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Oxygen Control of Intracellular Distribution of Mitochondria in Muscle Fibers

B. Pathi¹, S. T. Kinsey², and B. R. Locke¹

¹Department of Chemical and Biomedical Engineering, Florida State University, FAMU-FSU College of Engineering, 2525 Pottsdamer Street, Tallahassee, Florida 32310

²Department of Biology and Marine Biology, University of North Carolina Wilmington, 601 South College Road, Wilmington, NC 28403-5915

Abstract

Mitochondrial density in skeletal muscle fibers is governed by the demand for aerobic ATP production, but the heterogeneous distribution of these mitochondria appears to be governed by constraints associated with oxygen diffusion. We propose that each muscle fiber has an optimal mitochondrial distribution at which it attains a near maximal rate of ATP consumption (R_{ATPase}) while mitochondria are exposed to a minimal oxygen concentration, thus minimizing reactive oxygen species (ROS) production. We developed a coupled reaction-diffusion/cellular automata (CA) mathematical model of mitochondrial function and considered 4 fiber types in mouse extensor digitorum longus (EDL) and soleus (SOL) muscle. The developed mathematical model uses a reaction - diffusion analysis of metabolites including oxygen, ATP, ADP, phosphate and phosphocreatine (PCr) involved in energy metabolism and mitochondrial function. A CA approach governing mitochondrial life cycles in response to the metabolic state of the fiber was superimposed and coupled to the reaction-diffusion approach. The model results show the sensitivity of important model outputs such as the R_{ATPase} , effectiveness factor (η) and average oxygen concentration available at each mitochondrion to local oxygen concentration in the fibers through variation in the CA model parameter θ_{det} , which defines the sensitivity of mitochondrial death to the oxygen concentration. The predicted optimal mitochondrial distributions matched previous experimental findings. Deviations from this optimal distribution corresponding to higher CA model parameter values (a more uniform mitochondrial distribution) lead to lower aerobic rates. In contrast, distributions corresponding to lower CA model parameter values (a more asymmetric distribution) lead to an increased exposure of mitochondria to oxygen, usually without substantial increases in aerobic rates, which would presumably result in increased ROS production and thus increased risks of cytotoxicity.

Keywords

cellular automata; effectiveness factor; muscle; optimal mitochondrial distribution; reaction-diffusion model

Introduction

Intracellular diffusion plays a prominent role in defining both the density and distribution of mitochondria in skeletal muscle fibers (Nyack et al., 2007; Hardy et al., 2009; 2010; Kinsey

et al., 2011). While most fibers operate under reaction control with respect to oxidative phosphorylation, diffusion control limits may constrain aerobic cell function and other properties (Dasika et al., 2011). Previous experimental and mathematical modeling work suggests muscle fibers exhibit a non-uniform distribution of mitochondria to attenuate reaction-diffusion constraints associated with aerobic metabolism (Boyle et al., 2003; Nyack et al., 2007; Hardy et al., 2009; 2010; Pathi et al., 2011; Kinsey et al., 2011). In our previous studies (Pathi et al., 2012) we proposed that the non-uniform distribution of mitochondria in muscle fibers arises because of the net effects of the processes governing mitochondrial biogenesis and degradation.

Mitochondrial biogenesis is a complex process that involves proteins derived from both the mitochondrial and nuclear genomes (Wallace 1999; Chan, 2006; Hood et al., 2006; Gottlieb and Carreira, 2010; Terman et al., 2010). A primary molecular pathway through which biogenesis proceeds is through the activation of AMP kinase (AMPK), which is triggered when there is a cellular energy deficit. For example, during exercise there is an increase in the [AMP]/[ATP] ratio, which activates AMPK (Hood et al., 2009). However, the regional information encoded by signaling molecules which trigger mitochondrial biogenesis, such as an increase in AMP near an existing mitochondrion is most likely lost because these signals have to be propagated through the nucleus via diffusion, and nuclear products must then diffuse back to the mitochondria. In addition, previous studies suggest very slight gradients in high energy phosphate molecules like AMP, which is in contrast to the sometimes steep gradients in oxygen (Jimenez et al., 2008; Schroeder et al., 2010; Dasika et al., 2011; Pathi et al., 2011).

Contrary to mitochondrial biogenesis, there is abundant evidence that mitochondrial degradation is a selective process wherein damaged mitochondria are selectively eliminated by targeted mitophagy, which can be triggered by hypoxia (Lemasters 2005; Kim et al., 2007; Hyde et al., 2010; Gottlieb and Carreira, 2010; Youle R.J. and Narendra D. P., 2011; Green D.R., Galluzzi L. et al., 2011). The findings of our previous studies in a range of muscle fiber types suggested that the non-homogeneous distributions of mitochondria arise due to spatially homogeneous signals for the birth of mitochondria and spatially variable mitochondrial death that is dependent on local oxygen concentration (Pathi et al., 2012). In the present study, as in our previous work, 'mitochondrial shifts' result from a change in distribution of the mitochondrial population due to the birth and death of mitochondria, rather than to the migration of individual mitochondria.

The mathematical modeling approach used in our previous studies (Pathi et al., 2012) is a hybrid cellular-automata (CA) reaction-diffusion approach wherein specific CA rules govern mitochondrial birth and death. The model parameter involved in the death rule, θ_{det} was found to be different for different mitochondrial volume densities ($V_{V(\text{MT})}$) in the fibers. Fibers with a $V_{V(\text{MT})} = 0.10$ required a $\theta_{\text{det}} = 0.55$ (aerobic fibers) and those with a $V_{V(\text{MT})} = 0.10$ required a $\theta_{\text{det}} = 0.35$ (anaerobic fibers) to match the experimental data. Various reasons were cited for the need to use the two different values of θ_{det} including the difference in the sensitivity of mitochondrial death to low oxygen in aerobic and anaerobic fibers, the simplicity of the probability function used, which may not fully capture the oxygen dependence of mitochondrial degradation, or the possibility that aerobic and anaerobic fibers are exposed to different boundary oxygen concentrations. Since the previous work showed that only specific values of the model parameter θ_{det} allowed the model to fit the experimental findings, in the present study we analyze the role of oxygen on mitochondrial distribution through variation of the θ_{det} parameter in 4 different fiber types in mouse. This analysis will provide the sensitivity of intracellular mitochondrial distribution to local oxygen concentration and will show the sensitivity of the important model outputs such as the rate of ATP consumption (R_{ATPase}), effectiveness factor (η) and average oxygen

concentration available at each mitochondrion ($\langle O_2^{\text{mito}} \rangle$) to the model parameter (θ_{det}). The present study also addresses two vital questions – (1) do muscle fibers have an optimized mitochondrial distribution? ; (2) why do all mitochondria in a fiber not migrate towards the periphery where there is an increased availability of oxygen? In addition, the steady state mitochondrial distributions in the muscle fibers were analyzed for the case of lower boundary oxygen concentration to simulate hypoxia without allowing redistribution of mitochondria in order to determine if under hypoxia these fibers exhibit similar trends in their model outputs i.e. R_{ATPase} , η and $\langle O_2^{\text{mito}} \rangle$. Such a detailed analysis of mitochondrial redistributions in muscle fibers will help further our understanding of the metabolic and structural organization of these fibers and facilitate development of novel tissue and metabolic engineering approaches.

Mathematical Methods

A coupled CA and reaction diffusion approach in a two dimensional domain representing a muscle fiber is used as in our previous work (Pathi et al., 2011; 2012). In brief, muscle fibers are represented by a circular domain surrounded by varying numbers of capillaries specific to the fiber under consideration. The circular domain is discretized in space and each grid space represents either a mitochondria or cytoplasm. The size of each grid is equivalent to the diameter of a mitochondrion which is assumed to be 1 μm . Each fiber is surrounded with varying number of capillaries each occupying 5 μm in the fiber boundary (Mathieu-Costello et al., 1991). An initial distribution of mitochondria was generated by randomly placing mitochondria in the grids using a random number generator and a counter for the total mitochondrial number until the desired mitochondrial volume density is achieved for the specified fiber type. The boundary oxygen concentration at the capillaries was set to 35 (μM) which corresponds to a partial pressure of approximately 20 Torr. In order to simulate hypoxia, the boundary oxygen concentration at the capillaries was set to 18 μM corresponding to a partial pressure of approximately 10 Torr. All the other initial metabolite concentrations were obtained from literature (Pathi et al., 2012). The parameters for mouse muscle fibers were taken from our previous work (Luedeke et al., 2004; Overton et al., 2009; Pathi et al., 2012).

The rate law derived by Dasika et al. 2011 was used to determine the oxygen consumption rate and the ATP production rate at each mitochondrion. The rate law expresses ATP production in the mitochondria as a function of the concentrations of oxygen, ADP and P_i and it closely approximates the full model of mitochondrial oxidative phosphorylation developed by Beard (2005). The ATP consumption by the myofibrillar ATPase was modeled with a single Michaelis-Menten expression dependent on the ATP concentration. The oxygen flux from the capillaries to the cytoplasm was governed by a membrane transfer coefficient (k_{mt}), 1100 $\mu\text{m s}^{-1}$ (Dasika et al., 2011; Pathi et al., 2011). The temporal and spatial variations of the concentrations of the different metabolites considered in the present study, including ATP, P_i , O_2 , and phosphocreatine (PCr), were determined from solution of the species continuity equation for a given species “j” by

$$\frac{\partial C_j}{\partial t} = D_j \left[\frac{\partial^2 C_j}{\partial X^2} + \frac{\partial^2 C_j}{\partial Y^2} \right] + R_j \quad (1)$$

where C_j is the concentration of species j, t is the time, D_j is the diffusion coefficient and R_j is the reaction rate term for species j. The sum of PCr and creatine (Cr) is assumed constant (i.e. $C_{\text{PCr}} + C_{\text{Cr}} = \text{constant}$) and the sum of ATP and ADP is also assumed constant (i.e. $C_{\text{ATP}} + C_{\text{ADP}} = \text{constant}$). The boundary conditions used are similar to those in our previous studies (Pathi et al., 2011). The effectiveness factor, η , was also computed in order to assess

diffusional constraints in the fiber for the different fiber types based on the methods described in our previous work (Dasika et al., 2011; Pathi et al., 2011). The free energy of ATP hydrolysis (ΔG) was evaluated based on Overton et al., 2009 as used in Pathi et al., 2012.

Cellular Automata (CA) Rules

The CA rules used in the present study are similar to those used in our previous work (Pathi et al., 2011; 2012). Spatially homogeneous mitochondrial biogenesis signals and regionally variable mitochondrial degradation was used. Mitochondrial biogenesis was assumed to be spatially homogeneous across the fiber because its synthesis depends on the expression of nuclear and mitochondrial genes. In contrast, mitochondrial degradation is spatially variable and likely higher in regions where there is low oxygen availability and high ROS production (Pathi et al., 2012). A fixed $V_{V(MT)}$ was assumed and with the death of a mitochondria in one region, a new mitochondrion was generated in a random location such that the total count of mitochondria remained constant (Pathi et al., 2011). A new mitochondrion could only arise in a grid space adjacent to an existing mitochondrion, simulating mitochondrial fission. It is important to note that although this approach is consistent with spatially uniform *signals* for biogenesis, as the mitochondria shift toward the fiber periphery the rate of biogenesis in this region will be higher due to the presence of more mitochondria capable of undergoing fission. The CA rule governing mitochondrial death is given by

$$P_{\text{death}} = \exp\left(-\left(\frac{C_{O_2}}{\theta_{\text{det}}}\right)^2\right) \quad (2)$$

where θ_{det} is an adjustable model parameter that represents the sensitivity of mitochondrial death to the oxygen concentration. A lower θ_{det} therefore leads to increased mitochondrial death near the core of the fiber and a more dramatic shift in mitochondrial distribution toward the periphery of the fiber. The primary focus of the present study is mouse muscle fibers found in the extensor digitorum longus (EDL), which is almost exclusively comprised of 2b and 2x fibers, and the soleus (SOL), which is almost completely comprised of type 2a and 1 fibers (Luedeke et al., 2004). The value of θ_{det} was varied (0.15, 0.35, 0.55, 0.75 and 0.95) in order to determine the sensitivity of the steady state distribution patterns of mitochondria and other factors including R_{ATPase} , η and the local oxygen concentration at each mitochondrion to the model parameter (θ_{det})

The step by step procedure in which the model is simulated is explained in detail in our previous work (Pathi et al., 2012). In brief, mitochondria were randomly placed in the discretized domain such that the total mitochondrial count aligns with the experimentally measured $V_{V(MT)}$ for the fiber type under consideration. Spatial locations in the domain that contain mitochondria produce ATP, and in these regions the cellular ATPase rate (R_{ATPase}) was set to zero. However, regions lacking mitochondria consume ATP and the mitochondrial rate of production (R_{ATPProd}) for these regions was set to zero. CA iterations were run until a final steady state mitochondrial distribution pattern was obtained. The metabolite concentrations reached a quasi-steady state for each CA iteration. An alternating direction implicit finite difference scheme was used to solve the system of second order parabolic partial differential equations (Eqns. (1)) (Peaceman and Rachford, 1955; Madzvamuse and Maini, 2007; Yang et al., 2005). The model was implemented using MATLAB Release 7.9 (The Math Works, Inc., 2009).

The $\langle O_2^{\text{mito}} \rangle$ was evaluated for each of the fibers by considering steady state mitochondrial distributions (i.e. after CA iterations) and their corresponding oxygen concentration profiles.

The local oxygen concentration obtained from the steady state oxygen concentration profiles in all the locations containing a mitochondrion are summed and the sum is divided by the total $V_{V(MT)}$ in the fiber to obtain $\langle O_2^{mito} \rangle$ available in each mitochondrion. This helps assess the averaged local availability of oxygen at each mitochondrion. Increased exposure of mitochondria to oxygen could lead to higher reactive oxygen species (ROS) production and potentially result in cellular damage or apoptosis (Finkel and Holbrook, 2000). Thus, cellular damage triggered due to excessive ROS production is potentially decreased by exposing intracellular mitochondria to optimal oxygen concentrations such that the cell maintains high aerobic rate while producing minimal ROS. In addition, as in our previous work (Pathi et al., 2012), the radial distribution of IM mitochondria is calculated by determining the area of mitochondria present in 4 μm width bands of the fiber starting from the center of the fiber and moving to the fiber periphery normalized by the area of the bands containing those mitochondria. The obtained information is used to evaluate the mean weighted radii (MWr). The MWr is evaluated in order to assess the shifts of the distribution corresponding to different values of θ_{det} . A larger value of MWr implies a higher redistribution of mitochondria towards the fiber periphery. The MWr is evaluated by

$$MWr_r = \frac{\sum_{i=1}^M N_i * r_i}{\sum_{i=1}^M N_i} \quad (3)$$

Where N_i is the mitochondrial volume density, r_i is the corresponding radii normalized to the internal radius of the muscle cell, R , and M is the total number of radial points.

Results and Discussions

Mitochondrial distributional patterns for θ_{det} values of 0.15, 0.35, 0.55, 0.75 and 0.95 for mouse 2x and 2b fibers (EDL) and 1 and 2a fibers (SOL) are discussed below. The measurements of fiber size, CAF and $V_{V(MT)}$ for these fibers were obtained from experimental findings (Pathi et al., 2012) as illustrated in Table 1. Figure 1 shows mitochondrial distribution patterns for EDL 2b fibers for various θ_{det} values. The initial random distribution of mitochondria (Fig. 1A) changes during the CA iterations as mitochondria shift toward the capillary boundaries until a steady state pattern is established (Fig. 1B – F). A low θ_{det} value of 0.15 indicates a higher sensitivity of death rate to oxygen and hence leads to a greater redistribution of mitochondria towards the fiber periphery compared to higher values. In this case, as shown in Fig. 1B, there are no mitochondria at the fiber core and all of the mitochondria are redistributed towards the fiber edges. When the value of θ_{det} is increased to 0.35, most of the mitochondria still migrate towards the capillaries as shown in Fig. 1C. However, for a value of 0.55 the steady state mitochondrial patterns (Fig. 1D) match the experimentally observed distribution (results shown in our previous work, Pathi et al., 2012) and the patterns indicate that most of the mitochondria migrate towards the capillaries with few mitochondria present in the fiber core. With further increases of θ_{det} to 0.75 (Fig. 1E) and 0.95 (Fig. 1F), the Gaussian distribution functions governing mitochondrial death become wider and mitochondria are less redistributed towards the fiber periphery. Such mitochondrial redistributions also lead to changes in the free energy for ATP hydrolysis (ΔG). The ΔG increases from -63.01 kJ/mol to -61.04 kJ/mol as θ_{det} changes from 0.15 to 0.95. which indicates that mitochondria clustered near the edge (smaller θ_{det}) are more energetically favorable (more negative ΔG).

A decrease in the spatial variation of oxygen is observed after redistribution (Fig. 2(B) – (F)) in comparison to that before redistribution (Fig. 2(A)). It is interesting to observe that as the value of θ_{det} increases from 0.15 to 0.95, the observed oxygen gradient increases because there is less redistribution of mitochondria towards the fiber periphery (Fig. 1). For a θ_{det}

value of 0.15, where almost all of the mitochondria migrate towards the fiber periphery, the observed oxygen gradient is the least (Fig. 2(B)). In contrast, the oxygen gradient as observed from the oxygen concentration profiles is maximal for a θ_{det} value of 0.95 (Fig. 2(F)). The average non-dimensional concentrations of several metabolites before and after the CA iterations are shown in Fig. 3. The concentration changes from the initial resting muscle values until a steady state is reached at the elevated rates of aerobic ATP demand before the CA iterations are shown in Fig. 3(A). These concentration changes are similar to the variation in the metabolite concentrations during rest-to-work transitions (Kushmerick and Meyer, 1985). Metabolite concentrations change for nearly 5 CA iterations until a steady state in mitochondrial distribution patterns were reached as is shown for different values of θ_{det} (Fig. 3(B) – (F)). Based on a half-life of mitochondria in muscle of about 7 days, each CA iteration corresponds to approximately one week (Booth and Holloszy, 1977; Hepple R.T., 2010). In contrast, the time-scale for the reaction-diffusion process is much shorter and therefore the reaction-diffusion analysis can be considered to be quasi-steady with respect to the overall CA iterations. The maximum increases in concentrations of O_2 , PCr, and ATP, occur for a θ_{det} value of 0.15 (Fig. 3(B)) and the least increase for θ_{det} of 0.95 (Fig. 3(F)).

In order to simulate acute hypoxemia, these EDL 2b fibers (with mitochondrial distributions obtained after CA iterations under normoxic conditions) were subjected to half of the original boundary oxygen concentration, i.e. $18 \mu\text{M}$ in the absence of further CA iterations. The corresponding oxygen concentration profiles are shown in Fig. 4. Similar to the normoxic case, for θ_{det} of 0.15, where almost all of the mitochondria are present at the fiber edges, the oxygen gradient observed from the oxygen concentration profiles is the least (Fig. 4(A)) and it is the highest corresponding to a θ_{det} value of 0.95 (Fig. 4(E)).

The smallest amongst the mammalian fibers considered in the present analysis were the EDL 2x fibers. Steady state mitochondrial distribution patterns for different θ_{det} values for these fibers are illustrated in Figure 5. The mitochondrial distributional patterns in these fibers showed similar trends as the EDL 2b fibers as the θ_{det} value was varied from 0.15 (Fig. 5B) to 0.95 (Fig. 5F). Figure 6 and Figure 7 illustrate mitochondrial distributional patterns for SOL 2a and 1 fibers, respectively, for different θ_{det} values. Similar to EDL 2b and 2x fibers, the initial mitochondrial distribution (Fig. 6A for SOL 2a and 7 A for SOL 1) changes differently for different θ_{det} values as the CA iterations proceed until they reach their respective steady state patterns (Fig. 6B – F for SOL 2a and 7 B – F for SOL 1). The maximum redistribution is observed for a θ_{det} value of 0.15 (Fig. 6 B for SOL 2a and Fig. 7B for SOL 1) and the least for a θ_{det} value of 0.95 (Fig. 6 F for SOL 2a and Fig. 7F for SOL 1). As discussed in our previous work (Pathi et al., 2012), the mitochondrial distribution pattern corresponding to a θ_{det} value of 0.55 aligns the best with experimental findings for mammalian fibers. Similar to EDL 2b fibers, ΔG increases are observed in each of these fiber types as well as θ_{det} changes from 0.15 to 0.95. The corresponding increases in ΔG for 2a, 1 and 2x fibers are from -62.10 kJ/mol to -60.01 kJ/mol , from -55.12 kJ/mol to -53.07 kJ/mol and from -57.10 kJ/mol to -54.58 kJ/mol , respectively. Again, the smaller values of θ_{det} have the smaller (more negative) ΔG and thus are more energetically favorable.

In order to analyze the functional benefits of the non-uniform mitochondrial distributions corresponding to different values of θ_{det} , the R_{ATPase} , η and $\langle \text{O}_2^{\text{mito}} \rangle$ were evaluated for the different fibers under consideration for each of these θ_{det} values for both the normoxic and hypoxic cases. The evaluated quantities, R_{ATPase} , η and $\langle \text{O}_2^{\text{mito}} \rangle$ are plotted against MWr in order to analyze the trends in their values as mitochondrial distribution shifts occur corresponding to different θ_{det} values (Fig. 8 and Fig. 9).

In EDL 2b fibers, it can be observed that for both normoxic and hypoxic cases, the R_{ATPase} and η reach limiting values near MWr of 0.71 irrespective of any increase in $\langle O_2^{mito} \rangle$ (Fig. 8A – C). The R_{ATPase} , η and $\langle O_2^{mito} \rangle$ for the original distribution (Fig. 8A – C) suggests the need for redistribution and the potential functional benefits of mitochondrial redistribution. A MWr of 0.71 corresponds to a θ_{det} value of 0.55 at which the experimentally observed mitochondrial distribution pattern fits best with the model output (Pathi et al., 2012). Model results (Fig. 8A – C) show that for the normoxic case, the increase in R_{ATPase} is greater as the MWr changes from 0.59 to 0.71 than the corresponding increase as the MWr changes from 0.71 to 0.89 (Fig. 8A), in contrast, the increase in $\langle O_2^{mito} \rangle$ (Fig. 8C) as the MWr changes from 0.59 to 0.71 is similar to the increase as the MWr changes from 0.71 to 0.89. This finding suggests that beyond MWr of 0.71, $\langle O_2^{mito} \rangle$ keeps increasing without substantial increases in R_{ATPase} . Hypoxic fibers also show trends similar to their normoxic counterparts. The corresponding η plot (Fig. 8B) for both the normoxic and hypoxic case also depicts similar trends. The model results suggest that EDL 2b fibers may achieve an optimal mitochondrial distribution corresponding to MWr of 0.71 or θ_{det} value of 0.55. A MWr lower than 0.71 leads to lower rates and MWr higher than 0.71 leads to higher oxygen concentrations in mitochondria that do not result in substantial increases in rates and which could lead to increased ROS production. In order to determine if similar trends were observed in other mammalian fiber types, analysis was performed for SOL 2a, 1 and EDL 2x fibers as discussed below.

As with the EDL 2b fibers, R_{ATPase} , η and $\langle O_2^{mito} \rangle$ were evaluated for the EDL 2x (Fig. 8D – F), SOL 2a (Fig. 9A – C) and SOL 1 (Fig. 9D – F) fibers as well. MWr of 0.69, 0.73 and 0.67 corresponds to θ_{det} value of 0.55 for EDL 2x, SOL 2a and SOL 1 fibers, respectively. The mitochondrial distributional patterns obtained using these MWr values fits best with the experimental data (Pathi et al., 2011b). These MWr values may reflect an optimal mitochondrial distribution in these fibers because they show trends similar to EDL 2b fibers. Figure 8D – F for 2x, Figure 9A – C for 2a and Figure 9D – F for 1a fibers show that the increase in R_{ATPase} and η is more pronounced when MWr is below 0.69 for 2x, 0.73 for 2a and 0.67 for 1 fibers respectively and increases to attain its potential optimal value (i.e., 0.69 for 2x, 0.73 for 2a and 0.67 for 1 fibers respectively) than when it increases beyond this probable optimal value. In contrast, $\langle O_2^{mito} \rangle$ increases for both the cases. Fibers exposed to reduced boundary oxygen concentration (i.e., hypoxic fibers) also exhibit similar trends (Fig. 9). Thus, beyond the MWr value (i.e., 0.69 for 2x, 0.73 for 2a and 0.67 for 1 fibers, respectively) which could potentially reflect the optimal mitochondrial distribution, any further redistribution exposes mitochondria in the fiber to increased local oxygen without substantial increases in its aerobic rates. Several theories have been proposed to explain the prominent role of mitochondria in ROS generation and in the mechanisms of ROS-mediated cell diseases (Kirkinezos and Moraes, 2001). Excess exposure of mitochondria to oxygen without significant increases in R_{ATPase} could lead to increased ROS production due to prolonged exposure to hyperoxia. Mounting evidence suggests that excess ROS production due to hyperoxia is cytotoxic and could potentially damage the muscle fiber (Amicarelli, F., Ragnelli, A.M., et al., 1999; Flandin P, Donati Y., et al., 2005). Thus, we propose that for all of the fibers considered in the present analysis, an optimal mitochondrial distribution exists at which these fibers attain a near maximal R_{ATPase} at a minimal $\langle O_2^{mito} \rangle$. These proposed optimal distributions obtained from the model match experimentally observed distributions (Pathi et al., 2012).

The present results are consistent with previous findings that redistribution of mitochondria towards the fiber periphery nearer to the capillaries alleviates diffusional constraints and benefits the fiber functionally (Pathi et al., 2011; 2012; Kinsey et al., 2011). However, the

present analysis suggests that extreme redistribution towards the edges might cause more harm to the fiber than good. Even though there is not enough evidence to suggest a cut-off oxygen concentration beyond which mitochondria could produce high amounts of ROS, the lack of functional benefits to the fiber with increased migration towards the edges while increasing the concentration of oxygen exposure in each mitochondrion provides a reason why not all mitochondria migrate towards the fiber periphery.

The present work examines and explains mitochondrial distribution patterns in different mammalian muscle fibers and investigates the role of oxygen on mitochondrial distribution through variation of the θ_{det} parameter. However, there are some limitations of the present model which can be addressed in future work. Firstly, the present paper did not involve an analysis of the variation of total mitochondrial number in response to varying metabolic conditions and instead assumed a fixed volume density of mitochondria for each of the fibers considered. This was reasonable since the central focus was to understand intracellular mitochondrial distribution. Muscle activities such as exercise have a significant impact on intracellular oxygen gradients and mitochondrial distributions (Muller 1976). Hence an understanding of the phenomenon which dictates this distribution is the central focus of the present work. However, an analysis of changes in both mitochondrial count and distribution in response to the metabolic state of the fiber is important to model phenomena like sustained exercise or extreme hypoxia wherein there occur changes in mitochondrial count and distribution (Holloszy 2008; Hood et al., 2006; Kayar et al., 1986). Secondly, the present work does not take into consideration any of the diseased states of the fibers or of the mitochondrion. In order to simulate a diseased state of the fiber, such as diabetes, changes need to be made in the mitochondrial rate law and the probability functions that are used to model the mitochondrial life cycle. Previous studies have shown a dramatic reduction in the electron transport chain activity in subsarcolemmal mitochondrial population of fibers suffering with Type 2 diabetes (Ritov et al., 2005). In addition, mitochondria in these fibers decrease in density and produce large amounts of ROS (Sivitz, 2010). We plan to take into consideration the above factors in future work. Thirdly, the rate law for mitochondrial oxygen consumption and ATP production used in the present study (Dasika et al., 2011) is an approximation of the full model of oxidative phosphorylation developed by Beard (2005). The developed model is for healthy mitochondria. However, this model could be altered to develop a new mitochondrial rate law with ROS production. The developed rate law could be used as in the present analysis to determine if muscle fibers that contain ROS producing mitochondria (i.e., using the new mitochondrial rate law) have different intracellular mitochondrial distributions and cellular aerobic rates in comparison to healthy muscle that contain mitochondria which don't produce ROS. Fourthly, the present work does not take into consideration the different ATPases in muscle. The myosin ATPase in the myofibrils and the SR Ca^{2+} ATPase in the SR that surrounds the myofibrils are the major ATP consuming enzymes in active muscle, and in our model would be the major contributors to the aggregate ATPase reaction. However, separating these ATPases would add additional complexity to the model without providing new insight regarding mitochondrial distribution, which was our principal focus. On the other hand, the model does account for the absolute capacity for ATP demand as well as spatial variation in these myofibrillar ATPases. Skeletal muscle fiber volume is predominantly composed of myofibrils, SR, and mitochondria, and the relative contribution of each component is dependent on muscle function (Rome and Lindstedt, 1998). Therefore, the density and distribution of myofibrillar ATPases (the myosin ATPase and the SR Ca^{2+} ATPase) are governed by the density and distribution of mitochondria, because they cannot occupy the same volume. Finally, the present work does not take into consideration any changes in fiber capillarity which could be a result of endurance training or chronic exercise (Muller 1976), although this could be considered in future work.

Conclusions

The present study utilizes a coupled CA/reaction-diffusion model to determine mitochondrial distribution patterns for mammalian muscle fibers (EDL 2b, 2x and SOL 2a, 1). The sensitivities of the mitochondrial distribution and the corresponding R_{ATPase} , η and O_2^{mito} to the CA model parameter θ_{det} in these fibers were determined. The model results suggest that an optimal mitochondrial distribution exists in each of the fibers examined. At higher values of the CA model parameter the mitochondria were more uniformly distributed in the cell with correspondingly lower rates of ATPase. At lower values of the CA model parameter, the mitochondria were more distributed to the edges of the cell with higher rates. However, if the CA model parameter is too low, further redistribution does not lead to correspondingly higher rates, but exposes the mitochondria to potentially damaging high levels of oxygen. These high local oxygen concentrations in the mitochondria may lead to reactive oxygen species and cell damage. The value of the model parameter that leads to the best fit of experimental data to the model occurs at the optimal condition where the rate is highest with the lowest oxygen concentration. Similar conclusions are also derived when the steady state mitochondrial distributions in the muscle fibers were treated with a lower boundary oxygen concentration to simulate acute hypoxemia without allowing redistribution of mitochondria. The present study along with our previous work (Pathi et al., 2011; 2012) provides valuable insights which could be used in various bioengineering applications to treat muscle diseases and to generate replacement muscle tissue.

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Figure 1.

Mitochondrial distribution patterns in EDL 2b fibers with boundary oxygen concentration of $35 \mu\text{M}$. (A) Original distribution prior to CA iterations and redistributed mitochondria using CA rules to reach a steady state for θ_{det} values of 0.15 (B), 0.35 (C), 0.55 (D), 0.75 (E) and 0.95 (F). Grid spaces containing mitochondria are represented in red.

Figure 2.

Oxygen concentration profiles corresponding to mitochondrial distribution patterns in EDL 2b fibers shown in Fig. 1. prior to CA iterations (C) and after redistribution (D).

Mitochondrial distribution patterns in EDL 2b fibers with boundary oxygen concentration of 35 μM . (A) Original distribution prior to CA iterations and redistributed mitochondria using CA rules to reach a steady state for θ_{det} values of 0.15 (B), 0.35 (C), 0.55 (D), 0.75 (E) and 0.95 (F).

Figure 3.

Non dimensionalized average concentrations of metabolites corresponding to mitochondrial distribution patterns in EDL 2b fibers shown in Fig. 1 with boundary oxygen concentration of 35 μM . Metabolite concentration corresponding to original distribution prior to CA iteration (A), Variation of non dimensionalized average concentration of metabolites with CA iterations to reach a steady state for θ_{det} values of 0.15 (B), 0.35 (C), 0.55 (D), 0.75 (E) and 0.95 (F).

Figure 4.

Oxygen concentration profiles corresponding to mitochondrial distribution patterns in EDL 2b fibers shown in Fig. 1 for redistributed mitochondria for θ_{det} values of 0.15 (A), 0.35 (B), 0.55 (C), 0.75 (D) and 0.95 (E) with boundary oxygen concentration of 18 μM .

Figure 5.

Mitochondrial distribution patterns in EDL 2x fibers with boundary oxygen concentration of $35 \mu\text{M}$. (A) Original distribution prior to CA iterations and redistributed mitochondria using CA rules to reach a steady state for θ_{det} values of 0.15 (B), 0.35 (C), 0.55 (D), 0.75 (E) and 0.95 (F). Grid spaces containing mitochondria are represented in red.

Figure 6.

Mitochondrial distribution patterns in SOL 2a fibers with boundary oxygen concentration of 35 μM . (A) Original distribution prior to CA iterations and redistributed mitochondria using CA rules to reach a steady state for θ_{det} values of 0.15 (B), 0.35 (C), 0.55 (D), 0.75 (E) and 0.95 (F). Grid spaces containing mitochondria are represented in red.

Figure 7. Mitochondrial distribution patterns in SOL 1 fibers with boundary oxygen concentration of $35 \mu\text{M}$. (A) Original distribution prior to CA iterations and redistributed mitochondria using CA rules to reach a steady state for θ_{det} values of 0.15 (B), 0.35 (C), 0.55 (D), 0.75 (E) and 0.95 (F). Grid spaces containing mitochondria are represented in red.

Figure 8. Model results showing R_{ATPase} , effectiveness factor (η) and average oxygen concentration per mitochondrion for EDL 2b (A, B and C) and EDL 2x (D, E and F) fibers for different values of MWr for both normoxic (i.e. 35.1 μ M) and hypoxic (i.e. 18 μ M) case.

Figure 9. Model results showing R_{ATPase} , effectiveness factor (η) and average oxygen concentration per mitochondrion for SOL 2a (A, B and C) and SOL 1 (D, E and F) fibers for different values of MWr for both normoxic (i.e. 35.1 μ M) and hypoxic (i.e. 18 μ M) case.

Table 1

Findings from microscopy showing Fiber types, n-values, fiber diameter, capillaries around a fiber (CAF), total mitochondrial volume density (Total $V_{v(MT)}$) along with the best fit thetadet (θ_{det}) value obtained from the mathematical model.

Fiber Type	n (tissue preparations)	n(images analyzed)	Fiber Diameter (μm)	CAF	Total $V_{v(MT)}$	Thetadet (θ_{det})
EDL 2b	6	34	48 ± 1.1	44 ± 0.3	0.137 ± 0.010	0.55
EDL 2x	6	34	30 ± 1.4	3.8 ± 0.2	0.257 ± 0.012	0.55
SOL 2a	6	31	42 ± 1.3	4.8 ± 0.3	0.141 ± 0.007	0.55
SOL 1	6	31	43 ± 1.6	4.7 ± 0.5	0.250 ± 0.016	0.55