRNA and protein expression (Irrcher *et al.* 2003,Pilegaard *et al.* 2003). p38MAPK not only phosphorylates and activates PGC-1α, but also drives the increased expression of PGC-1α in response to exercise. This conclusion was reached by observing increased PGC-1α promoter activity when the p38 MAPK pathway was stimulated but not in the presence of specific p38 MAPK inhibitors (Akimoto *et al.* 2005). Activating transcription factor 2 (ATF2) and myocyte enhancer factor 2 (MEF2) are upstream transcription factors of the PGC-1α gene, both of which are stimulated by phosphorylation by p38 MAPK (Cao *et al.* 2004,Zhao *et al.* 1999). As illustrated in figure 3, exercise stimulates p38 MAPK, thereby activating PGC-1α transcription factors, leading to increases PGC-1α expression and subsequent mitochondrial biogenesis.

Exercise and Aging

Amidst the plethora of documented detriments of aging, physical inactivity represents a less-well studied but no less important hallmark of the aging process. The nematode worm may seem like an unlikely model for human aging, but we now know that *Caenorhabditis elegans*, like humans, exhibit a progressive deterioration in muscle as they age (Herndon *et al.* 2002). Remarkably, the nervous system of the worms remains intact, and it seems that sarcopenia occurs in parallel and possibly as a consequence of decreased locomotor activity as they age. Age-related declines in physical activity have also been reported in rodents (Holloszy 1997) and humans (Black *et al.* 1996), leading investigators to posit that physical inactivity is a major determinant of many physiological detriments ascribed to aging. The issue of physical activity is particularly important in human studies of aging because of substantial inter-individual variability, but free living habitual physical activity patterns are difficult to accurately quantify and are seldom reported or used as covariates.

Physical activity, being both a predictor of age-related mitochondrial dysfunction and potent activator of mitochondrial biogenesis, may be an effective tool to prevent, reverse, or delay the onset of sarcopenia. Although exercise has never been shown to increase maximal lifespan, the potential benefit in combating age-related morbidities has prompted many investigators to examine adaptations to endurance training in older adults. The general consensus is that older adults maintain their ability to adapt to endurance exercise, as evidenced by robust increases in VO_2 peak (Coggan *et al.* 1992,Short *et al.* 2003), mitochondrial enzyme activities (Coggan *et al.* 1992,Menshikova *et al.* 2006,Short *et al.* 2003), mitochondrial content (Jubrias *et al.* 2001,Menshikova *et al.* 2006), protein synthesis rates (Balagopal *et al.* 2001), mtDNA copy number (Menshikova *et al.* 2006), and gene transcripts for mitochondrial proteins (Short *et al.* 2003). However, the overall enthusiasm for exercise as a prescription for aging is somewhat dampened by some reports showing

more modest effects of exercise in older adults that are blunted when compared to responses in young (Aniansson *et al.* 1980,Jubrias *et al.* 2001,Orlander & Aniansson 1980). As discussed elsewhere (Coggan *et al.* 1992), these data may reflect decreased adaptation of older muscle to a training stimulus or simply insufficient training volume to reveal their full potential. Additional studies are needed to assess whether older adults are able to adapt to low-volume intense interval training, which has been recently shown to induce adaptations similar to endurance training in young humans (Gibala *et al.* 2009). Cross-sectional studies of chronically endurance trained young and older adults provide some insight into this issue. Proctor et al. found that oxidative capacity, determined by activities of citrate synthase and succinate dehydrogenase, was lower with age in sedentary individuals, but similar in endurance trained young and older men (Proctor *et al.* 1995). Similarly, mitochondrial respiratory chain activity was similar in young and elderly endurance athletes (Brierley *et al.* 1996). These seminal observations were recently confirmed by our lab in a study of young (18-30 years) and older (59-76) men who were either sedentary or highly endurance trained (Lanza *et al.* 2008). Mitochondria were isolated from biopsy tissue from vastus lateralis muscle, and ATP synthesis rates were measured by luciferase bioluminescence. The data in Figure 4 show that mitochondrial oxidative capacity was lower with age in sedentary but not in endurance trained individuals, consistent with the notion that physical (in)activity is a major determinant of mitochondria-based changes with age. However, of interest, exercise could not completely prevent the age-related reduction in mtDNA abundance (Figure 4). Since genes are ultimately expressed as proteins, we measured the relative abundance of numerous proteins related to muscle energy metabolism by first labeling peptides using iTRAQ reagent, followed by separation and analysis by liquid chromatography tandem mass spectrometry. We found that 27 proteins involved in oxidative and non-oxidative ATP production in skeletal muscle were significantly reduced with age in sedentary individuals, but this age effect was nearly absent in endurance trained young and older men (Figure 5). Three subunits of cytochrome c oxidase and an anapleurotic TCA cycle enzyme remained significantly reduced in endurance trained older adults. Interestingly, this study also found that the expression of the class III histone deacetylase SIRT3, was reduced with aging, but similarly elevated in young and older endurance trained individuals (Lanza *et al.* 2008). Thus, it seems that exercise may activate some of the signaling pathways that are linked with the lifespan-extending effects of caloric restriction. In sum, although exercise may delay or mask some of the functional changes to the aging mitochondrion, there are some changes at the level of mtDNA abundance and protein expression that even regular vigorous endurance exercise cannot entirely prevent. This notion is confirmed by a recent study *in vivo* in which oxidative capacity (by ^{31}P-MRS) was found to be well-preserved with old age in the tibialis anterior but 35% lower in older compared with young runners (Larsen *et al.* 2009). Thus, it seems that while physical activity is an important determinant of sarcopenia and related dysfunctions, there is a component of aging that is an inevitable function of chronological age. The less robust increase in PGC-alpha and TFAM expression in highly trained older people than younger people (49) may explain why older people could not regain all mitochondrial capacity as young people by exercise.

HORMONES AND MITOCHONDRIAL BIOGENESIS

Thus far our discussion has focused mainly on the role of exercise in stimulating mitochondrial biogenesis through intracellular signals such as AMP and calcium. It is important to also consider the role of hormones in maintaining energetic homeostasis in their target tissues. Hormones such as insulin, growth hormone, and thyroid hormone accomplish this homeostasis, in part, by stimulating mitochondrial biogenesis.

Insulin

Insulin is a major postprandial hormone with strong influence on energy metabolism (Nair *et al.* 1984). One of the first clues pointing to insulin as a mediator of mitochondrial function came in a study of muscle protein synthesis in miniature swine (Boirie *et al.* 2001). An 8 hour infusion of insulin (0.7 mU/kg/min) significantly increased the fractional synthesis rate of mitochondrial proteins in skeletal muscle, measured using L-[1-13C]leucine as a tracer. In contrast, the synthesis rates of contractile and sarcoplasmic proteins remained unchanged. Insulin's effect on mitochondrial protein synthesis is mediated, at least in part, through its influence on gene expression as it has been shown to increase mRNA transcripts for cytochrome oxidase 1 and NADH dehydrogenase 1 (Huang *et al.* 1999). As protein synthesis rates increase in response to insulin, so too should the abundance of mitochondrial proteins and overall muscle oxidative capacity. Shortly after Boirie's initial report from swine, these findings were confirmed in humans (Stump *et al.* 2003). As with swine, insulin infusion increased muscle mitochondrial protein synthesis rates, and consistent with Huang et al., gene transcript levels of NADH dehydrogenase and cytochrome c oxidase also increased. Furthermore, mitochondrial oxidative capacity was clearly elevated with insulin infusion, as indicated by increased citrate synthase activity, cytochrome oxidase activity, and mitochondrial ATP production rates (Stump *et al.* 2003). However, this insulin effect in humans has been shown only when amino acids and glucose levels are maintained. This functional response may be attributed to increased expression of mitochondrial proteins; however, it is also possible that ATP production rates could increase acutely as a result of changes in the efficiency of the respiratory chain or allosteric interactions that increase mitochondrial enzyme activities. Subsequent studies in c-peptide negative type 1 diabetic subjects revealed that insulin deprivation significantly reduced many mitochondrial gene transcripts and ATP synthesis rates (Karakelides *et al.* 2007). Although the precise molecular mechanism requires further study, these studies uncover a new role for insulin as a regulator of skeletal muscle mitochondrial function. Thus far, our discussion has focused largely on the role of insulin in stimulating gene expression, the fractional synthesis rates of mitochondrial proteins, and acute stimulation of mitochondrial ATP synthesis. In addition to insulin's anabolic role in regulating mitochondrial protein expression, there is evidence to support a role for insulin in suppressing proteolysis independently of the presence of amino acids (Gelfand & Barrett 1987); an effect which seems to be diminished with age (Rennie 2009). Thus, insulin's ability to enhance mitochondrial function is likely to be due to the combined effects of acute activation of mitochondrial enzymes, increased mitochondrial protein synthesis, and suppressed mitochondrial protein degradation.

Insulin's role in regulating mitochondrial function has provided some inroads to understanding the tenuous relationship between insulin sensitivity and mitochondrial function. Altered mitochondrial physiology is often proposed to be a cause of insulin resistance in populations such as obese (Simoneau & Kelley 1997), type 2 diabetic (Kelley *et al.* 2002), and elderly (Petersen *et al.* 2003,Short *et al.* 2005). Now that insulin has emerged as a regulator of mitochondrial biogenesis, it is not unreasonable to expect that this signaling pathway may be blunted in insulin resistant individuals. In other words, decreased mitochondrial content and function may be a consequence of impaired insulin signaling rather than the reverse. This alternative view is supported by a report that insulin increased muscle mitochondrial ATP synthesis in non-diabetic controls but not in individuals with type 2 diabetes (Stump *et al.* 2003). Similarly, insulin increased mitochondrial protein synthesis in non-diabetic people (Stump *et al.* 2003) but not in people with type 2 diabetes (Halvatsiotis *et al.* 2002). A follow-up study revealed that mitochondrial ATP synthesis rates were similar in type 2 diabetic and nondiabetic people at lower (i.e., postabsorptive) insulin concentrations, but increasing insulin to postabsorptive levels stimulated mitochondrial ATP synthesis in non-diabetic people only (Asmann *et al.* 2006). These

results were mirrored by insulin's effect on PGC-1α expression, which was blunted in type 2 diabetic individuals at high physiological insulin levels (Asmann *et al.* 2006). In sum, these data are consistent with the notion that mitochondrial function is altered in type 2 diabetes because of altered insulin signaling pathways related to mitochondrial biogenesis rather than an intrinsic mitochondrial abnormality. Several other reports demonstrate complete dissociation between mitochondrial function and insulin sensitivity, casting even more shadows on the precise relationship between the two variables. For example, Asian Indians with severe insulin resistance have higher mitochondrial DNA abundance and ATP production rate (Nair *et al.* 2008) and experimental fat overfeeding increases mitochondrial biogenesis while causing insulin resistance (Hancock *et al.* 2008). These studies underscore that there must be many factors other than insulin that are involved in mitochondrial biogenesis and function.

Thyroid hormone and growth hormone

Thyroid hormones have long been recognized as potent mediators of mitochondrial function (Winder & Holloszy 1977). Much of the early work in this area was conducted by the Italian group of Paradies and Ruggiero who found that mitochondria from hyperthyroid rats exhibited increased enzyme activities, substrate oxidation, and respiration rates (Paradies *et al.* 1994,Paradies & Ruggiero 1988,Paradies & Ruggiero 1990). Exogenous thyroid hormone T4 increases mitochondrial volume density increases in both liver and muscle in rats (Goldenthal *et al.* 2004,Wooten & Cascarano 1980). Thyroid hormone also increases the expression of uncoupling proteins (UCP2, UCP3) (Jekabsons *et al.* 1999,Lanni *et al.* 1997), which are believed to alter mitochondrial efficiency by dissipating the proton gradient across the inner mitochondrial membrane. It is logical that UCPs and other mitochondrial proteins would be co-regulated to maintain energetic homeostasis in the face of increased proton leak. Until recently, it was unclear whether thyroid hormone increased ATP production, or simply increased fuel oxidation to maintain an adequate proton motive force. We found that 14 days of T3 treatment in rats increased UCP2 and UCP3 expression as well as mitochondrial ATP synthesis rates in soleus muscle, heart, and liver (Short *et al.* 2001). These data demonstrate that thyroid hormone increases mitochondrial capacity for ATP synthesis in oxidative tissues, rather than simply increasing substrate oxidation to account for dissipation of the transmembrane proton gradient. Thyroid hormone appears to regulate mitochondrial function, in part, by increasing the expression of mitochondrial proteins. A follow-up study from our group showed that T3 administration robustly increased mitochondrial protein synthesis rates (Short *et al.* 2007); an effect that could be explained by increased transcription of genes encoding mitochondrial proteins such as cytochrome c oxidase (Short *et al.* 2001,Wiesner *et al.* 1992). Since mitochondrial DNA contains promoter regions with response elements for thyroid hormone receptors (Enriquez *et al.* 1999), it is probable that thyroid hormone affects mitochondrial protein expression by regulating the transcription of mitochondrial genes. Thyroid hormone also appears to influence mitochondrial function independently of protein expression. Our studies of T3 administration in rats showed that T3 increases mitochondrial protein synthesis in the soleus and plantaris muscles, but ATP synthesis rates increased in the oxidative but not the glycolytic tissues (Short *et al.* 2001,Short *et al.* 2007), suggesting that thyroid hormone may regulate mitochondrial function through other mechanisms. Cardiolipin, which is increased by T3 (Brand *et al.* 1992) is known to exert allosteric control of several enzymes involved in substrate transport such as carnitine acylcarnitine translocase (Paradies *et al.* 1996). Thyroid hormone 3,5-Diiodothyronine (T2) also has been suggested abolish the allosteric inhibition of cytochrome oxidase by ATP (Arnold & Kadenbach 1999). Thus, although thyroid hormone clearly influenced mitochondrial function, the underlying mechanism is multifaceted and involved regulation of the translational rate of gene transcripts as well as post-translational effects on the activities of numerous enzymes that require further study.

The effects of growth hormone (GH) on mitochondrial function are less well-studied, but there is accumulating evidence that similar to thyroid hormone, GH may exhibit some regulatory control over mitochondrial biogenesis and function. We measured muscle mitochondrial function in humans infused for 14 hours with either saline or GH (Short *et al.* 2008). Acute GH infusion significantly increased mitochondrial function, measured by citrate synthase activity and ATP synthesis rates in mitochondria isolated from skeletal muscle. These functional changes were accompanied by significantly increased mRNA transcripts for cytochrome c oxidase and TFAM, and non-significant increases in PGC-1α and NRF-1. Unlike thyroid hormone, these effects occurred without changing mitochondrial protein synthesis rates (Short *et al.* 2008). Additional studies are needed to fully elucidate the effects of GH on mitochondrial function, particularly in consideration of previous findings that acute GH administration to trained cyclists results in decreased exercise performance and increased plasma lactate levels (Lange *et al.* 2002). Although it is difficult to draw conclusions from studies of sedentary versus highly trained subjects, these paradoxical observations suggest that GH may induce mitochondrial biogenesis without a commensurate increase in exercise capacity, possibly because of effects of GH on insulin action and substrate metabolism that may limit exercise performance..

Conclusion

For more than a billion years, mitochondria have resided within their host cells, providing regulatory control of energy homeostasis, cellular signaling, and apoptosis. Reports of age-related declines in mitochondrial function have spawned a vast body of literature devoted to understanding the underlying mechanisms. These mechanisms involve decreased abundance of mtDNA, reduced expression of mRNA encoded by both nuclear and mitochondrial genes, and reduced synthesis and expression of mitochondrial proteins, ultimately resulting in decreased function of the whole organelle. Research into potential therapies to prevent, reverse, or delay the onset of age-related detriments have been fueled by recent advances in understanding the regulation of mitochondrial biogenesis by transcription factors and transcriptional co-activators that govern the coordinated expression of two distinct genomes. As with many physiological processes that must be tightly regulated, mitochondrial biogenesis appears to be controlled in part by hormones such as insulin, thyroid hormone, and growth hormone. Other intracellular signals linked with exercise (i.e., Ca^{2+}, AMP) initiate a signaling pathway involving PGC-1α to induce mitochondrial proliferation and increased function of existing mitochondria. The beneficial effects of exercise on combating age-related mitochondrial dysfunction are well-documented, and although physical inactivity is emerging as a major determinant of mitochondrial dysfunction, exercise cannot ameliorate all signs of aging in the mitochondrion.

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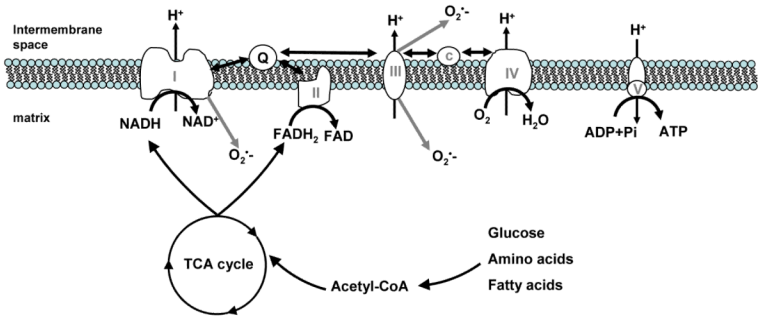

Figure 1.

Mitochondrial ATP synthesis is coupled to fuel oxidation in the tricarboxylic acid (TCA) cycle. Acetyl CoA, derived from glucose, amino acids and fatty acids, is oxidized in the TCA cycle to produce reducing equivalents in the form of NADH and $FADH_2$. NADH is oxidized by complex I (HADH dehydrogenase), while complex II (succinate dehydrogenase) oxidizes succinate to reduce FADH to $FADH_2$. Electron flow from complexes I and II converge on a mobile electron carrier, Coenzyme Q, which transfers electrons to complex III (coenzyme Q-cytochrome c reductase). A second mobile electron carrier, cytochrome c, transfers electrons to complex IV (cytochrome c oxidase). Passage of electron pairs through respiratory chain complexes I, III, and IV results in proton transport across the inner mitochondrial membrane. The resulting proton gradient is used to drive the phosphorylation of ADP at complex V (ATP synthase). Gray arrows indicate sites of superoxide ($O_2^{\cdot -}$) production at complexes I and III.

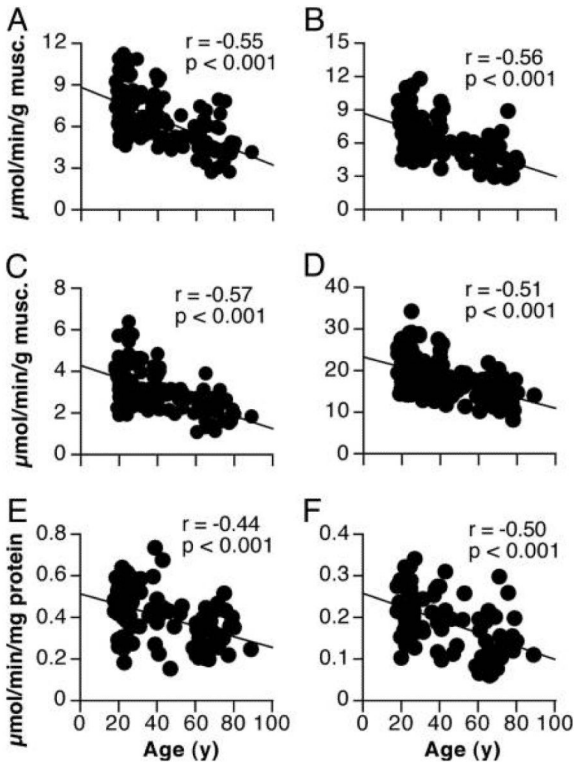

Figure 2.

ATP synthesis rates decreased with aging when expressed per muscle tissue weight (Panels A,B,C) or per mg mitochondrial protein (Panel E and F). Citrate synthase activity also decreased with age (Panel D). Figure reproduced with permission from (Short et al., PNAS, 2005).

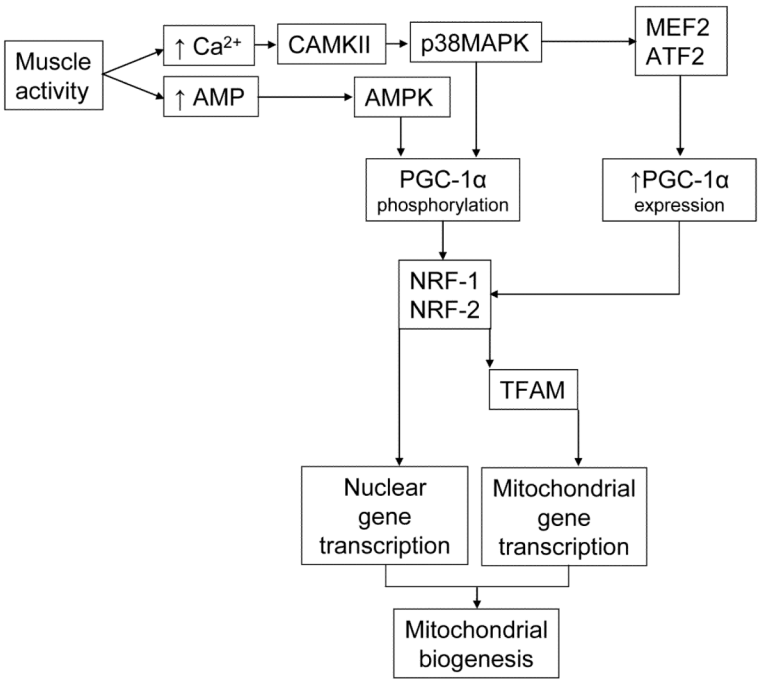

Figure 3.

Muscle activity stimulates mitochondrial biogenesis through a rapid increase in PGC-1α activity as well as a more chronic increase in PGC-1α expression. Increased intracellular calcium and AMP levels during exercise respectively activate calcium-calmodulin dependent protein kinase (CAMKII) and AMP-activated protein kinase (AMPK). The activation of both protein kinases leads to phosphorylation and activation of PGC-1α subsequent activation of nuclear transcription factors NRF-1 and NRF-2, which increase the transcription of nuclear-encoded mitochondrial genes. NRFs also stimulate the expression of the mitochondrial transcription factor (TFAM) which increases the expression of mitochondrial genes encoded by the mitochondrial genome. The more chronic increase in PGC-1α expression occurs as a result of activation of MEF2 and ATF2 by P38MAPK.

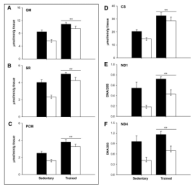

Figure 4.
Mitochondrial ATP production rates were decreased in sedentary older compared to young subjects, but were similar in highly endurance trained young and older adults using substrates glutamate + malate (GM, panel A), succinate + rotenone (SR, panel B), and palmitoyl-l-carnitine + malate (PCM, panel C). Similar results were observed for citrate synthase activity (panel D). Mitochondrial DNA copy number corresponding to NADH dehydrogenase subunits 1 (panel E) and 4 (panel F) were lower in sedentary older adults. Endurance exercise increased mtDNA copy number in both age groups, but could not resolve the age-related decrement. Data are presented as means ± SEM. *Pairwise comparisons revealed significant ($P < 0.05$) effects of age within activity groups; **significant ($P < 0.05$) main effects of training. ■, young; □, older. Reproduced with permission from Lanza et al., Diabetes, 2008.

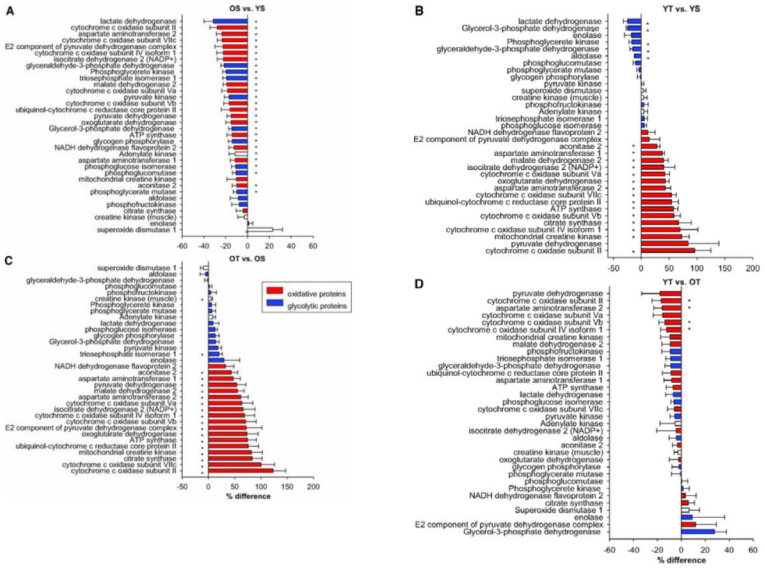

Figure 5.

The relative abundance of numerous proteins involved in oxidative ATP synthesis in skeletal muscle decreased with age in sedentary adults (panel A) and increased with exercise training in young (panel B) and older (panel C). A comparison of trained young and older adults revealed that the majority, but not all, age-related differences in protein expression were resolved (panel D). Data are presented as means ± SEM. *Significant ($\boldsymbol{P} < 0.05$) differences in relative expression. Reproduced with permission from Lanza et al., Diabetes, 2008.

Table 1

Effects of aging on mitochondrial function in humans

Reference	Muscle group	Measurement	Age effect
In vitro studies			
Barrientos et al., 1996 (Barrientos *et al.* 1996)	Vastus lateralis	Isolated mitochondria respiration	↔
Boffoli et al., 1996 (Boffoli *et al.* 1996)	Vastus lateralis	Enzyme activity	↓
Brierley et al., 1996 (Brierley *et al.* 1996)	Vastus lateralis	Isolated mitochondria respiration	↔
Chretien et al., 1998 (Chretien *et al.* 1998)	Deltoid		↔
Coggan et al., 1992 (Coggan *et al.* 1992)	Lateral gastrocnemius	Enzyme activity	↓
Houmard et al., 1998 (Houmard *et al.* 1998)	Vastus lateralis Lateral gastrocnemius	Enzyme activity	↓ ↔
Hutter et al., 2007 (Hutter *et al.* 2007)	Vastus Lateralis	Permeabilized fiber respiration	↔
Lanza et al., 2008 (Lanza *et al.* 2008)	Vastus lateralis	Enzyme activity Isolated mitochondria ATP synthesis	↓ ↓
McCully et al., 1993 (McCully *et al.* 1993)	Lateral gastrocnemius	Enzyme activity	↓
Meredith et al., 1989 (Meredith *et al.* 1989)	Vastus lateralis	Tissue homogenate respiration	↓
Pastoris et al., 2000 (Pastoris *et al.* 2000)	Vastus lateralis Gluteus maximus Rectus abdominus	Enzyme activity	↓ ↔ ↔
Proctor et al., 1995 (Proctor *et al.* 1995)	Vastus lateralis	Enzyme activity	↓
Rasmussen et al., 2003 (Rasmussen *et al.* 2003a)	Vastus lateralis	Isolated mitochondria respiration	↔
Rasmussen et al., 2003 (Rasmussen *et al.* 2003b)	Vastus lateralis	Isolated mitochondria respiration	↔
Rooyackers et al., 1996 (Rooyackers *et al.* 1996)	Vastus lateralis	Enzyme activity	↓
Short et al., 2005 (Short *et al.* 2005)	Vastus lateralis	Isolated mitochondria ATP synthesis	↓
Short et al., 2003 (Short *et al.* 2003)	Vastus lateralis	Enzyme activity	↓
Tonkonogi et al., 2003 (Tonkonogi *et al.* 2003)	Vastus lateralis	Isolated mitochondria and permeabilized fiber respiration	↓ ↓
Trounce et al., 1989 (Trounce *et al.* 1989)	Vastus lateralis	Isolated mitochondria respiration	↔
In vivo studies			
Amara et al., 2007 (Amara *et al.* 2007)	Tibialis anterior First dorsal interosseus	^{31}P-MRS PCr kinetics Near-infrared spectroscopy	↔ ↓
Chilibeck et al., 1998 (Chilibeck *et al.* 1998)	Gastrocnemius	^{31}P-MRS PCr kinetics	↔
Conley et al., 2000 (Conley *et al.* 2000)	Vastus lateralis	^{31}P-MRS PCr kinetics	↓
Kent-braun and Ng, 2000 (Kent-Braun & Ng 2000)	Tibialis anterior	^{31}P-MRS PCr kinetics	↔
Lanza et al., 2005 (Lanza *et al.* 2005)	Tibialis anterior	^{31}P-MRS PCr kinetics	↔
Lanza et al., 2007 (Lanza *et al.* 2007)	Tibialis anterior	^{31}P-MRS PCr kinetics	↔
Larsen et al., 2009 (Larsen *et al.* 2009)	Tibialis anterior Vastus lateralis	^{31}P-MRS PCr kinetics	↔ ↓

Reference	Muscle group	Measurement	Age effect
McCully et al., 1991 (McCully *et al.* 1991)	Lateral gastrocnemius	^{31}P-MRS PCr kinetics	↓
McCully et al., 1993 (McCully *et al.* 1993)	Lateral Gastrocnemius	^{31}P-MRS PCr kinetics	↓
Petersen et al., 2003 (Petersen *et al.* 2003)	Soleus	Basal oxidation (^{13}C-MRS) and phosphorylation (^{31}P-MRS)	↓
Schunk et al., 1999 (Schunk *et al.* 1999)	Vastus lateralis	^{31}P-MRS PCr kinetics	↔
Taylor et al., 1997 (Taylor *et al.* 1997)	Calf	^{31}P-MRS PCr kinetics	↔