RAPID REPORT

Changes in mitochondrial morphology and organization can enhance energy supply from mitochondrial oxidative phosphorylation in diabetic cardiomyopathy

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Jarosz J, Ghosh S, Delbridge LM, Petzer A, Hickey AJ, Crampin EJ, Hanssen E, Rajagopal V. Changes in mitochondrial morphology and organization can enhance energy supply from mitochondrial oxidative phosphorylation in diabetic cardiomyopathy. Am J Physiol Cell Physiol 312: C190–C197, 2017. First published November 30, 2016; doi:10.1152/ajpcell.00298.2016.—Diabetic cardiomyopathy is in part induced by metabolic dysfunction within cardiac mitochondria. It is characterized by metabolic alterations such as an overdependence on fatty acid oxidation (6) and subsequent mitochondrial inefficiencies (26) characterized by increased reactive oxidative species (ROS) production (21, 24) and decreased ATP and phosphocreatine (PCr) concentrations (17). These metabolic alterations coincide with changes to the subcellular ultrastructure, such as increased lipid and glycogen deposition, loss of myofibrils, and changes to mitochondrial morphology and arrangement within the cell (2, 3, 8, 11).

With regard to mitochondria, some studies report mitochondrial swelling (10, 15, 29) while others show decreased mitochondrial size (7); reports of increased mitochondrial volume fraction (15, 32) sit alongside findings of decreased mitochondrial density (14, 30). Most studies agree that structural remodeling of the diabetic cardiomyocyte progresses with time (15, 16). A positive correlation is also apparent between mitochondrial fission and ultrastructure alterations in energy metabolism during diabetes (25, 31, 41).

In this study we collected transmission electron microscopy (TEM) images of left ventricular myocytes from age-matched control and Sprague-Dawley (SD) rat models of Type 1 diabetes and measured differences in mitochondrial morphology and spatial organization. We performed these measurements on TEM images of the entire transverse section of the cell as opposed to small regions of the longitudinal section of the cell that were typically analyzed by previous studies. The cardiomyocyte is ~20 μm in diameter and 100 μm in length. Therefore, high-magnification TEM images of longitudinal sections typically show small regions of interest. Our data enabled us to determine whether the previously reported alterations were highly localized within the cell. The data also enabled us to determine whether the columnar arrangement of mitochondria in between the contractile machinery had also changed due to structural remodeling.

Although a strong correlation between ultrastructural remodeling and metabolic malfunction is well established, the relative impact of these two changes on overall energy supply and demand within the diabetic myocyte is yet to be determined. Our aim is to test the hypothesis that structural remodeling can act as a compensatory mechanism for inefficient mitochondrial ATP synthesis. Knowledge of the precise nature of mitochondrial remodeling events would enhance drug development strategies that target mitochondrial biogenesis and ATP synthesis (38). Since structural remodeling and mitochondrial
dysfunction are tightly coupled in animal models, computational models are ideal tools that can decouple these interactions in-silico for investigations into feed forward and feedback mechanisms between structure and function. Therefore, using a computational model of cardiac oxidative phosphorylation (OXPHOS) energy metabolism, we investigated whether the measured structural changes provided any compensation for mitochondrial OXPHOS inefficiencies.

MATERIALS AND METHODS

Animals, fixation protocol, and electron microscopy. All animal procedures followed guidelines approved by the University of Auckland Animal Ethics Committee (for animal procedures conducted in Auckland, Application No. R826).

Six-week-old male rats (250 g body wt) were injected with a single dose of streptozotocin (STZ; 55 mg/100 g body wt) in saline to induce diabetes or an equal volume of saline for controls. The animals were euthanized at 9 wk postinjection, and hearts were excised, chemically fixed (2.5% gluteraldehyde, 2% formaldehyde, and 50 mM CaCl₂ in 0.15 M sodium cacodylate buffer), and processed for standard TEM.

Seventy-nanometer (nm)-thick sections of tissue from the control and diabetic resin blocks (from the left ventricular mid-wall) were sectioned and imaged using a FEI Tecnai F30 microscope equipped with a Gatan US1000 camera. Montages of several tissue regions were acquired using SerialEM and reconstructed with IMOD (19). The images had 2.3 × 2.3 nm pixel resolution. Transverse-view two-dimensional micrographs of seven control and seven diabetic cardiomyocytes were selected across a collection of electron micrographs from three control and three diabetic SD rats for quantitative comparisons. Figure 1, A and B, shows typical transverse sections of control and diabetic myocytes, respectively.

Image segmentation and measurement of spatial characteristics. Transverse-sectional electron micrographs were segmented for the sarcolemma and boundaries of mitochondria with the open-source EM image processing software package IMOD, as shown in Fig. 1. Mitochondria were labeled individually and within contiguous groupings of one or more mitochondria appearing to be in contact (mitochondrial clusters). Individual mitochondria in the EM images were segmented by drawing closed contours around their outer membrane. Mitochondrial clusters were subsequently labeled by joining the contour between individual mitochondria appearing to be in contact (Fig. 1C).

Fig. 1. Transverse view of mitochondrial organization in left ventricular myocytes of control and streptozotocin (STZ) rat hearts. A: myocyte transverse section from a control animal with outlines of the sarcolemma boundary (red) and mitochondria outer membrane boundary (green). B: myocyte transverse section from a STZ-induced Type 1 diabetic rat heart. C: magnified view of small region in control myocyte showing individual mitochondria outlined in green (left) and clusters outlined in yellow (right).
Mitochondrial morphology was described with cross-sectional area and shape parameters. Cluster morphology was similarly quantified to describe mitochondrial clustering. The size of a mitochondrion (or cluster) was defined as the cross-sectional area of the segmented mitochondrion (or cluster), which was then normalized to cell area.

Solidity is a measure of the convexity of a region and was calculated as the ratio of mitochondrial (or cluster) area to that of the smallest convex polygon that could enclose it.

Elongation of each region is related to eccentricity and was measured by the extent to which it aligned with its equivalent major axis:

\[
\text{Elongation} = 1 - \frac{A}{0.25\pi L^2}
\]

where \( A \) is the unnormalized area of the region and \( L \) is the major axis length of an ellipse with the same normalized second central moments as the region. These measures were extracted for each of the images using MATLAB’s regionprops binary image function.

Surface-to-volume ratio (\( \gamma \)) of individual and clusters of mitochondria was estimated by calculating the ratio between the perimeter and cross-sectional area of each mitochondrion or cluster.

In addition to the above morphological metrics, the density of mitochondria (or clusters) and the spatial distribution of the mitochondria (or clusters) relative to cell center, termed dispersion or radius of gyration (\( r \)), were also calculated.

Compartmental modeling of energy metabolism. Subsequent to the morphological analysis, we used an experimentally validated mathematical model of mitochondrial metabolism in cardiomyocytes to investigate the impact of measured morphological changes on mitochondrial function. We used a mixed-compartment model of energy transfer in cardiomyocytes based on Ref. 1. The model, composing of three compartments: myofibrillar (mitochondrial inner membrane (IMS)), and matrix space, incorporates key reactions involved in energy transfer between the myofibril and IMS compartments, such as the PCR shuttle, ATP consumption during cross-bridge kinetics, and the adenylate kinase reaction (see Fig. 3B). The model represents the rate of ATP synthesis, \( v_{\text{synthesis}} \), within the matrix using a kinetic scheme derived from Ref. 1.

\[
v_{\text{synthesis}} = \frac{v_{f_{\text{max}}} \text{ADP} \times \text{Pi}}{K_d \times K_p \times \text{Den}} - \frac{v_{i_{\text{max}}} \text{ATP}}{K_i \times \text{Den}}
\]

where \( v_{f_{\text{max}}} \) and \( v_{i_{\text{max}}} \) represent maximum ATP synthesis rate (forward reaction) and maximum ATP hydrolysis rate (backward direction), respectively. ATP, ADP, and Pi (inorganic phosphate) are the concentrations of respective metabolites in the mitochondrial matrix. All other constants in the equation represent various rate constants from Ref. 1. We assumed that diffusion of metabolites is rapid in the myofibrillar compartment, leading to a uniform distribution of metabolites in the myofibril. Fluxes of various metabolites (\( j_{\text{menergy}}^{\text{IMS}} \)) across the mitochondrial outer membrane, from myofibril to the IMS, are described by modifying similar equations previously derived by (4). We incorporate a parameter \( \gamma \), which is the ratio of the outer membrane surface area to mitochondrial volume:

\[
j_{\text{menergy}}^{\text{IMS}} = \frac{\gamma P_{\text{ext}}}{j_{\text{IMS}}} (\text{Met}_{\text{myo}} - \text{Met}_{\text{IMS}})
\]

where \( P_{\text{ext}} \) denotes the permeability of outer membrane to a metabolite, while \( \text{Met}_{\text{myo}} \) and \( \text{Met}_{\text{IMS}} \) are the concentration of a metabolite in myofibril and IMS compartments, respectively. The constant \( f_{\text{IMS}} \) denotes the volume fraction of the IMS with respect to the mitochondrial volume, which was set to 0.25 based on Ref. 37. The rate of ATP hydrolysis (\( j_{\text{hydrolysis}}^{\text{IMS}} \)) due to cross-bridge cycling in the myofibrillar region was provided as an input to the model, while the model calculated all other reaction rates and metabolite concentrations as functions of time. The model equations were defined in CellML and solved using OpenCOR (12). The validity of the model was confirmed when the simulation reproduced the results from previous computational and experimental studies (37).

Using this model, we investigated the impact of changing the surface-to-volume ratio (\( \gamma \)), as estimated from our electron microscopy data, in the context of diabetic cardiomyopathy where mitochondrial ATP synthesis rate, \( v_{f_{\text{max}}} \), is impaired.

RESULTS

Alterations in ultrastructure are consistent with increased mitochondrial fission in diabetic cardiomyopathy. Differences (\( P < 0.05 \)) in size, shape and density occurred between diabetic and control myocytes. Figure 2A shows that at 9 wk post STZ-induced diabetes the myocytes had smaller mitochondria (20% change relative to control myocytes) and diabetic heart mitochondria were ~10% longer with 2.5% less solidity than control heart mitochondria. Numerical density, described by the frequency of individual mitochondria per unit area, was 53% greater in diabetes (Fig. 2C).

Mitochondria form larger clusters on transverse section of the diabetic cardiomyocyte. Columns of mitochondria are distributed uniformly through the cell volume, interspersed between myofibrils for optimal energy supply (34). Mitochondria are known to dynamically fuse and form networks to increase ATP production at high workloads (27). As there is a decreased capacity to produce ATP in diabetic cardiomyopathy (18, 26, 36), we investigated whether the overall spatial arrangement of mitochondria within the cell cross-section area is altered in diabetes. Figure 2B shows that diabetic mitochondrial clusters were larger (60% more mitochondria per cluster), more elongated (9%), and less convex (35%) than control mitochondria clusters. Figure 2D is a cumulative distribution plot of the radius of gyration between the cell center and each of the mitochondria (Fig. 2D, left) and mitochondrial clusters (Fig. 2D, right). The figures show that the distribution of the mitochondria and their clusters were not significantly different between the control and diabetic states. Figure 2E provides a conceptual sketch that summarizes our morphological and organizational findings in this study.

Average mitochondrial cluster surface area is substantially increased in diabetes. As the size and shape of mitochondrial clusters changed within diabetic cells, so did its perimeter-to-cross-sectional area ratio. The mean perimeter of mitochondrial cluster, normalized to its cross-sectional area, increased by 22.5% in diabetic cardiomyocytes (Fig. 3A, 6.6 \( \mu \text{m}^{-1} \)) in control vs. 8.2 \( \mu \text{m}^{-1} \) in diabetic cells). In what follows, we used a mathematical model of mitochondrial metabolism to investigate the effect of this change, via \( \gamma \), on the OXPHOS system when ATP synthesis, \( v_{f_{\text{max}}} \), is impaired.

Increased mitochondrial cluster surface area can elevate metabolite concentrations in the cytosol. Experimental investigations into the relationship between structure and function can only provide correlations between them under different conditions. Here, using biophysical simulations we examined the impact of increased mitochondrial clustering on energy metabolism. A recent study by Picard et al. (28) showed that adjacent mitochondria that are in contact with each other exhibit coordinated cristae organization. The study suggests that subcellular content such as calcium and protons can pass through the inter-mitochondrial junctions, Kurz et al. (20) have
Fig. 2. Changes in mitochondrial morphology and spatial arrangement in STZ-induced Type 1 diabetic Sprague-Dawley (SD) rats. A: cumulative distribution plots of individual mitochondrial size, elongation (relative major axis length) and solidity (measure of convexity). B: cumulative distribution plots of the size, elongation and solidity of mitochondria clusters. C: spatial density of individual mitochondria (left) and clusters of mitochondria (right) in control and diabetic cell transverse sections. D: spatial distribution of individual mitochondria (left) and clusters of mitochondria (right) relative to the center of the cell. E: sketch summarizing the morphological and organizational changes to mitochondria (colored in dark grey) in diabetic rats measured in this study.
also shown that mitochondria can synchronize their membrane potentials and exhibit similar amplitudes. We therefore assumed that mitochondria within a cluster are metabolically coupled with each other and ATP consumed by the myofibrils must diffuse only through the surfaces that are in contact with the myofibril space. Under these assumptions, we simulated three scenarios using the two measured values of control and diabetic mitochondrial cluster surface area normalized to its volume (γ) and two values of $v_{\text{max}}$ representing mitochondrial ATP synthesis capacity in control and diabetic myocytes (see Table 1). These simulations were used to answer the question: does the increased surface area of mitochondrial clusters provide any feedback to energy metabolism? The three scenarios were as follows:

1) Scenario 1: control metabolic capacity, control surface area (CC).
2) Scenario 2: diabetic metabolic capacity, control surface area (DC).
3) Scenario 3: diabetic metabolic capacity, diabetic surface area (DD).

A 10% depression in $v_{\text{max}}$, the maximum rate of ATP synthesis through OXPHOS, was used to simulate the diabetic condition and was motivated by previous a study (18), which reported an ~10% downregulation in major subunits of OXPHOS Complex V (ATP synthase).

To establish a common reference point for comparison we input the same ATP consumption rate profile ($\varphi_{\text{hydrolysis}}$), indicative of the force production, for all three scenarios. The input simulated a change from low-workload (3,000 μM/M/s) to high-workload ATP consumption rate (6,000 μM/M/s) (37) over a period of 50 s at a pacing frequency of 5.55 Hz. (see Fig. 3C).

Based on the assumptions of the model the resulting steady-state $\varphi_{\text{synthesis}}$ matched the $\varphi_{\text{hydrolysis}}$ provided as an input (Fig. 3C, top right). However, the average concentration of various metabolites differed between the simulations to meet the same ATP consumption demand.

Comparing the results from the simulation set CC and DC in Fig. 3C, bottom left, we found that decreasing $v_{\text{max}}$ elevated the ADP-to-ATP ratio in the myofibril region. However, when the depressed $v_{\text{max}}$ was coupled with increased value of γ in scenario DD, these ratios decreased by 7% relative to the DC scenario (58% relative to CC). These results indicate that increased mitochondrial cluster surface area can play a compensatory role by increasing the ATP levels in the myofibril region.

Increased mitochondrial cluster outer membrane surface area can reduce mitochondrial oxidative phosphorylation efficiency. Further examination of mitochondrial matrix steady-state ADP-to-ATP metabolite levels under the three scenarios revealed that increasing γ has the inverse response on metabolite levels inside the mitochondrial matrix (Fig. 3C, bottom right). At the highest value of γ, the ADP-to-ATP ratio increased above the effects of depressing the $v_{\text{max}}$ alone (see DC vs. DD). Since in all the simulations the same ATP synthesis rate was maintained, an increased ADP-to-ATP ratio points toward depressed mitochondrial efficiency. Analysis of other compartments of the model also showed that enhanced metabolite transport across the increased outer surface area facilitates these alterations by changing the steady-state equilibrium of metabolites between mitochondria and the myofibrils.

**DISCUSSION**

We have measured and compared transverse-section-wide changes in cardiomyocyte mitochondrial organization and morphology in diabetes and control rats. Diabetic cells had increased mitochondrial density that was complemented by a decrease in average size of individual mitochondria in diabetes. Such alterations are consistent with mitochondrial fission in diabetic cardiomyocytes (22, 39). Although alterations in mitochondrial spatial organization may not result solely from fission processes, our results show that fragmented mitochondria have a greater tendency to form into contiguous clusters, extending further across the transverse section of the diabetic cardiomyocyte. The absence of differences in the cell-wide columnar arrangement of the mitochondria suggests that fission may occur within the existing columns of mitochondria, but there is no evidence that new connections between mitochondrial columns have formed.

An experimentally validated biophysical model provided novel insights into how increased clustering of mitochondria, and therefore increased mitochondrial-cluster surface area, could feedback into energy metabolism. Our analysis showed that increased mitochondrial cluster outer membrane surface area may slightly reduce (7%) the myofibrilar ADP-to-ATP ratio. Minor changes to availability of ATP or ADP can have significant implications on overall functioning of the cell (24). For instance, reduced myofibril ADP and ADP-to-ATP ratio can lead to improved performance of sarcoplasmic reticulum Ca$^{2+}$-ATPase pumps (35).

These alterations are facilitated by increased metabolite transport between mitochondria and myofibrils due to the increased outer membrane surface area. The increased outer membrane surface area also caused an increase in the ADP-to-ATP ratio in the mitochondrial matrix, required to maintain the same rate of $\varphi_{\text{synthesis}}$. This is indicative of reduced mitochondrial efficiency, consistent with previous experimental studies (40, 41).

Other ultrastructural changes that have not been examined in this study could also affect energy metabolism. For example, reduction in the number of cristae folds could play an important role in regulating metabolism (13, 23). Not all mitochondria within a cluster may be functioning to their fullest capacity. Fusion and fission processes are needed not only for enhancement of metabolism but also removal and repair of damaged mitochondria (9). A spatial model of mitochondrial biogenesis and metabolism would enable us to probe questions of spatio-temporal regulation of mitochondria. This study has shown for the first time through a mixed-compartment model...
that a structural change (that can be incorporated in the model) can impact on energy metabolism.

Future work will need to quantify the effect of other alterations observed in the diabetic cardiomyocyte. With these additional data, the exact nature of the interactions between the changes in biochemical/metabolic pathways and cell ultrastructure will be better understood in the diabetic heart. A more comprehensive knowledge of these molecular processes would increase the efficacy of drug discovery strategies.
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Disclosures

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

Author Contributions


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