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Mitochondrial functional specialization in glycolytic and oxidative muscle fibers: tailoring the organelle for optimal function

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Picard M, Hepple RT, Burelle Y. Mitochondrial functional specialization in glycolytic and oxidative muscle fibers: tailoring the organelle for optimal function. *Am J Physiol Cell Physiol* 302: C629–C641, 2012. First published October 26, 2011; doi:10.1152/ajpcell.00368.2011.—In skeletal muscle, two major types of muscle fibers exist: slow-twitch oxidative (type I) fibers designed for low-intensity long-lasting contractions, and fast-twitch glycolytic (type II) fibers designed for high-intensity short-duration contractions. Such a wide range of capabilities has emerged through the selection across fiber types of a narrow set of molecular characteristics suitable to achieve a specific contractile phenotype. In this article we review evidence supporting the existence of distinct functional phenotypes in mitochondria from slow and fast fibers that may be required to ensure optimal muscle function. This includes differences with respect to energy substrate preferences, regulation of oxidative phosphorylation, dynamics of reactive oxygen species, handling of Ca²⁺, and regulation of cell death. The potential physiological implications on muscle function and the putative mechanisms responsible for establishing and maintaining distinct mitochondrial phenotype across fiber types are also discussed.

mitochondria; reactive oxygen species; calcium retention capacity; oxidative capacity

SKELETAL MUSCLE FIBERS are fantastic molecular and metabolic machines that have developed through evolution a large scope of contractile properties, ranging from slow contracting, low-powered fibers designed for endurance, to fast contracting, high-powered fibers designed for short bursts of high-intensity work. Such a wide range of functional specialization has emerged through the selection across fiber types of optimal cytoarchitectural configurations, and expression of specific isoforms for most molecular components of myofibers including, among others, sarcomeres, excitation-contraction coupling machinery, and energy metabolism pathways. Importantly, in this process, evolution seems to have favored coadaptation whereby only a very narrow combination of molecular characteristics appear suitable to achieve a specific contractile phenotype (42).

At the level of energy metabolism, one of the classical and distinctive features differentiating fiber types is mitochondrial volume density, slow twitch type I fibers typically displaying a two- to threefold higher mitochondrial density and substantially lower capacity for nonoxidative ATP synthesis compared with fast twitch type II fibers. While this difference in mitochondrial quantity was for a long time considered the main factor that varied substantially across fiber types, studies demonstrating the existence of fiber type-specific differences in

mitochondrial respiratory properties, and in mechanisms coupling mitochondria to sites of ATP consumption, have progressively contributed to change this view.

More recently, studies from our laboratory (79) and others (2) have shown that marked differences also exist between fast and slow fibers with respect to the metabolism of reactive oxygen species (ROS) and the regulation of the permeability transition pore (PTP) by Ca²⁺, indicating that mitochondrial specialization across fiber types extends to several key functions of these organelles. Overall, these results have led to the suggestion that specific mitochondrial phenotypes exist in slow and fast fibers and may be required to ensure optimal muscle function (2, 79).

The observation that not all mitochondria are created equal in muscle currently raises important questions. For instance, major advances have been made over the past decade in our understanding of mitochondrial biogenesis with the discovery of key triggering signals, and the identification of several transcription factors and coactivators including peroxisome proliferator-activated receptor (PPAR)- γ coactivator-1 α (PGC-1 α), nuclear respiratory factor (NRF)-1, NRF2, and PPARs (43). However, because mitochondrial biogenesis has been mainly considered from a quantitative perspective, the signaling events and molecular mechanisms by which mitochondria acquire fiber type-specific phenotypes remain largely unknown. In addition, the impact of these different mitochondrial functional phenotypes on myofiber physiology itself remains unclear. This is particularly important considering that differ-

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ences in mitochondrial function may influence a number of cellular variables including cytosolic Ca^{2+} , redox state of pyridine nucleotide pools, level of reactive oxygen and nitrogen species, as well as cell death signaling. Finally, the existence of distinct mitochondrial functional phenotypes in slow and fast muscle fibers in normal muscle could have an important impact on how we judge whether mitochondria are involved in muscle dysfunction in a number of pathological states in which changes in fiber type are suspected or known to occur.

Building on the above-mentioned concept of coadaptation of muscle properties, this review will provide an overview of the experimental evidence currently available to support the existence of mitochondrial functional specialization between fiber types. We will focus on three functional subcategories for which data are available, namely: 1) respiratory properties and regulation of energy exchange; 2) metabolism of ROS; and 3) regulation of the PTP, particularly in relation to Ca^{2+} . For each of these subsystems, we will discuss the potential physiological implications on muscle function, and when possible, the molecular mechanisms that may underlie mitochondrial specialization. We supplement our discussion of the current knowledge by suggesting research avenues that will contribute to our expanding understanding of the mechanisms underlying the creation and maintenance of specific mitochondrial phenotypes between different muscle fiber types.

Mitochondrial Functional Specialization in Skeletal Muscle

Respiratory properties and coupling to cellular ATPases. Several studies have compared different intrinsic respiratory properties of mitochondria in slow and fast skeletal muscle including 1) respiratory capacities with various combinations of substrates; 2) activities of the TCA cycle, β -oxidation pathway, and respiratory chain enzymes; 3) coupling efficiency between respiration and phosphorylation, proton conductance, as well as membrane properties; and 4) regulation of respiration by ADP and mitochondrial coupling to cellular ATPases through various mechanisms, such as the creatine kinase shuttle.

RESPIRATORY CAPACITY AND ENZYMOLOGY. Maximal respiratory capacity of mitochondria from predominantly fast versus slow muscles has been measured in several species including cats, rabbits, rats, and fish. In general, studies performed on isolated mitochondria have reported little to no fiber type difference for maximal ADP-stimulated respiration in the presence of substrates feeding the respiratory chain at the level of complex I (e.g., pyruvate-malate, glutamate-malate, 2 oxoglutarate), complex II (succinate) as well as complex IV (46, 61, 101, 120). Data from our laboratory (79) (Fig. 1A) and others (6, 85) have shown that these results hold true in saponin permeabilized fiber bundles where mitochondrial morphology is preserved (83). These similar findings from both isolated and permeabilized preparations demonstrate that the lack of fiber type differences in isolated organelles (where only 20–40% of total muscle mitochondria are retrieved after homogenization) is not confounded by the possibility of selection bias during mechanical isolation of mitochondria.

This relative functional similarity is in good agreement with results from several studies indicating little differences in the content/maximal activity of components of the oxidative phos-

phorylation machinery of mitochondria across fiber types (Fig. 1B). For instance, in mitochondria from fish muscle (61), the activities of all respiratory chain complexes as well as ATP synthase are similar across fiber types. In cats (101), the activity of complex IV is similar in mitochondria from the soleus (>95% slow twitch) and gracilis (>70% fast twitch), while in rats (5), mitochondrial cytochrome content ($c+c_1$ and $a+a_3$) and activities of complex III and IV are similar in mitochondria from the white gastrocnemius (exclusively type IIb/x) and the soleus [\sim 90% type I; (5)]. More recently, Balaban's group has generated proteomic evidence that supports the lack of substantial differences in the molecular composition and capacity of the oxidative phosphorylation (OXPHOS) machinery between red and white skeletal muscle (33). Care should, however, be taken before generalizing this interpretation to the entire mitochondrial proteome since only a limited number of mitochondrial proteins were captured in this analysis (358 proteins out of more than 1,000 proteins composing the mammalian mitochondrial proteome).

In contrast, substantial differences exist between mitochondria from slow and fast muscle with respect to their capacity to oxidize fatty acids and glycerol-3-phosphate (Fig. 1C). Indeed, in both rat and rabbit, mitochondria from slow-oxidative muscles (soleus) display a higher state 3 respiration in the presence of palmitoyl-carnitine compared with mitochondria from glycolytic muscles [e.g., extensor digitorum longus (EDL), gracilis, or white gastrocnemius] (6, 46, 70, 85). This difference is suggested to be due to the greater activity of β -oxidation enzymes such as hydroxyacyl-CoA dehydrogenase (46, 70) and potentially to the activity of the carnitine-acylcarnitine translocase CPTII, located on the matrix side of the inner mitochondrial membrane (85). Overall, these characteristics are clearly consistent with the overall metabolic phenotype of slow muscles, which derive a significant portion of their energy from the oxidation of fatty acids and express higher levels of proteins involved in sarcolemmal fatty acid transport (e.g., FAT/CD36, FATP, FABP_{pm}) and intracellular binding (FABP) compared with fast muscles (34).

Conversely, mitochondria from fast muscle (e.g., gracilis and white gastrocnemius) have a four- to tenfold higher state 3 respiration in the presence of glycerol-3-phosphate (6, 46, 85) compared with mitochondria from slow-oxidative muscle (Fig. 1C), likely due to the greater activity of mitochondrial glycerol-3-phosphate dehydrogenase (46). These data strongly suggest that mitochondria within glycolytic fibers rely on two shuttles (e.g., α -glycerophosphate and malate-aspartate shuttles) to import cytosolic reducing equivalents, compared with only one (e.g., malate-aspartate shuttle) in mitochondria from slow-oxidative fibers. This specific feature of fast muscle mitochondria may be important to limit the accumulation of cytosolic reducing equivalents, and of glycolytic intermediates during short bursts of contractions.

Few data are currently available on the molecular mechanisms that could underlie the establishment of differences in oxidative capacities for lipids and glycolysis-derived substrates across muscle types. Thyroid hormones were suggested to play a role based on the fact that they influence the metabolic profile of skeletal muscles in a muscle-type-specific manner (37, 99). In support of this hypothesis, T3 supplementation in rats was shown to induce an increase in the capacity to oxidize glycerol-3-phosphate and a concomitant reduction in the capacity to

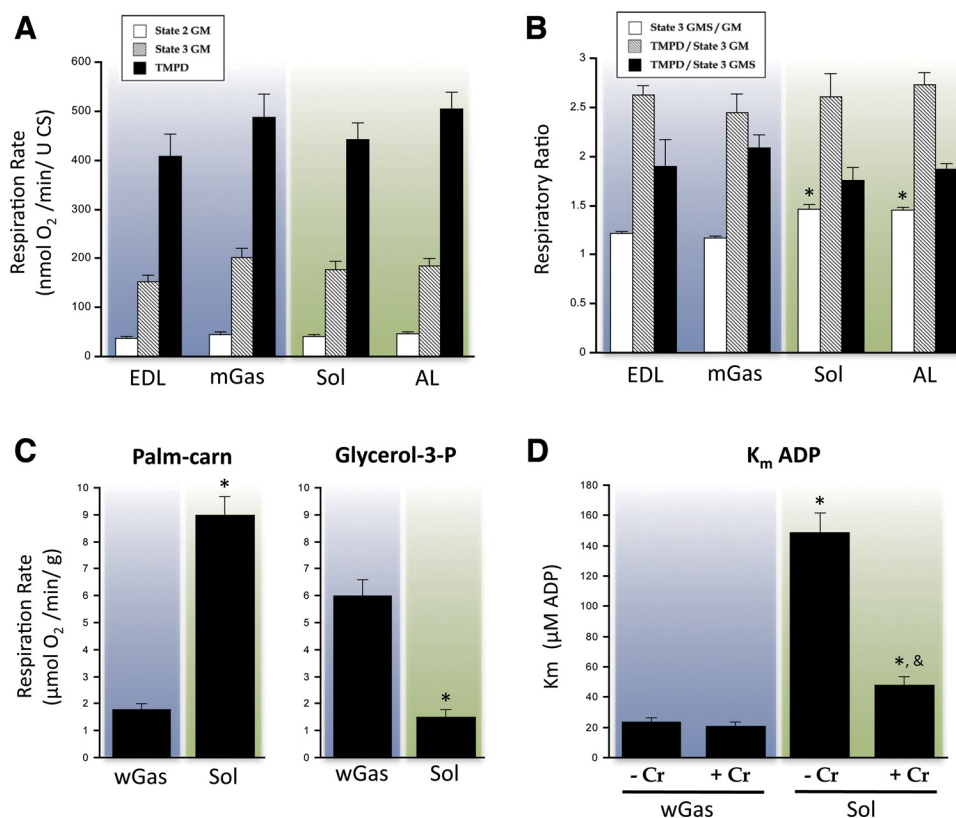


Fig. 1. Quantitative and qualitative respiratory parameters measured in permeabilized myofibers. *A*: mitochondrial respiratory rates normalized for the mitochondrial volume marker citrate synthase (CS) under different conditions do not differ between the fast-type glycolytic extensor digitorum longus (EDL) and mixed gastrocnemius (mGas) muscles (blue shading), and the slow-type oxidative soleus (Sol) and adductor longus (AL) muscles (green shading). State 2 GM, complex I-driven respiration with glutamate (2 mM) and malate (10 mM); state 3 GM, GM + ADP (2 mM); TMPD, complex IV-driven respiration with tetramethylphenylenediamine (0.5 mM) and ascorbate (5 mM). *B*: respiratory ratios representing the relative activity of different components of the respiratory chain. State 3 GMS, complex I + II driven respiration with GM and succinate (10 mM). *C*: maximal respiratory rate with the lipid substrate palmitoyl-carnitine (Palm-carn) is fourfold lower in the glycolytic white gastrocnemius (wGas) muscle than in the oxidative Sol, whereas maximal respiratory rate with the substrate glycerol-3-phosphate (glycerol-3-P) is threefold higher in wGas than in Sol. *D*: apparent affinity for ADP (K_m) is lower in wGas than in Sol. The fast wGas K_m for ADP is not sensitive to the addition of creatine (+Cr, 20 mM), whereas the slow Sol K_m for ADP is reduced by 70% with +Cr. Data shown in *A* and *B* are from Ref. 82. All measurements performed on rat muscle were as in Picard et al. (81, 82) (*A* and *B*); Ponsot et al. (85) (*C*), and Burelle and Hochachka (18) (*D*). *Statistical significance ($P < 0.05$) from glycolytic muscles; &statistical significance from no creatine (-Cr) conditions ($P < 0.05$). P values were obtained from unpaired *t*-test assuming unequal variance between groups. Data are presented as means \pm SE; $n = 8$ –12 per group.

oxidize octanoyl-carnitine (6), producing the profile observed in fast-glycolytic fibers. However, this effect of T3 was observed in slow-oxidative muscles, which express significant amounts of thyroid receptors, but not in fast-glycolytic muscles, in which thyroid hormone receptors are less abundant (6). Therefore, it is difficult to explain how physiological levels of T3 could underlie the difference in substrate-specific oxidative capacities between slow and fast muscles since the impact of T3 should, if anything, bring slow and fast mitochondria closer together in terms of their substrate preference than would otherwise exist in the absence of T3.

COUPLING EFFICIENCY, PROTON CONDUCTANCE, AND MEMBRANE PROPERTIES. Very few studies have investigated whether variations across fiber types exist with respect to coupling efficiency of oxidative phosphorylation. This parameter, known as the P/O ratio, is conventionally determined by measuring the amount of oxygen required to rephosphorylate a known amount of ADP. It is well established that the P/O ratio decreases as respiration is progressively reduced from maximal ADP-stimulated respiration to submaximal respiration rates (35). The main factor responsible for this phenomenon is the

increasing contribution of the proton leak of the inner membrane to respiration as the rate of oxidative phosphorylation is progressively lowered (15). Early studies comparing P/O ratios in mitochondria isolated from different skeletal muscle fibers in the rat failed to detect differences when using pyruvate or palmitoyl-carnitine as substrate (74). In these experiments, P/O values were only measured at maximal respiration rates in the presence of saturating amounts of ADP, which did not allow exclusion of differences at more physiologically relevant submaximal rates of respiration.

However, more recent studies (70) comparing P/O ratios over the entire range of respiratory capacity in mitochondria isolated from the rat soleus and the fast EDL also reported no significant difference with pyruvate or palmitoyl-carnitine as respiratory substrates. On the other hand, direct measurement of proton leak kinetics in fish muscle showed that proton leak was greater in mitochondria from white muscle compared with mitochondria from red muscle (61). Since this difference was only apparent when leak values were normalized per unit of complex IV activity, but not when expressed per milligram of total mitochondrial proteins, the authors argued that under

some circumstances, normalization to a marker of the respiratory chain capacity may thus be more appropriate than total protein, particularly for functions related to the inner membrane (61). In addition, this study reported greater membrane fluidity in mitochondria from red muscle compared with their counterparts from white muscle, possibly due to variations in phospholipid profile (e.g., chain length, saturation, cardiolipin content) (61). However, no information is available on the phospholipid profile in mitochondria across fiber types, and it remains unclear how this could affect the *in vivo* activity of membrane bound proteins. Taken as a whole, these data thus suggest that while fiber type differences may exist with respect to mitochondrial membrane properties and proton leak, whether this results in fiber type differences in mitochondrial coupling efficiency remains to be demonstrated conclusively.

REGULATION OF RESPIRATION BY ADP AND MITOCHONDRIAL COUPLING TO CELLULAR ATPASES. One of the most striking differences between mitochondria from fast-glycolytic and slow-oxidative fibers concerns the sensitivity of respiration to ADP, and the mechanisms coupling mitochondrial ATP supply to subcellular sites of ATP consumption (53, 57, 93, 96–98). These properties were largely uncovered following the development of saponin-permeabilized fibers, which allowed study of mitochondria in a relatively preserved cytoarchitectural environment (58, 93, 98). Using this approach, several studies, including ours, have shown that mitochondria in slow-oxidative muscles, such as the heart and the soleus, display an apparent K_m (Michaelis-Menten constant) for exogenous ADP in range of 200–500 μM , which is approximately 10-fold higher than K_m values measured in isolated mitochondria (53, 57, 96, 97). In contrast, mitochondria within fast-twitch glycolytic fibers display a K_m for ADP between 10 and 30 μM , closer to that observed in isolated mitochondria (Fig. 1D) (53, 57, 96, 97). A low permeability of the outer mitochondrial membrane (OMM) to ADP is suggested as one of the mechanisms contributing to the high K_m value observed in mitochondria from slow-oxidative fibers (53, 57, 96, 97). This is mainly based on the observation that disruption of the OMM using a well-controlled hypo-osmotic shock lowers the K_m for ADP to the values observed in isolated mitochondria and in mitochondria from fast-glycolytic fibers (57). The molecular mechanisms underlying fiber type-specific OMM permeability to ADP are currently unknown but could involve differences in the conductance and isoform expression of voltage-dependent anion channel (VDACs), which are responsible for the transport of a number of solutes across the MOM including adenylates (24, 32, 53, 57, 60, 93, 96, 97). Furthermore, recent evidence demonstrates that mitochondrial affinity for ADP is modulated by the contractile state of myofibers (76), with contraction lowering the K_m for ADP. This effect was more pronounced in fast fibers, suggesting that different mechanisms linking contractile state and mitochondrial energy exchange may exist between slow and fast muscles (76).

The other factor likely explaining the low sensitivity of mitochondria to exogenous ADP in slow-oxidative muscle may be compartmentalization of energy exchange, which restricts the access of exogenous ADP to mitochondria. Indeed, functional units, termed intracellular energetic units (ICEUs), have been well described in the heart (16, 50, 84, 93, 95, 102), and some evidence for their existence in the soleus muscle has been obtained (102). Within these ICEUs, ATP and ADP are focally

released and directly transferred by channeling between mitochondria on the one hand, and sarcoplasmic reticulum (SR)- Ca^{2+} and myofibrillar (MF)- Mg^{2+} ATPases on the other hand (16, 50, 84, 93, 95, 102). Three lines of evidence, obtained in permeabilized fibers, have led to this conclusion. First, in these fibers, SR- Ca^{2+} loading capacity and capacity to relax rigor tension are much higher when supported by ATP generated by oxidative phosphorylation compared with exogenous ATP added to the incubation medium (50). This indicates that mitochondria-derived ATP has a preferential access to SR- Ca^{2+} /MF- Mg^{2+} ATPases (50). Second, and in line with these results, similar mitochondrial respiration rates can be achieved with 40 times less ADP if ADP is derived from SR- Ca^{2+} /MF- Mg^{2+} ATPase activity, compared with when it is added directly in the incubation medium (95, 102). And third, even in the presence of a powerful exogenous ADP trap system in the incubation media, ADP produced endogenously by the hydrolysis of ATP can still stimulate mitochondrial respiration, providing direct evidence for a compartmentalization of energy and a regulatory signal between mitochondria and SR- Ca^{2+} /MF- Mg^{2+} ATPases in slow-oxidative muscle (16, 95, 102).

Currently, the factors involved in this form of compartmentalization of energy and regulatory signal exchange are unclear. One hypothesis is that it is related to the structural arrangement of mitochondria around myofibrils (72, 84, 95). Indeed, in oxidative muscle fibers, mitochondria appear to be clustered at sites of high ATP demand and are organized into highly ordered elongated structures forming contacts with the SR and having extensive branching across the A-band area of the sarcomere where Mg^{2+} ATPases are most abundant (see Fig. 3A) (72, 95). This configuration provides the physical proximity between mitochondria and SR- Ca^{2+} /MF- Mg^{2+} ATPases that is required to observe ICEUs (72, 84, 93, 95). In contrast, in fast-glycolytic muscle fibers in which mitochondria are less abundant, mostly located at the level of Z-lines with no trans A-band branches (72), the spatial configuration is less compatible with the formation of ICEUs (72, 95). In fact, Ventura-Clapier's group reported that ICEUs were absent in the mouse white gastrocnemius muscle (51). Moreover, they elegantly demonstrated that in the white gastrocnemius muscle of mice deficient in the sarcomeric creatine kinase isoform (MM-CK), a compensatory proliferation and spatial reconfiguration of mitochondria occurs, which coincides with the emergence of direct energy channeling within ICEUs (51), thus providing strong evidence for the role of mitochondrial spatial configuration in the development of ICEUs, and more generally on mitochondrial functional specialization across fiber types. Obviously, because specialization of mitochondrial energy exchange across fiber types is intimately linked to the overall design of slow and fast fibers, the molecular regulation underlying this specialization is likely to involve fundamental signaling factors with broad impact on myogenesis (71, 94).

Another noticeable difference between mitochondria from slow-oxidative and fast-glycolytic fibers is the functional coupling between mitochondrial ATP production and sites of ATP consumption through the creatine kinase (CK) system (Fig. 1D). In slow-oxidative muscle, mitochondria express sarcomeric mitochondrial CK (sMt-CK), an isoform located in the intermembrane space, which is functionally coupled with the ATP/ADP exchanger (ANT) and VDAC channels (115). sMt-CK thus uses ATP produced in the mitochondria to re-

generate ADP locally near the ANT, thereby exerting a strong control on oxidative phosphorylation. Together with cytosolic CK isoforms located at the vicinity of several key ATPases (see Ref. 115 for review), sMt-CK also ensures efficient energy and signal transfer through reversible phosphotransfer reactions (96, 115). In contrast, this CK shuttle system is non-existent in fast-glycolytic muscle, due to the low levels of sMt-CK (87). However, the mechanisms underlying the fiber type-specific expression of sMt-CK, one of the major factors responsible for the presence of CK shuttle in slow muscles, still remains unknown.

Taken together, the data available thus indicate that major differences exist between slow-oxidative and fast-glycolytic muscle fibers with respect to the mechanisms coupling mitochondria to sites of ATP consumption. This is likely explainable by the necessity in oxidative fibers to have efficient energy delivery despite the strong diffusional constraints imposed by the highly organized and densely packed intracellular environment, contrasting with glycolytic fibers that rely much less on mitochondria for ATP production. Again, however, the signaling events and molecular mechanisms underlying this specialization remain largely unknown.

Metabolism of reactive oxygen species. Three studies from our laboratories (79, 80, 82) and another (2) have demonstrated the existence of very significant differences in the mitochondrial metabolism of ROS across fiber types. In these experiments, net mitochondrial H_2O_2 release was measured using Amplex red both in permeabilized fibers (2, 79, 80, 82) and in isolated mitochondria (79). Results showed that H_2O_2 release was two- to threefold higher from mitochondria in white gastrocnemius compared with the soleus muscle under basal state 2 respiration in the presence of complex I or complex II substrates. As a consequence, free radical leak expressed as a percentage of total electron flux through the respiratory chain was 3.5-fold greater in mitochondria from the white gastrocnemius compared with that of the soleus muscle (79). A broader examination of two fast dominant versus two slow dominant muscles confirms the existence of this fiber type difference in H_2O_2 release (Fig. 2A) (82).

Currently, the mechanisms underlying this substantial difference in H_2O_2 release are not fully understood. Because Amplex red detects H_2O_2 that diffuses outside mitochondria, this could be due to 1) a greater production of H_2O_2 , and/or 2) a lower endogenous H_2O_2 scavenging capacity in mitochondria from glycolytic muscle compared with mitochondria from oxidative muscle (Fig. 2, B–D) (see Refs. 14 and 110 for reviews).

H_2O_2 production in mitochondria is largely determined by superoxide production ($\text{O}_2^{\cdot-}$), which occurs mainly at the level of complexes I and III of the respiratory chain (14, 110). Studies in isolated mitochondria indicate that membrane potential ($\Delta\Psi$) is a key determinant of $\text{O}_2^{\cdot-}$ production at these sites, which increases exponentially in response to small increases in $\Delta\Psi$, particularly in the upper range values [i.e., 175–185 mV (39, 55, 107, 112)]. However, as discussed in the section *Coupling efficiency, proton conductance and membrane properties*, indices of proton leak and direct measures of $\Delta\Psi$ suggest that membrane potential is not higher in mitochondria from glycolytic muscle, and may in fact tend to be lower than in mitochondria from oxidative muscle (61). Although $\Delta\Psi$ is a major determinant of $\text{O}_2^{\cdot-}$ production, other mecha-

nisms could contribute, including variations in the redox state of mitochondria (7), the stoichiometry-activity ratios of the respiratory chain complexes (59), and the susceptibility to proton pump slipping (52). However, whether fiber type differences in these properties exist and translate into different rates of $\text{O}_2^{\cdot-}$ production has not been studied. It should be noted that, although Mn-SOD activity is essential for the conversion of superoxide into H_2O_2 , differences in the activity of this enzyme are unlikely to account for variations in net mitochondrial H_2O_2 release across fiber types. Indeed, previous work by Van Remmen and colleagues showed that large variations in Mn-SOD content in transgenic animals (heterozygous knock out or overexpression of Mn-SOD) do not translate into measurable changes in net H_2O_2 release in skeletal muscle mitochondria using the Amplex red system, reflecting the fact that this enzyme is in excess capacity relative to $\text{O}_2^{\cdot-}$ production (48, 66).

In contrast, H_2O_2 scavenging by endogenous antioxidant systems was recently shown to have a significant impact on mitochondrial H_2O_2 release in the Amplex red system (109). In this recent study, chemical depletion of GSH in isolated skeletal muscle mitochondria was shown to increase net H_2O_2 release two- to threefold. This was observed with respiratory substrates and respiratory chain inhibitors targeting different sites, and yielding different rates of $\text{O}_2^{\cdot-}$ production (109). While the main objective of this study was to provide a correction method to better estimate $\text{O}_2^{\cdot-}$ production from measurements of H_2O_2 efflux, the data presented clearly suggest that fiber type differences in mitochondrial H_2O_2 release could be attributable to variations in endogenous H_2O_2 scavenging capabilities (109). In fact, direct measurements in permeabilized muscle fibers have shown that the capacity of mitochondria from fast-glycolytic fibers to scavenge an exogenous H_2O_2 load is approximately 40–50% lower compared with mitochondria from slow-oxidative fibers (2).

In addition, the activities of important antioxidant enzymes are significantly lower in glycolytic muscle compared with oxidative muscle, even when expressed per unit of citrate synthase activity to take into account differences in mitochondrial contents (Fig. 2, B–D). This is particularly striking for glutathione peroxidase, the main mitochondrial H_2O_2 scavenging enzyme, which is on average 88% (range: 77–95) lower in glycolytic compared with oxidative muscles. Taken together, the data available thus suggest that H_2O_2 buffering capacity per mitochondrial unit differs considerably in glycolytic compared with oxidative fibers and may account for differences in H_2O_2 emitting potential.

Although the mechanism underlying this difference is unclear, it likely involves fiber type differences in the expression level of PGC-1 α and PGC-1 β . These transcriptional coactivators, in addition to their effect on mitochondrial biogenesis, were shown to regulate the expression level of many ROS detoxifying enzymes mRNA (SOD1, SOD2, Gpx1, catalase) both at baseline and in response to H_2O_2 (106), thus allowing to scale the activity of antioxidant systems to the mitochondrial biomass (Fig. 2E). However, additional mechanisms (i.e., signal amplification in slow muscle; epigenetic silencing in fast muscles) must exist to account for the net greater abundance of antioxidant relative to mitochondrial mass (per citrate synthase) in slow muscles than in fast muscles.

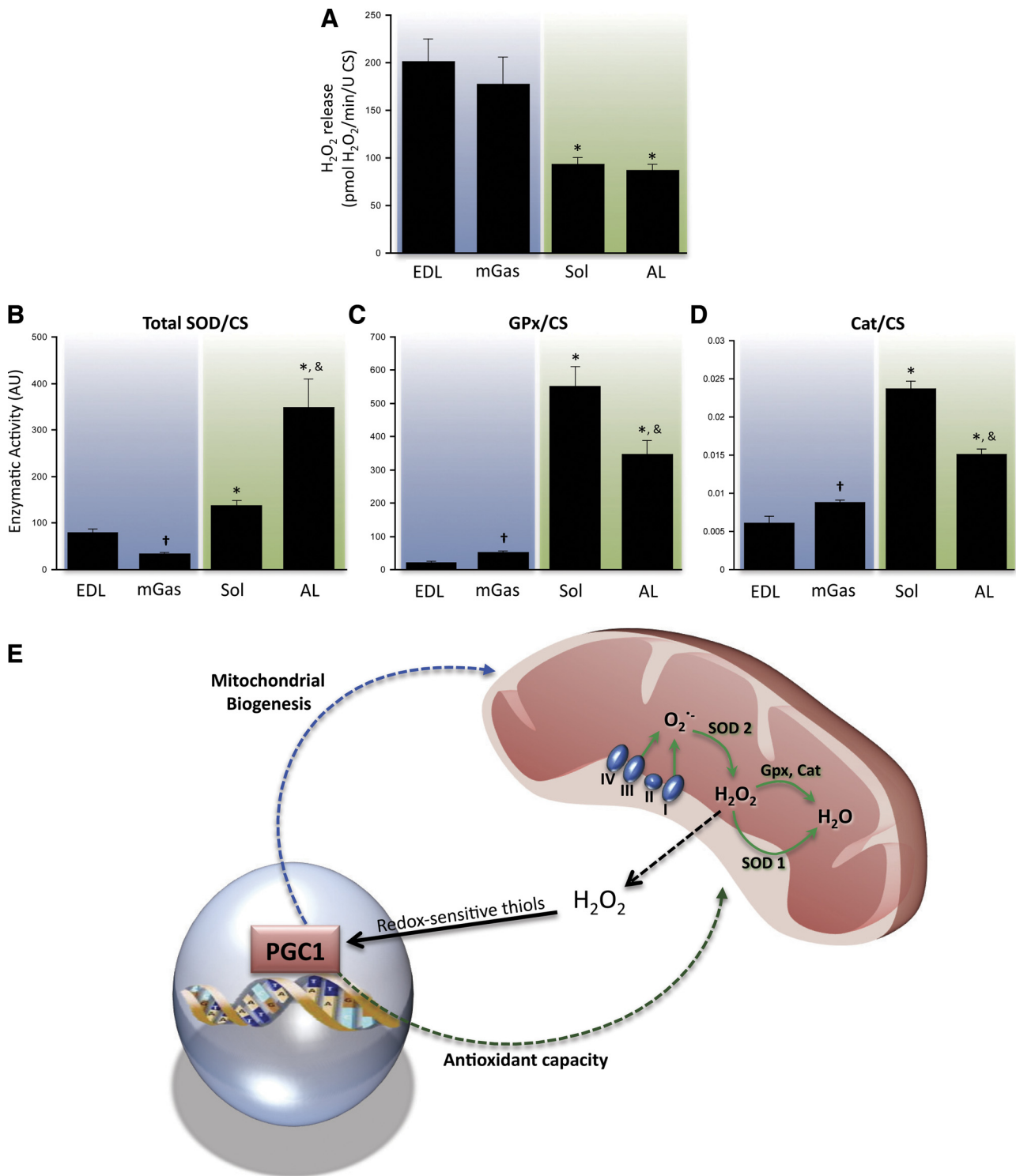


Fig. 2. Reactive oxygen species (ROS) metabolism differs between glycolytic EDL and mGas muscles (blue shading), and the slow-type oxidative Sol and AL muscles (green shading). *A*: mitochondrial hydrogen peroxide (H₂O₂) release measured with the Amplex Red system, normalized for citrate synthase, is higher in glycolytic EDL and mGas muscles than in oxidative Sol and AL muscles. *B–D*: when normalized for mitochondrial content, activity of endogenous antioxidant enzymes manganese and copper-zinc superoxide dismutases (total SOD, U/mg protein) (*B*), glutathione peroxidase (GPx, $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g protein}^{-1}$) (*C*), and catalase (Cat, K/g protein) (*D*) is lower in fast glycolytic muscles. AU, arbitrary units. *E*: overlap among signaling pathways driving mitochondrial biogenesis and antioxidant defenses involves PGC-1 α , which coactivates the transcription of both types of genes under the influence of redox-sensitive signaling pathways (106). Data shown in *A–D* are from Ref. 82. *Statistical significance ($P < 0.05$) from glycolytic muscles; &statistical significance from Sol muscle ($P < 0.05$); †statistical significance from EDL muscle ($P < 0.05$). *P* values were obtained from unpaired *t*-test assuming unequal variance between groups. Data are presented as means \pm SE; $n = 8$ per group.

From a physiological perspective, there is a clear rationale for scaling antioxidant defenses to mitochondrial content. In oxidative muscle with a large mitochondrial biomass, this allows protection against oxidative stress (106). Conversely, in fast muscle, greater ROS production per mitochondrial unit may be required to maintain proper redox-dependent signaling despite low mitochondrial content. In addition, greater capacity to generate mitochondrial ROS may contribute, together with other factors, to trigger adaptive mitochondrial biogenesis when glycolytic muscles are recruited more frequently. Although strong experimental evidence in support of this hypothesis is still lacking, it is nonetheless consistent with data from several recent studies showing that 1) enhanced ROS signaling in muscle from SOD1 knockout mice results in mitochondrial proliferation (47); 2) overexpression of SOD1 in type IIB fibers blocks training-induced mitochondrial biogenesis (65); and 3) exogenous antioxidant supplementation in humans blunts the benefits of exercise training in terms of insulin sensitivity and response of mitochondrial biogenesis signaling (90, 108). Overall specialization of mitochondrial ROS metabolism, rather than a simple 1:1 scaling between mitochondrial content and antioxidant capacity, thus appears to be important for normal function of glycolytic and oxidative fibers.

PTP regulation and relationship with cellular Ca^{2+} dynamics. The mitochondrial permeability transition was initially described in isolated mitochondria as a sudden increase of the inner membrane permeability to solutes in the presence of a high calcium concentration ($[Ca^{2+}]$) (40). Although initially thought to be due to unspecific membrane damage, it is now widely accepted that this phenomenon is actually caused by the opening of the PTP, a nonspecific multiconductance proteinaceous channel of the inner membrane (25, 27, 36, 41, 54, 122). Prolonged opening of the PTP in the large-conductance mode leads to equilibration of ions and solutes of $<1,500$ Da size across mitochondrial membranes, collapse of $\Delta\Psi$, mitochondrial swelling, and ATP hydrolysis by the F_0F_1 ATPase. This sequence of events has drawn considerable attention to the PTP as an important player in apoptotic and necrotic cell death. However, importantly, an increasing number of studies indicate that transient opening of the PTP (i.e., pore flickering) in a low-conductance mode that is permeable to ions, but not to larger molecules, likely serves physiological regulatory purposes, by fine-tuning $\Delta\Psi$ (55) and by acting as a fast Ca^{2+} release channel that would regulate mitochondrial Ca^{2+} levels and mitochondrial Ca^{2+} -sensitive dehydrogenases, and participate in the amplification/propagation of Ca^{2+} signals arising from the endoplasmic (ER)-sarcoplasmic (SR) reticulum located near mitochondria (44, 49, 91, 117).

In skeletal muscle, large variations exist in the amount, size, and spatial configuration of mitochondria relative to the SR and myofibrils (Fig. 3A) (72). Moreover, cellular Ca^{2+} dynamics are known to differ considerably across fiber types both in amplitude and in frequency (10, 20, 21), which likely expose mitochondria to different levels of Ca^{2+} (Fig. 3A). Considering the importance of Ca^{2+} in the regulation of PTP opening, we determined whether the sensitivity of the Ca^{2+} -induced PTP opening differed across fiber types. In this study, we in fact observed that the Ca^{2+} threshold for PTP opening was approximately threefold higher in permeabilized fibers from glycolytic (white gastrocnemius) compared with oxidative muscle (soleus) (79) when exposed to $30 \mu M Ca^{2+}$, which corre-

sponds to the estimated concentration of Ca^{2+} in the SR-mitochondria microdomains upon maximal stimulation of ryanodine receptors (92). A similar phenomenon was also observed in isolated mitochondria from these muscles by our group (79), and recently confirmed by others (67). A broader examination of two fast dominant versus two slow dominant muscles also confirms the existence of this fiber type difference in PTP sensitivity to Ca^{2+} (Fig. 3, B and C).

The factors responsible for this large difference in PTP sensitivity are not fully understood. Currently available data suggest that it is not related to differences in the expression of putative protein modulators of the PTP such as cyclophilin-D, ANT-1, and VDACs, or to differences in respiratory properties and $\Delta\Psi$ (79). On the other hand, endogenous Ca^{2+} levels in mitochondria from glycolytic muscle are lower than those measured in mitochondria from oxidative muscle (79), likely due to the lower free intracellular $[Ca^{2+}]$ prevailing in glycolytic versus oxidative fibers at rest (20, 21, 31). However, we observed that this could only account partly for the large difference in resistance to PTP opening between fiber types, suggesting that additional mechanisms are involved (79). In this study, it was also striking to note that the greater resistance of mitochondria from glycolytic muscle to Ca^{2+} -induced PTP opening occurred despite the fact that ROS emission, which promotes PTP opening (11, 56, 111), was substantially higher in mitochondria from glycolytic muscle than in mitochondria from oxidative muscle (see Fig. 2A and Fig. 3, A and B). This further supports specificity of PTP regulation across fiber types.

From a physiological perspective, these data suggest that different Ca^{2+} thresholds for PTP opening represent one of the optimization mechanisms required to ensure optimal mitochondrial function across fiber types. More specifically, in fast-glycolytic muscle, mitochondria are likely exposed to large increases in matrix $[Ca^{2+}]$ compared with mitochondria from slow-oxidative muscle due to: 1) the large and rapid Ca^{2+} surges that occur during contractions (21); 2) the physical proximity of mitochondria to large SR Ca^{2+} stores (64, 72, 102); and 3) the small mitochondrial volume density and thus the low distribution volume for Ca^{2+} in the matrix (12) (Fig. 3D). In fast-glycolytic fibers, a higher Ca^{2+} threshold for PTP opening may ensure proper operation of the PTP in the flickering mode [which regulates $\Delta\Psi$, ROS production and Ca^{2+} signaling waves (17, 28, 64)], and prevention of accidental switch to the high-conductance mode involved in cell death signaling. Further work is, however, required to validate this hypothesis.

On the other hand, the implication of this phenomenon in muscle pathology remains unclear. Indeed, under certain conditions such as exposure to bupivacaine, the greater resistance to PTP opening observed in fast-glycolytic fibers could clearly contribute to explain why mitochondrial dysfunction and myotoxicity appear to be less important in these fibers than in those with a slow-twitch phenotype (45). On the other hand, the resistance of fast-glycolytic fibers to toxic insults is in apparent contradiction with the observation that type II fibers appear to be more affected than type I fibers in other pathological states such as ischemia-reperfusion (23, 118) and Duchenne muscular dystrophy (116), in which PTP opening plays a role in injury (19, 27, 69). A likely explanation for this phenomenon is that alterations in cellular factors that promote PTP opening

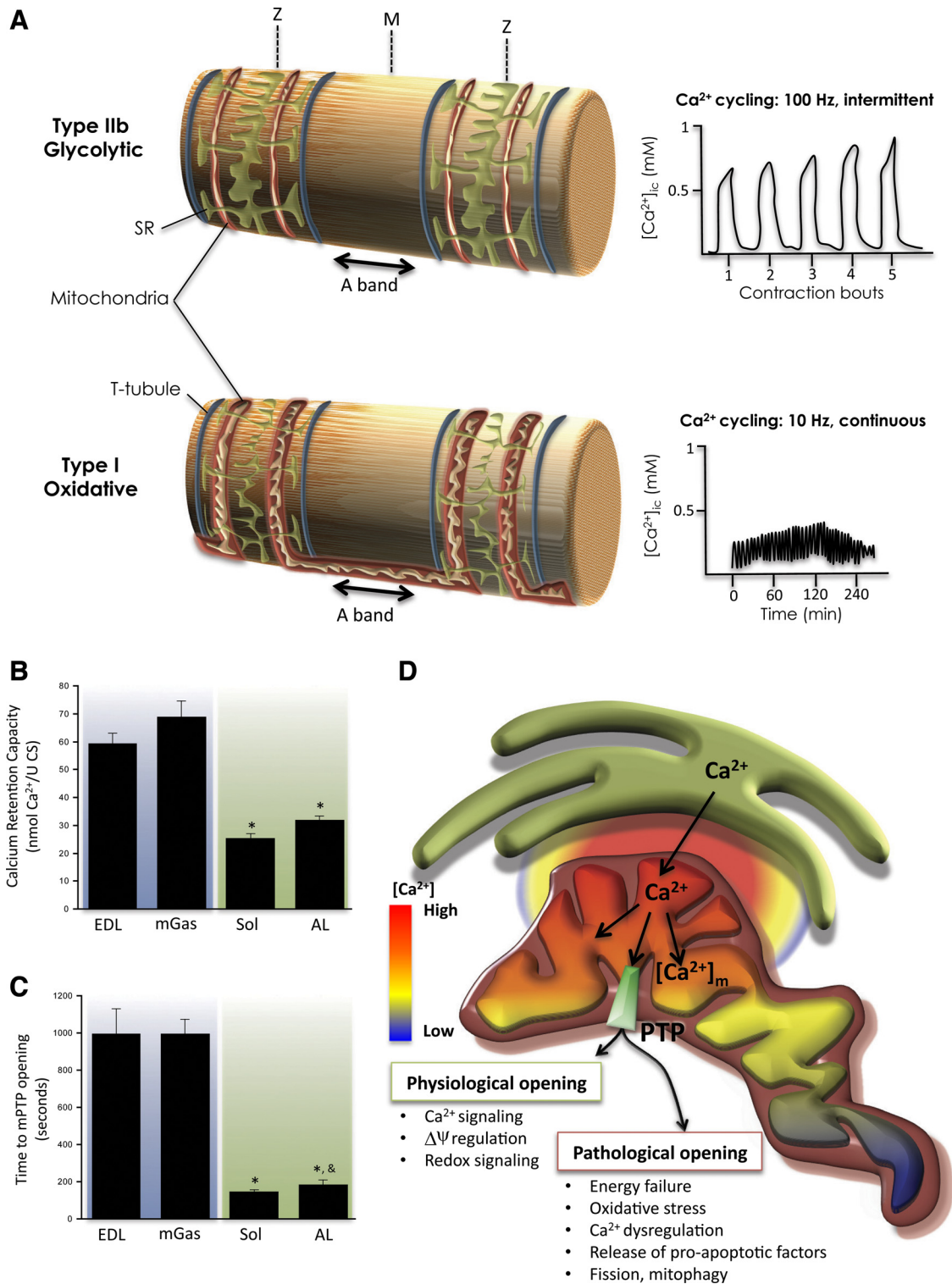


Fig. 3. Considerable differences in cytoarchitecture and intracellular Ca²⁺ transients exist between type I oxidative and type II glycolytic muscle fibers. *A*: schematic representation of myofibrils from fast type IIb and slow type I fibers. [Ca²⁺]_{ic}, intracellular calcium concentration. [Adapted from Ogata and Yamasaki (72)]. *B*: mitochondria uptake considerable amounts of Ca²⁺ released from the sarcoplasmic reticulum (SR) during muscle contraction. Excess matrix [Ca²⁺] triggers the opening of the permeability transition pore (PTP), which has important functional consequences. PTP opening events are classified under two conductance states: physiological and pathological opening. *C*: the amount of mitochondrial Ca²⁺ uptake required to trigger PTP opening—calcium retention capacity, measured with the Ca²⁺ dye Calcium green—is higher in glycolytic EDL and mGas muscles than in oxidative Sol and AL muscles. *D*: upon external Ca²⁺ challenge, the time required to trigger opening of the PTP is higher in EDL and mGas than in Sol and AL. Data shown in *B* and *C* are from Ref. 82. All data were obtained as described in Picard et al. (79, 81). *Statistical significance ($P < 0.05$) from glycolytic muscles; &statistical significance from Sol muscle ($P < 0.05$). *P* values were obtained from unpaired *t*-test assuming unequal variance between groups. Data are presented as means ± SE; $n = 8$ per group.

are greater in fast fibers than in slow fibers as a result of these pathological states and overwhelm the capacity of mitochondria to resist to permeability transition. Clearly, the involvement of mitochondria in cell death depends on the convergence of several factors, which make it difficult to predict their involvement in disease outcome.

Potential mechanisms underlying mitochondrial functional specialization. Very little is known about the signaling mechanisms that account for the striking phenotypic differences described above. However, recent findings provide initial insights into this process and allow us to speculate about the most probable mechanisms responsible for establishing and maintaining distinct mitochondrial phenotypes within fast-glycolytic and slow-oxidative muscle fibers.

Before we outline these mechanisms, we must address the fact that mitochondrial functional differences in muscle cells of different fiber type could potentially be due to different proportions of subsarcolemmal (SS) and intermyofibrillar (IMF) mitochondria that populate myofibers. SS mitochondria are densely packed beneath the plasma membrane, whereas IMF mitochondria are distributed between myofibrils, as shown in Fig. 3A. Compared with fast fibers, slow fibers contain a similar volume of IMF mitochondria, but contain significantly more SS mitochondria. Interestingly, these two geographically different populations of mitochondria contain different levels of key metabolic enzymes (30) and have different functional properties (1, 22, 73, 77, 103). Compared with IMF, SS mitochondria have been shown to have a lower oxidative capacity (22, 73, 77, 103), to produce more ROS (1, 22, 103) and to be more sensitive to Ca^{2+} -induced PTP (1). However, slow fibers contain a greater proportion of SS mitochondria, have the same oxidative capacity, and produce less ROS than fast fibers, which is inconsistent with the intrinsic properties of SS relative to IMF mitochondria. Therefore, we conclude that variations in the proportions of SS and IMF mitochondria between fiber types do not account for fiber type differences in mitochondrial function.

Most of our knowledge regarding mitochondrial biogenesis—the synthesis of new mitochondria—relates to *trans*-acting transcription factors [i.e., peroxisome proliferator-activated receptors (PPARs), nuclear respiratory factors (NRF1, NRF2), estrogen-related receptor- α], and coactivators such as PPAR- γ coactivator- α and β (PGC-1 α , PGC-1 β) (38, 88, 100). These nuclear transcriptional elements are mostly known to regulate mitochondrial content (i.e., volume density) in muscle (119). As such, in slow-twitch oxidative muscle where mitochondrial mass is two to three times that of fast-twitch glycolytic muscle, PGC-1 α is constitutively expressed at higher levels (62), and higher still in the tissue that is most dense in mitochondria, the heart (29). This tissue-specific difference in PGC-1 α and in PPARs, which regulate the expression of many enzymes of fat metabolism (e.g., β -oxidation cycle) (4), may therefore contribute to explain differences in mitochondrial mass and substrate specificity among fiber types. However, for the most part, the discriminating functional differences among mitochondria from muscles of different fiber type composition illustrated in Figs. 1–3 are unlikely to be solely explained by variations of *trans*-acting elements.

Instead, epigenetic mechanisms may prove key determinants of mitochondrial specialization across fiber types, by fine-tuning sensitivity/responsivity of the nuclear DNA to the various transcriptional agents mentioned above. Epigenetics refers to a set of heritable but plastic mechanisms capable of stably modulating gene expression in response to environmental cues (68, 121). DNA methylation (104) and posttranslational modifications of histones are among the most heavily studied epigenetic mechanisms (86). These molecular mechanisms acting on DNA can efficiently silence target nuclear genes, such as specific myosin heavy chain subtypes (75). DNA methylation may play a particularly important role in muscle fiber differentiation and specialization, as it does during satellite cell activation (105) and in the differentiation of other tissues from embryonic stages (89).

Fiber type-specific responsiveness to given stimuli or transcription factors and coactivators may likewise be determined by specific epigenetic marks. For example, despite the fact that PGC-1 α coregulates both mitochondrial mass and antioxidant enzymes (106), the proportions between mitochondrial mass and antioxidant enzyme activity are not 1:1 among mitochondria from oxidative and glycolytic muscles (Fig. 2). Similarly, transcriptional responses to thyroid hormones, as well as their effect on mitochondrial function, differ between slow- and fast-twitch muscles (6), suggesting that *cis*-acting epigenetic mechanisms may modulate the ability to express important mitochondrial genes (63) including PGC-1 α itself (9). Thus, fiber type-specific epigenetic marks are likely to regulate fiber-type specific proteome signatures, including mitochondrial gene expression.

It is noteworthy that several substrates necessary for epigenetic modifications, including *s*-adenosyl-L-methionine (SAM), AcCoA, NAD^+ , and ATP, are derived from mitochondrial metabolism (114), which may constitute an essential evolutionary mechanism to stably link cellular energetic demands, mitochondrial function, and the nuclear epigenome. This would ensure an optimal match of myofiber function and mitochondrial phenotype. Likewise, fundamental signaling factors with broad impact on myogenesis (e.g., fluctuations in $[\text{Ca}^{2+}]$) and circadian rhythms (3) may contribute to the functional specialization of mitochondria and the establishment of a cytoarchitectural environment conducive to optimal compartmentalization of energy exchange. This type of mechanism, whereby epigenetic modifications of the nuclear and mitochondrial genomes establish cell-specific programs based on intracellular cues, may go a long way in explaining differences in specific mitochondrial features observed across fiber types. Tools capable of measuring genome-wide methylation profiles (13), proteomics (8, 33), posttranslational modifications of mitochondrial proteins (26, 78), and metabolomic profiling (113) may prove valuable in deciphering the origin of these mitochondrial phenotypic variations across cell types.

Summary and Conclusion

In conclusion, while volume density is clearly the most evident mitochondrial characteristic differentiating oxidative from glycolytic fibers, increasing evidence indicates that mitochondrial specialization across fiber types is ob-

served for several key functions of these organelles, including: 1) capacities to oxidize lipid substrates and glycerol-3-phosphate that reflect the general metabolic orientation of fibers; 2) organization of energy exchange mechanisms between mitochondria and various cellular ATPases to optimize energy exchange according to the metabolic profile of fibers and their specific cytoarchitectural organization; 3) ROS-emitting potential per mitochondrial unit which differs perhaps because of the necessity to balance protection against oxidative stress and maintenance of proper ROS-mediated cellular signaling; and 4) resistance to Ca^{2+} -induced PTP opening which ensures physiological opening of the PTP without accidental activation of cell death. Overall, these results thus suggest that mitochondrial functional specialization exists between fiber types in accordance with the principle of coadaptation (42) and that these phenotypes may be required to ensure optimal muscle function (79). However, the molecular mechanisms that establish and maintain such diverse mitochondrial phenotypes across fiber types are still unclear. Deciphering the biological mechanisms controlling mitochondrial function in skeletal muscle and in other cell types should enhance our ability to design tools and interventions capable of optimizing mitochondrial function in different situations and across the life span.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.P., R.T.H., and Y.B. conception and design of the research; M.P. performed the experiments; M.P. analyzed the data; M.P. and Y.B. interpreted the results of the experiments; M.P. and Y.B. prepared the figures; M.P. and Y.B. drafted the manuscript; M.P., R.T.H., and Y.B. edited and revised the manuscript; M.P., R.T.H., and Y.B. approved the final version of the manuscript.

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