

Is Skeletal Muscle Oxidative Capacity Decreased in Old Age?

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Abstract

In humans, decreases in cardiac output play an important role in the age-related decrease in whole-body oxidative capacity. What remains less clear is whether a decline in skeletal muscle oxidative capacity is also an inevitable consequence of aging, as a number of other factors that could affect oxidative capacity also change with age, including: physical activity, health status, fibre-type composition, rates of protein synthesis and muscle blood supply. Both *in vitro* studies using muscle biopsy tissue and *in vivo* studies using ^{31}P -magnetic resonance spectroscopy are used to study muscular oxidative capacity. Using these methodologies, researchers have found age-associated reductions in the oxidative capacities of specific muscles. In most cases, however, the influence of physical activity has not been adequately controlled, making it difficult to evaluate the effects of age itself from those of lifestyle changes associated with aging.

Upon critical evaluation of the existing literature, the following picture regarding the effect of age on muscle oxidative capacity appears: although the maximum level of muscular oxidative capacity attainable through training may decline with age, much of the age-associated decline in oxidative function is related to the reductions in fitness and/or habitual physical activity that typically occur in this population. Future studies in this area must account for the health and activity status of their study participants.

The rate of oxygen delivery to working muscles, the oxygen-carrying capacity of the blood, and the amount of oxygen extracted from the blood and utilised by working muscles are all key determinants in maximal oxygen consumption ($\dot{V}\text{O}_{2\text{max}}$). An age-associated decline in $\dot{V}\text{O}_{2\text{max}}$ has been demonstrated in humans, even after accounting for changes in whole-body and appendicular fat-free muscle mass.^[1] Clearly, decreases in cardiac output play an important role in the age-related decrease in whole-body oxidative capacity. What remains less clear, however, is whether a decline in skeletal muscle oxidative capacity is also an inevitable consequence of aging.

Skeletal muscle oxidative capacity reflects the capacity for a working muscle to regenerate adeno-

sine triphosphate (ATP) through aerobic metabolic pathways.^[2] Several investigators, using a variety of methods, have reported an age-associated decline in oxidative capacity,^[3-6] while others have observed no such loss in oxidative function.^[2,7,8]

There are a number of important factors that must be addressed when considering the effect of old age on human skeletal muscle oxidative capacity. For example, habitual physical activity would be expected to play an important role in determining the extent to which oxidative capacity changes with age,^[1] yet activity is frequently neglected in these studies. Likewise, the health status, including medications and comorbidities, of the study participants is often not reported. Finally, the age ranges vary a great deal among studies; in some cases, 'old' sub-

jects are in their fifties, while in other cases the studies include participants who are well into their eighties. It seems reasonable to expect that age-related changes in oxidative capacity might vary greatly across such a wide range of ages. The goal of this review is to address these considerations and to examine the results of existing studies of oxidative capacity in aging humans, in an effort to come to a consensus on the issue of the effect of aging on skeletal muscle oxidative capacity.

1. *In Vitro* Studies of Oxidative Capacity

One common method used to evaluate muscle oxidative capacity involves assaying the activity of enzymes involved in oxidative metabolic pathways. The enzymes most often used to evaluate oxidative capacity are citrate synthase (CS), succinate dehydrogenase (SDH) and cytochrome-c oxidase (COX). These are mitochondrial enzymes, the first two of which are associated with the tricarboxylic acid cycle, and the last of which is associated with the electron transport chain. Less often, enzymes associated with lipid oxidation, such as β -hydroxyacyl-CoA dehydrogenase, have been examined.

These studies typically involve open or percutaneous biopsy of muscle tissue, most often from the vastus lateralis muscle (VL). The sample is quickly frozen and later homogenised, after which the activity of specific enzymes is determined, usually through fluorometric methods. These assays provide information about the maximal activity of specific enzymes under conditions of saturating substrate availability. Unfortunately, the tissue samples used in these preparations are typically very small, and may not accurately reflect the capacity of the entire muscle under investigation, especially given the possibility of regional fibre-type differences within muscles.^[9,10]

Several studies have shown that the enzymatic activities of CS,^[4,6,11-13] SDH,^[4,14] COX^[13] and β -hydroxyacyl-CoA dehydrogenase,^[4] as well as oxidation of pyruvate by crude muscle homogenate,^[15] all decline as age increases in sedentary individuals. It must be noted, however, that in all of these studies, physical activity either was not controlled or was correlated to the oxidative enzyme activities (table I). Thus, the question remains, as many of the investigators themselves have stated, to what extent

does aging itself, rather than the reduction in physical activity associated with age, contribute to the decline in oxidative capacity?

Some resolution to this question may be found by examining the results of these studies more closely. Two of the studies found that the age-associated declines in enzymatic activity in sedentary individuals were muscle-specific.^[11,12] For example, Houmard et al.,^[11] found that CS activity was reduced in the lateral gastrocnemius, but not the VL of older individuals. Pastoris and colleagues^[12] demonstrated an age-related decline in the CS activity of the VL, but not the gluteus maximus or rectus abdominus muscles. It has been suggested that the gastrocnemius is recruited to a greater extent than the quadriceps during human locomotion^[18] and thus the gastrocnemius might be expected to be affected more by a decline in activity. Likewise, in a truly sedentary population, the gluteus maximus and rectus abdominus muscles are probably not recruited very often, even by young individuals, and thus these muscles might not be affected by a decline in everyday activity.

In another study, investigators compared older (mean age ~58 years) and young (mean age ~25 years) men who were either sedentary or endurance-trained.^[14] No differences in CS or SDH activity were found between the trained old and young groups. Both CS and SDH activity were reduced with age in the sedentary group, but this decline attained statistical significance only for SDH activity in type IIa fibres, suggesting a possible role for fibre-type in the observed changes. The lack of a difference in oxidative capacity between trained older and trained young study participants suggests that physical activity plays a larger role than aging in differences in oxidative capacity. Furthermore, based on the concept of the orderly recruitment of motor units,^[19,20] the fibre-type-specific decline in SDH activity seen in the untrained older men may also be the product of differences in activity. The type IIa fibres, which would be recruited during contractions requiring intermediate amounts of force, might be expected to exhibit a decline in age if the amount or intensity of physical activity was reduced enough to affect the degree of their recruitment during everyday activity. This issue is further complicated by shifts in muscle fibre-type composi-

Table I. Enzymatic studies of oxidative capacity in aging

Study	Age (y)	No./sex of study participants	Muscle(s)	Parameter(s) of oxidative capacity	Activity measure	Activity/fitness difference	Age effect on enzymatic capacity	Activity effect on oxidative capacity
Barrientos et al. ^[16]	15–95	132 M and F	Quads	Isolated mitochondrial substrate oxidation	Survey	NA	Uncorrected: R– Adjusted for activity: NS	NA
Brierley et al. ^[17]	21–95	51	Quads	Isolated mitochondrial substrate oxidation	PA score ^a Handgrip strength	NA	Age vs overall mitochondrial function: R– Corrected for PA or handgrip strength: NS	PA score: R+ Handgrip strength: R+
Coggan et al. ^[4]	O = 64 ± 1 Y = 24 ± 1	10 M, 10 F 10 M, 10 F	LG	CS β-H-CoA D SDH	NA	NA	CS: O < Y β-H-CoA D: O < Y SDH: O < Y	NA
Houmard et al. ^[11]	18–80	55 M	VL LG	CS	Treadmill VO _{2max}	Age vs VO _{2max} : R–	CS: O < Y for LG O ≈ Y for VL	NA
McCully et al. ^[6]	O = 66 ± 6 Y = 28 ± 7	5 M, 1 F 4 M	LG	CS	Cycle VO _{2max}	Age vs VO _{2max} : R–	Age vs CS: R–	VO _{2max} vs CS: R+
Meredith et al. ^[15]	O = 65 ± 3 Y = 24 ± 2	5 M, 5 F 5 M, 5 F	VL	Pyruvate oxidation by muscle homogenate	Cycle VO _{2max}	O < Y	O < Y	NA
Pastoris et al. ^[12]	15–91	32 M, 44 F	VL GM RA	CS MDH SDH COX	Survey	NA ^b	Age vs CS: R– for VL, only	NA
Proctor et al. ^[14]	O = 51–62 ^c Y = 21–30	M M	VL	SDH CS	Treadmill VO _{2max}	O < Y Trained > untrained	SDH: O < Y; untrained type IIa fibres only CS: NS	CS: trained > untrained SDH: trained > untrained

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Table 1. Contd

Study	Age (y)	No./sex of study participants	Muscle(s)	Parameter(s) of oxidative capacity	Activity measure	Activity/fitness difference	Age effect on enzymatic capacity	Activity effect on oxidative capacity
Rooyackers et al. ^[13]	O = 73 ± 2 Mid. = 53 ± 2 Y = 24 ± 1	7 M, 9 F 7 M, 7 F 6 M, 6 F	VL	CS and COX from both muscle homogenate and isolated mitochondria	Treadmill VO _{2max}	Age vs VO _{2max} : R ⁻	Age vs CS: R ⁻ Age vs COX: R ⁻ R ⁻ O < Y for CS, not COX ↓ Overall rate of mitochondrial synthesis	CS: R+ COX: R+

a Modified Allied Dunbar score.

b Although study participants were surveyed, no specific use of this information was presented.

c These investigators included trained and untrained groups of old and young study participants.

COX = cytochrome-c oxidase; CS = citrate synthase; F = females; GM = gluteus maximus; LG = lateral gastrocnemius; M = males; MDH = malate dehydrogenase; Mid. = middle-aged; NA = measurement was not performed; NS = correlation between variables was not significant; O = old; PA = physical activity; Quads = quadriceps; R⁻ = negative correlation between variables, per linear regression; R⁺ = positive correlation between variables, per linear regression; RA = rectus abdominus; SDH = succinate dehydrogenase; Survey = unspecified self-report of study participant activity; VL = vastus lateralis; VO_{2max} = maximal oxygen consumption; y = young; β-H-CoA D = β-hydroxyacyl-CoA dehydrogenase; ↓ = decrease; ≈ = signifies no significant difference between groups.

tion that are believed to occur with aging (see section 3).

Together, the studies of enzymatic activity generally suggest that there is a decline in oxidative capacity with age. However, this decline appears to be largely due to a drop in physical activity that occurs with aging, rather than to aging in and of itself. This conclusion is supported by the observation that oxidative capacity was not reduced in sufficiently active elders.^[14]

Age-associated changes in oxidative enzyme capacities, determined from homogenates of muscle biopsy tissue, may reflect alterations both in the capacity of individual mitochondria for oxidative phosphorylation and in the mitochondrial content of the muscle. To address this issue, a number of investigators have studied isolated muscle mitochondria. Muscle samples for the isolation of mitochondria are collected in much the same manner as those used for enzymatic analysis, and they are subject to the same limitations, but are further complicated by the need for a greater amount of muscle tissue due to the relatively low mitochondrial yield. Newer methodologies promising an improved yield are being developed and refined.^[21]

It has been suggested that detrimental mutations of mitochondrial DNA (mtDNA) accumulate with increasing age and may contribute to an impairment of mitochondrial function (for recent reviews see Brierley et al.^[17] and Short and Nair^[22]). Recently, an age-related reduction in mtDNA, apparently independent of peak oxygen consumption and habitual physical activity, was observed in tissue from the vastus lateralis muscle.^[23] An age-associated decline in mitochondrial protein synthesis has also been reported, but it is important to note that there was no control for activity in that particular study.^[13] Barrientos et al.^[16] found a significant negative correlation between isolated mitochondrial substrate oxidation and age (15–95 years). However, this relationship disappeared when tobacco use and physical activity were included as factors in a multivariate analysis. Brierley et al.^[17] found that mitochondrial substrate oxidation was significantly related to physical activity, but not age. Interestingly, they also observed a clear increase in the number of COX-deficient fibres in study participants aged 64 years and older, suggesting that synthesis of COX

was reduced with age. These investigators speculated that skeletal muscle was sufficiently adaptable to maintain function in the face of age-related changes in mtDNA. It should be noted that all of the mitochondrial studies described here used muscle tissue from the VL.

Rates of mitochondrial protein synthesis tend to be higher than those of mixed muscle protein synthesis,^[13] which implies a high rate of turnover of oxidative enzymes, as they constitute the bulk of mitochondrial protein. With age, the rate of mitochondrial protein synthesis in sedentary individuals has been shown to decline from young to middle age and remain low into old age. Interestingly, no further decline was found from middle to old age, despite continued reductions in CS and COX activities.^[13] These investigators suggested that this discrepancy might be due to increased mitochondrial breakdown or accumulation of mitochondrial protein damage, possibly due to reduced protein turnover with increasing age.

2. *In Vivo* Studies of Oxidative Capacity

In the past decade, much of the study of oxidative capacity in aging has made use of a non-invasive alternative to muscle biopsy methods – phosphorus magnetic resonance spectroscopy (³¹P-MRS) [table II]. Since it was first used to examine human muscle,^[24] MRS has proven to be a valuable and reliable tool for the study of muscle metabolism. Spectroscopy allows investigators to track real-time changes in the relative concentrations of those compounds involved in high-energy phosphate metabolism (ATP, phosphocreatine [PCr], inorganic phosphate [Pi]), as well as changes in muscle pH (by monitoring the chemical shift between PCr and Pi). For a more detailed review of MRS methods, see Kent-Braun et al.^[25] and Sapega et al.^[26]

Spectroscopy samples a much larger region of a muscle than does biopsy. Because it is non-invasive, MRS permits excellent temporal resolution during and after exercise. Furthermore, MRS provides a more functional approximation of oxidative capacity than enzymatic assays, because it measures oxidative capacity in exercising whole muscle, rather than under isolated conditions optimised for enzymatic function. There are, however, limitations associated with MRS. It provides information only

on the relative concentrations of metabolites, and as such, spectra must be referenced to some external standard, or combined with muscle biopsies to obtain true metabolite levels. In addition, MRS is volume-dependent, meaning that the spectra produced are averages of the high-energy phosphates sampled within the sensitive volume of the coil. Inactive muscle within this volume will blunt the metabolic changes detected by the coil. Likewise, the fibre-type composition of the muscle cannot be discerned with MRS.

The most common method of using MRS to evaluate oxidative capacity involves tracking PCr recovery kinetics after muscle contraction. The recovery of PCr has been shown to be an oxidative process^[29,30] that follows a monoexponential time course that is typically fit by the equation:

$$[\text{PCr}]_t = [\text{PCr}]_o + (\Delta[\text{PCr}](1 - e^{(-t/\tau)}))$$

(Eq. 1)

where $[\text{PCr}]_t$ is the PCr concentration at a given timepoint during recovery, $[\text{PCr}]_o$ is the PCr level at the onset of recovery, $\Delta[\text{PCr}]$ is the change in PCr concentration from rest to the onset of recovery, t = time, and τ is a time constant.^[29] Occasionally, the value of τ itself is used as the measure of oxidative capacity, but more often the time constant of PCr recovery ($k_{\text{PCr}} = 1/\tau$) or the half-life of PCr recovery ($t_{1/2} = \ln[2]\tau$) is reported. Alternatively, the theoretical maximum rate of oxidative phosphorylation (V_{max}) can be calculated by multiplying the resting $[\text{PCr}]$ by k_{PCr} . All of these variables provide essentially the same information and are rooted in the concept that PCr is recovered solely through oxidative phosphorylation. These measures have all been significantly correlated to CS activity, although the coefficient of determination (r^2) values ranged from 0.24–0.41 in one study^[31] to as high as 0.58 in another.^[6] Interestingly, a significant correlation was not found between any of these variables and COX activity.^[31] These values are generally thought to be independent of exercise intensity, but they are sensitive to pH^[32-34] and oxygen availability.^[35]

Other MRS measures that have been used to evaluate oxidative capacity in aging humans include the slope of work versus $[\text{Pi}]/[\text{PCr}]$ during steady-state exercise^[36,37] and the inflection point at which the slope in $[\text{Pi}]/[\text{PCr}]$ rapidly increases during exercise protocols of incrementally increasing intensi-

Table II. *In vivo* phosphorous magnetic resonance spectroscopy (³¹P-MRS) studies of oxidative capacity in aging

Study	Age (y)	No./sex of study participants	Muscle(s)	MRS parameter	Activity measure	Activity/fitness difference	Age effect on oxidative capacity	Activity effect on oxidative capacity
Chillbeck et al. ^[27]	O = 70 ± 4	2M, 8F	Plantar flexors	Pi/PCr inflection point	Survey	NA	'Breakpoint': O ≈ Y Slope after break: Y > O	NA
	Y = 26 ± 2	7M, 6F						
Chillbeck et al. ^[7]	O = 67 ± 7	2M, 10F	Plantar flexors	τ	Cycle	VO _{2max} : O < Y	O ≈ Y	NA
	Y = 27 ± 2	6M, 4F			VO _{2max}			
Coggan et al. ^[3]	O, untrained = 62 ± 2	6M	Plantar flexors	Pi/PCr slope	Treadmill	Training status	O > Y	Trained > untrained
	O, trained = 63 ± 3				VO _{2max}			
	Y, untrained = 25 ± 2							
	Y, trained = 27 ± 4							
Conley et al. ^[5]	O = 69 ± 6	18M, 22F	VL	k _{PCr}	Survey	NA	O < Y	NA
	Y = 39 ± 8	6M, 3F						
Kent-Braun and Ng ^[2]	O = 75 ± 5	9M, 9F	TA	t _{1/2}	Accelerometry	Accelerometry: O ≈ Y	O ≈ Y	NA
	Y = 33 ± 5	10M, 9F			SPAQ			
McCully et al. ^[28]	O = 67 ± 2	0M, 5F	LG	τ	NA	NA	VO < Y and O	NA
	VO = 80 ± 5	5M, 15F			Only health status			
	Y = 25 ± 5	2M, 3F						
McCully et al. ^[6]	O = 66 ± 6	5M, 1F	LG	V _{max}	Cycle	Age vs VO _{2max} : R ⁻	Age vs V _{max} : R ⁻	V _{max} vs VO _{2max} : R ⁺
	Y = 28 ± 7	4M			VO _{2max}			
Schunk et al. ^[8]	O = 61 ± 6	6M, 4F	VL	t _{1/2}	Survey	NA	O ≈ Y	NA
	Y = 27 ± 4	11M, 11F						

F = females; **k_{PCr}** = time constant of PCr recovery; **LG** = lateral gastrocnemius muscle; **M** = males; **NA** = measurement was not performed; **O** = old; **Pi/PCr** = inorganic phosphate/phosphocreatine; **R⁻** = negative correlation between variables, per linear regression; **R⁺** = positive correlation between variables, per linear regression; **SPAQ** = Stanford Physical Activity Questionnaire; **Survey** = unspecified self-report of study participant activity; **TA** = tibialis anterior muscle; **t_{1/2}** = half-life; **VL** = vastus lateralis muscle; **V_{max}** = maximum rate of oxidative phosphorylation; **VO** = very old; **VO_{2max}** = maximal oxygen consumption; **Y** = young; τ = time constant; ≈ indicates no significant difference between groups.

ties.^[38] During incremental exercise in which a steady-state is achieved at each work load, the initial slope of work versus $[Pi]/[PCr]$ is linear and low. This slope reflects the capacity of the mitochondria to keep pace with the energy demands on the muscle, and is thus considered an index of the oxidative potential of the muscle.^[36-38] As exercise progresses, $[Pi]/[PCr]$ begins to increase more rapidly, the slope of work versus $[Pi]/[PCr]$ becomes markedly steeper, and eventually the 'steady-state' is lost. The work level at which this inflection point in the slope of work versus $[Pi]/[PCr]$ occurs is also considered an indicator of oxidative capacity; it is well-correlated with the initial slope ($r^2 = 0.78$),^[38] and it occurs at a higher work rate following endurance training.^[39] Both the initial slope of work versus $[Pi]/[PCr]$ ^[3] and the percentage of peak power at which the break point occurs^[27] have been correlated significantly with CS activity in the lateral gastrocnemius, although the r^2 values were fairly low ($r^2 = 0.30-0.40$, and 0.35 , respectively).

Studies using MRS to evaluate changes in oxidative capacity in the gastrocnemius muscles found that it did indeed decline with age, whether it was evaluated using the rate constant of PCr resynthesis^[6,28] or the initial slope of work versus $[Pi]/[PCr]$.^[3] These results were confirmed in more recent studies that evaluated the VL muscle using k_{PCr} ^[5] and the gastrocnemius using the $[Pi]/[PCr]$ inflection point.^[27] However, just as with the earlier enzymatic studies of oxidative capacity, the effect of physical activity on the measures of oxidative capacity was unclear. Two studies^[3,6] found significant fitness or physical activity effects, as well as age effects. In the third,^[5] all of the study participants were vaguely described as 'recreationally active', but no specific measurements of activity were made, leaving open the possibility that the old ($n = 40$) and young ($n = 9$) groups differed in this regard.

Experiments that have attempted to study old and young study participant groups of similar habitual physical activity or included physical activity as a specific control in their analyses have shown no change in oxidative capacity as a result of aging alone. This result has been demonstrated using $t_{1/2}$ of PCr in the VL^[8] and tibialis anterior^[2] muscles and also using τ in the gastrocnemius muscle.^[7] Thus, it would appear that declines in *in vivo* oxidative capa-

city of skeletal muscle with aging are the result of reductions in habitual physical activity rather than of aging *per se*. This suggestion is supported by the recent observation of marked increases in V_{max} in the quadriceps muscle of older study participants following 6 months of endurance or resistance training,^[40] a result consistent with those of Coggan and colleagues,^[3] who showed that chronically-trained older study participants had greater oxidative capacity than those who were untrained and older, and similar oxidative capacities to untrained young study participants. However, the oxidative capacities of the trained older study participants did not match those of comparably trained young study participants, suggesting that aging may lower the upper limit of attainable improvements in muscular oxidative capacity.

3. Muscle Fibre-Type Changes with Age

Both 31P-MRS and enzymatic assays (unless they are performed on single muscle fibres) can be affected by the fibre type distribution of the muscle(s) studied. A number of studies have suggested that there is a preferential reduction in the size of type II fibres with age.^[4,6,11,13,14,27,41] However, an age-related increase in co-expression of myosin isoforms within a given fibre type has also been reported.^[42] Thus, for a given biopsy or MRS muscle sample, there is likely to be a greater percentage of muscle tissue that contains the slow myosin heavy chain isoform in older adults. How this difference would affect measures of oxidative capacity is unclear, as there is a large variability in oxidative enzymes within a given fibre type.^[43,44] In addition, other studies^[45,46] have demonstrated age-related declines in shortening velocity, a parameter that is likely to influence ATP consumption within a given fibre type. This result suggests that changes in fibre types may not completely account for changes in oxidative capacity.

4. Changes in Muscle Capillarisation with Age

Age-related decreases in cardiac output can impair oxygen delivery to muscle during whole-body exercise, but most of the studies presented in this review have focused on the oxidative capacity of

single muscles. During single-muscle exercise, it is unlikely that the need for oxygen would exceed the delivery capacity (i.e. cardiac output) in the elderly. However, an age-related decline in muscle capillarisation could theoretically restrict oxygen delivery within the muscle to the point of affecting MRS results. There are some data to suggest that skeletal muscle capillarisation declines with age, as evaluated by capillary density (capillaries/mm²),^[4] capillary/muscle fibre ratio^[4] and number of capillary contacts per fibre.^[4,14] In contrast, at least one study has shown no effect of age on capillary density^[14] or capillary/muscle fibre ratio, although habitual physical activity may again play a role in the change observed with age.^[47] Furthermore, this study found no effect of age on the muscle fibre area per capillary contact,^[14] the parameter which, it might be argued, is most related to oxygen delivery. Overall, it appears that changes in capillary morphology likely do not play an important role in age-related alterations of oxidative capacity of skeletal muscle, although they may be related to measures of whole-body oxygen kinetics.^[48]

5. Conclusion

It appears that there is a decline in muscle oxidative capacity with increasing age, both *in vitro* and *in vivo*. It also appears, however, that most of this decline is related to the reductions in fitness and/or habitual physical activity that typically occur in the aging population. Much work remains to be done to determine the mechanisms by which aging and activity interact to affect muscle function. From a clinical standpoint, finding optimal exercise regimens to address specific muscular deficits is an important goal that has yet to be achieved. As suggested in this review, however, future research directed at determining the effects of the aging process itself on muscle function should include an objective, sensitive measure of physical activity.

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