CALL FOR PAPERS | Mitochondria in Cardiovascular Physiology and Disease

Pressure overload differentially affects respiratory capacity in interfibrillar and subsarcolemmal mitochondria

Michael Schwarzer, Andrea Schrepper, Paulo A. Amorim, Moritz Osterholt, and Torsten Doenst
Department of Cardiothoracic Surgery, Jena University Hospital, Friedrich Schiller University of Jena, Jena, Germany
Submitted 18 September 2012; accepted in final form 10 December 2012

Schwarzer M, Schrepper A, Amorim P, Osterholt M, Doenst T. Pressure overload differentially affects respiratory capacity in interfibrillar and subsarcolemmal mitochondria. Am J Physiol Heart Circ Physiol 304: H529–H537, 2013. First published December 15, 2012; doi:10.1152/ajpheart.00699.2012.—Years ago a debate arose as to whether two functionally different mitochondrial subpopulations, subsarcolemmal mitochondria (SSM) and interfibrillar mitochondria (IFM), exist in heart muscle. Nowadays potential differences are often ignored. Presumably, SSM are providing ATP for basic cell function, whereas IFM provide energy for the contractile apparatus. We speculated that two distinguishable subpopulations exist that are differentially affected by pressure overload. Male Sprague-Dawley rats were subjected to transverse aortic constriction for 20 wk or sham operation. Contractile function was assessed by echocardiography. Heart tissue was analyzed by electron microscopy. Mitochondria were isolated by differential centrifugation, and respiratory capacity was analyzed using a Clark electrode. Pressure overload induced left ventricular hypertrophy with increased posterior wall diameter and impaired contractile function. Mitochondrial state 3 respiration in control was 50% higher in IFM than in SSM. Pressure overload significantly impaired respiratory rates in both IFM and SSM, but in SSM to a lower extent. As a result, there were no differences between SSM and IFM after 20 wk of pressure overload. Pressure overload reduced total citrate synthase activity, suggesting reduced total mitochondrial content. Electron microscopy revealed normal morphology of mitochondria but reduced total mitochondrial volume density. In conclusion, IFM showed higher respiratory capacity in the healthy rat heart and a greater depression of respiratory capacity by pressure overload than SSM. The differences in respiratory capacity of cardiac IFM and SSM in healthy hearts are eliminated with pressure overload-induced heart failure. The strong effect of pressure overload on IFM together with the simultaneous appearance of mitochondrial and contractile dysfunction may support the notion of IFM primarily producing ATP for contractile function.

METHODS

Animals. Male Sprague-Dawley rats (40–50 g) were obtained from Charles River (Sulzfeld, Germany) and were fed ad libitum at 21°C with a light cycle of 12 h. The use of animals was consistent with the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996), and the experimental protocols were approved by the local Animal Welfare Committee of the University of Leipzig and Jena, Germany (AZ: 24-9168.11TVV36/06; 22-2684-04-02-056/10).

Materials. Chemicals were obtained from Sigma-Aldrich (Deisenhofen, Germany), Merck (Darmstadt, Germany), Serva (Heidelberg, Germany), Bayer (Leverkusen, Germany), and Narkodorm-n (Neumünster, Germany).

Surgical interventions. The model of heart failure (42) has been described in detail before. Animals of 40–50 g were anesthetized with intraperitoneal ketamine (50 mg/kg) and xylazine (10 mg/kg), intubated with 16-gauge tubing, and ventilated with room air (1 ml/100 g, 96/min). A partial median sternotomy and thymectomy were performed. After dissection of the aortic arch, a tantalum clip (0.35 mm internal diameter; Pilling-Weck, Kernen, Germany) was placed on the aorta between the brachiocephalic trunk and the left common carotid artery. The sternotomy was closed with interrupted sutures and the skin closed with running sutures. After vital signs were reestablished, rats were extubated and kept on warming pads for the recovery
Table 1. HW, BW, and lung weight as well as TL of animals after 20 wk of PO compared with control

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>20 wk of PO</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>BW, g</td>
<td>426 ± 3</td>
<td>426 ± 3</td>
</tr>
<tr>
<td>HW, g wet wt</td>
<td>1.22 ± 0.03</td>
<td>2.53 ± 0.09***</td>
</tr>
<tr>
<td>HW/BW, mg/g</td>
<td>2.84 ± 0.07</td>
<td>5.97 ± 0.09***</td>
</tr>
<tr>
<td>TL, mm</td>
<td>40.5 ± 0.3</td>
<td>41.2 ± 0.4</td>
</tr>
<tr>
<td>HW/TL, mg/mm</td>
<td>31.0 ± 1.0</td>
<td>61.6 ± 2.3***</td>
</tr>
<tr>
<td>Lung weight, g</td>
<td>1.69 ± 0.11</td>
<td>4.49 ± 0.35***</td>
</tr>
<tr>
<td>LBI, g/kg</td>
<td>3.88 ± 0.25</td>
<td>11.05 ± 0.94***</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>14.9 ± 0.8</td>
<td>14.8 ± 0.6</td>
</tr>
<tr>
<td>LiverBI, g/kg</td>
<td>33.7 ± 1.0</td>
<td>36.4 ± 1.6</td>
</tr>
</tbody>
</table>

Data are means ± SE. HW, heart weight; BW, body weight; TL, tibia length; LBI, lung weight-to-BW index; LiverBI, Liver weight-to-BW index. *P < 0.05, **P < 0.01, and ***P < 0.001 compared with matched controls after 20 wk of pressure overload (PO) by aortic constriction.

Complex III activity was determined as antimycin-A-sensitive reduction of cytochrome c (27), using decylubiquinol as substrate, which was prepared as previously described (23). Cytochrome c oxidase (complex IV) was measured as the oxidation of reduced cytochrome c according to the method of Wharton and Tzagaloff (50).

Mitochondrial oxidative phosphorylation. Oxygen consumption of isolated mitochondria was measured using a Clark-type oxygen electrode (Stratham Kelvin) at 25°C (31, 47) as linearity of respiratory measurements has been described between 22 and 41°C (41). Mitochondria were incubated in a solution consisting of 80 mM KCl, 50 mM MOPS, 1 mM EGTA, 5 mM KH2PO4, and 1 mg/ml fatty acid-free bovine serum albumin at pH 7.4. The rate of oxidative phosphorylation was measured using glutamate, pyruvate and malate, palmitoyl-carnitine and malate, or succinate and rotenone as substrates and ADP as stimulus. The ADP-stimulated oxygen consumption (state 3) and the ADP-limited oxygen consumption (state 4) in the respiratory chamber and the ADP-to-O ratio (ADP added per oxygen consumed) were determined as previously described (7, 13).

Electron microscopy. Left ventricular samples were taken from freshly excised hearts, washed, fixed in 2.5% glutaraldehyde/1% paraformaldehyde, postfixed in 2% osmium tetroxide, embedded in resin, and sectioned. Mitochondrial volume density and number were quantified by stereology in a blinded fashion using the point counting method (6), which determines volume occupied by mitochondria in the tissue assessed.

Mitochondrial size and internal complexity. To index mitochondrial subpopulation size and complexity, we performed flow cytometry analyses using a fluorescence-activated cell sorting (FACS) Calibur (Becton Dickinson, Heidelberg, Germany) as previously described (10). Individual parameters (size and complexity) were assessed using specific light sources (laser and photomultiplier tube) and specific detectors. Before the initial assessments, gating parameters were established to exclude debris as follows. Freshly isolated, healthy, functional mitochondria were divided into several fractions. Fractions were 1) kept on ice, 2) repeatedly frozen and thawed in liquid nitrogen, or 3) subjected to detergent treatment. The fractions were analyzed in the flow cytometer, and settings were chosen to exclude damaged mitochondria (freeze/thaw or detergent treated). Thus, with the use of these settings, only healthy mitochondria were analyzed. The evaluated settings were kept for all investigations. Once the gating parameters were established, functional freshly isolated mitochondrial tree diluted in buffer and analyzed <2 h after isolation. Gated events (100,000 events/sample) were subsequently examined using the forward scatter detector (FSC; 488-nm argon laser and diode detector) and sideward scatter detector (SSC; photomultiplier tube and 90° collection lens) and represented in FSC versus SSC density plots. The geometric mean (in arbitrary units), representing FSC (linear scale), was used as an indicator of size, whereas values from SSC (logarithmic scale) were used to indicate complexity in the subpopulations. Although FSC is proportional to the individual mitochondria particle size, the absolute value remains an arbitrary unit.

Table 2. Echocardiographic parameters of hearts subjected to PO

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>20 wk of PO</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>LVPWD, mm</td>
<td>1.98 ± 0.24</td>
<td>2.71 ± 0.19*</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>7.98 ± 0.27</td>
<td>9.44 ± 0.46*</td>
</tr>
<tr>
<td>EF, %</td>
<td>68.2 ± 2.0</td>
<td>47.8 ± 1.6***</td>
</tr>
<tr>
<td>FS, %</td>
<td>39.8 ± 1.6</td>
<td>25.4 ± 1.0***</td>
</tr>
</tbody>
</table>

Data are means ± SE. LVPWD, left ventricular posterior wall thickness in diastole; LVEDD, left ventricular end-diastolic dimension; EF, ejection fraction; FS, fractional shortening. *P < 0.05 and ***P < 0.001 compared with sham-operated animals after 20 wk of PO by aortic constriction.

H530 DIFFERENCES BETWEEN IFM AND SSM IN PO HF

**AJP-Heart Circ Physiol** • doi:10.1152/ajpheart.00699.2012 • www.ajpheart.org
Statistical analysis. Data are presented as means ± SE. Data were analyzed using two-way ANOVA or a Student’s t-test where appropriate. Post hoc comparisons among the groups were performed using the Bonferroni method. Differences among groups were considered statistically significant if $P < 0.05$, and main effects for population and surgery were given in figures and tables.

**RESULTS**

Table 1 shows animal weights and organ weights after 20 wk of pressure overload. Heart weight increased as did heart weight-to-body weight and heart weight-to-tibia length ratios, both signaling cardiac hypertrophy. Increased lung weight-to-body weight

Fig. 1. Maximal mitochondrial respiration (state 3, ADP stimulated) in sham-operated animals (white bars) and heart failure (black bars) of isolated mitochondria [interfibrillar mitochondria (IFM) and subsarcolemmal mitochondria (SSM)] with pyruvate and malate (A), palmitoyl-carnitine and malate (B), glutamate (C), or succinate and rotenone (D) as substrates is shown. 2,4-Dinitrophenol-uncoupled mitochondrial respiration with glutamate (E) and succinate/rotenone (F) is shown. Data are means ± SE; $n = 9$ to 10. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ for treatment (T), population (P), and interaction (I) or compared with respective sham-operated group; ns, nonsignificant; †$P < 0.05$ compared with IFM group of same treatment.
Fig. 3. Citrate synthase activity per heart weight (A) and mitochondrial yield (B) of sham-operated animals (white bars) and heart failure animals (black bars) is shown. Data are means ± SE; n = 9 to 10. **P < 0.01 compared with sham-operated group. There are no significant differences in IFM and SSM yield regarding T, P, and I.

Index indicated impairment in left ventricular contractile function. There was no difference in liver weight-to-body weight ratio, suggesting no significant impairment in right ventricular function.

Table 2 shows echocardiographic parameters. After 20 wk of pressure overload, hearts were hypertrophied, dilated, and showed reduced systolic function as illustrated by increased left ventricular posterior wall thickness in diastole and left ventricular end-diastolic dimension and decreased ejection fraction and fractional shortening, indicating heart failure.

Figure 1 shows higher maximal ADP-stimulated mitochondrial respiration for IFM than subsarcolemmal SSM with different complex I substrates (pyruvate, Fig. 1A; palmitoyl-carnitine, Fig. 1B; and glutamate, Fig. 1C). Mitochondrial respiration (state 3) with the complex II substrate succinate also showed differences between IFM and SSM (Fig. 1D). With contractile dysfunction, IFM’s respiratory capacity was significantly impaired with complex I substrates. SSM’s respiratory capacity was also significantly impaired, but the reduction was less pronounced compared with that of IFM (reduction IFM vs. SSM glutamate, 70 vs. 57%; pyruvate/malate, 72 vs. 55%; and palmitoyl-carnitine/malate, 59 vs. 40%). This impairment eliminated the differences in respiratory capacity found between IFM and SSM in control animals. Complex II substrate-dependent mitochondrial respiration remained unchanged even in pressure overload-induced contractile dysfunction. We further assessed uncoupled (2,4-dinitrophenol) respiration with glutamate and with succinate/rotenone as substrates. The results were similar as with state 3 respiration, indicating that the observed changes seem unrelated to the phosphorylation system or ADP-to-ATP transport processes.

Figure 2 shows ADP-to-O ratios and state 4 (ADP limited) respiration of isolated mitochondria. ADP-to-O ratios were not different between IFM and SSM and did not change in heart failure, indicating normal coupling of ATP production to O2 consumption. In pressure overload, state 4 respiration was reduced with pyruvate malate in IFM only and with glutamate in IFM and SSM. There was no difference in state 4 respiration between population with pyruvate, palmitoyl-carnitine, or succinate as substrates. These changes were minor compared with the effect observed with state 3 respiration.
Table 3. Citrate synthase activities of isolated mitochondrial subpopulations of hearts subjected to PO

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>20 wk of PO</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSM</td>
<td>17.8 ± 3.5</td>
<td>14.6 ± 1.2</td>
</tr>
<tr>
<td>IFM</td>
<td>29.0 ± 4.6†‡</td>
<td>17.7 ± 0.9**</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 4–6. IFM, interfibrillar mitochondria. P values for treatment, 0.010; population, 0.011; and interaction, 0.117. †P < 0.01 compared with control; ‡P < 0.05 compared with subsarcolemmal mitochondria (SSM).

Figure 3 shows citrate synthase activity related to heart weight. Total citrate synthase activity in heart homogenate as a marker for mitochondrial activity was significantly reduced in heart failure (Fig. 3A). Mitochondrial recovery was not different for isolation of IFM, SSM, or total mitochondria (Fig. 3B). Table 3 shows citrate synthase activities of the two subpopulations following isolation. In pressure overload heart failure, IFM present with reduced citrate synthase activity. This might indicate a reduced mitochondrial activity in this population.

Table 4 shows activities of isolated complexes of the electron transport chain in isolated mitochondrial populations. Activity of complex I was reduced in pressure overload heart failure in both SSM and IFM comparably (SSM, −29%; and IFM, −23%). Activities of other complexes (II–IV) were not different with pressure overload.

Figure 4 shows mitochondrial number and volume density in heart tissue as assessed by electron microscopy. Mitochondrial number remained unchanged in heart failure, whereas mitochondrial volume density, representing the fraction of tissue occupied by mitochondria, was reduced.

Figure 5 shows the results of FACS analysis of isolated mitochondria. FSC measures mitochondrial size, and SSC measures sideward reflection by mitochondria that is caused by the internal structure and describes mitochondrial complexity. SSM had greater size and higher complexity compared with IFM. Whereas IFM and SSM tended to show a reduction in size in heart failure, mitochondrial internal complexity remained unaffected.

DISCUSSION

We demonstrate in this study that differences in SSM and IFM in healthy hearts are differentially affected in pressure overload, with IFM showing a greater depression in respiratory capacity than SSM when subjected to pressure overload. The correlation of the strong effect of pressure overload on IFM, together with the simultaneous appearance of mitochondrial and contractile dysfunction, may indicate support for the hypothesis of IFM primarily producing ATP for contractile function.

Our current analysis revives an old but still relevant question with new methodology. In our study, we found higher respiratory capacity in IFM than in SSM, whereas ADP-to-O ratios and ADP-limited (state 4) respiration were comparable in both populations. Thus our findings are consistent with previous results (20, 34). Additionally, we describe for the first time that cardiac IFM are smaller in size than SSM in rats. Complexity measured by FACS indicated reduced internal structural complexity in IFM compared with SSM. This reflects the differences in internal structure described with electron microscopy (37) and is consistent with previous results in mice (10). Differential effects on respiratory capacity of cardiac mitochondrial subpopulations have been described in diabetes (9, 10, 36), with ischemia-reperfusion (21), in aging (14), in cardiomyopathic hamsters (18), with fasting (32) calcium stress (16), and in volume overload (48). Whereas type 2 diabetes, calcium stress, fasting, and volume overload in the heart seem to affect only respiratory capacity of SSM (9, 16, 32, 36, 48) and type I diabetes and cardiomyopathy only IFM (10, 18), ischemia and aging affect both populations differentially with a stronger influence of ischemia on SSM (21) and greater differences observed in IFM with aging (14). Other investigations found protein expression changes in exercise in SSM (22) or cardiolipin depletion in ischemic SSM (28). These results do not allow predicting what changes may be expected in other disease models. In our rat model of pressure overload, the two mitochondrial populations were found to be different, with IFM being smaller than SSM, having lower internal complexity but displaying higher maximal respiratory rates. In addition, pressure overload differentially affected respiratory capacity of the two subpopulations with IFMs respiratory capacity suffering more under pressure overload than SSMs. The result is similar respiratory capacity under conditions of chronic pressure overload.

This information is important because it has strong potential implications. As mentioned in the introduction, differences between SSM and IFM are often not addressed in recent publications. However, neglecting these differences may affect the interpretation of the results. For example, Picard et al. (35) investigated mitochondrial function in skeletal muscle and compared respiratory rates of isolated mitochondria and skinned fibers in young and aged rats. These authors presumably isolated a mixed population of SSM and IFM. They describe reduced mitochondrial respiratory capacity in isolated mitochondria but not in permeabilized fibers and conclude that functional impairment is exaggerated in isolated mitochondria. However, this work omitted any consideration that respiration in permeabilized fibers is limited by diffusion with intracellular structures.

Table 4. Activities of isolated complexes of the respiratory chain from cardiac mitochondria of hearts subjected to PO

<table>
<thead>
<tr>
<th>Complex</th>
<th>Control</th>
<th>20 wk of PO</th>
<th>Control</th>
<th>20 wk of PO</th>
<th>P&lt;sub&gt;mean&lt;/sub&gt;</th>
<th>P&lt;sub&gt;pop&lt;/sub&gt;</th>
<th>P&lt;sub&gt;inter&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex I, nmol·min&lt;sup&gt;−1&lt;/sup&gt;·mg protein&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>976 ± 54</td>
<td>751 ± 47*</td>
<td>1114 ± 90</td>
<td>785 ± 46***</td>
<td>&lt;0.001</td>
<td>0.156</td>
<td>0.384</td>
</tr>
<tr>
<td>Complex II, nmol·min&lt;sup&gt;−1&lt;/sup&gt;·mg protein&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>49.8 ± 7.3</td>
<td>49.0 ± 4.2</td>
<td>53.4 ± 7.6</td>
<td>54.6 ± 4.3</td>
<td>0.971</td>
<td>0.416</td>
<td>0.865</td>
</tr>
<tr>
<td>Complex III, nmol·min&lt;sup&gt;−1&lt;/sup&gt;·mg protein&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>8.94 ± 0.49</td>
<td>8.33 ± 0.68</td>
<td>9.65 ± 1.29</td>
<td>7.35 ± 1.13</td>
<td>0.164</td>
<td>0.899</td>
<td>0.411</td>
</tr>
<tr>
<td>Complex IV, nmol·min&lt;sup&gt;−1&lt;/sup&gt;·mg protein&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>16.33 ± 1.91</td>
<td>14.54 ± 0.78</td>
<td>17.96 ± 1.25</td>
<td>13.76 ± 1.39*</td>
<td>0.043</td>
<td>0.746</td>
<td>0.400</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 5–10. Two-way ANOVA was performed to assess for effects of treatment (P<sub>mean</sub>), population (P<sub>pop</sub>), and interaction (P<sub>inter</sub>). *P < 0.05 and ***P < 0.001 compared with sham-operated animals after 20 wk of pressure overload by aortic constriction.
Fig. 4. Electron microscopy pictures of control animals (A) and in heart failure (B) are shown. Mitochondrial number (C) and volume density (D) as determined with electron microscopy of sham-operated animals (white bars) and in heart failure (black bars) are shown. PO, pressure overload. Data are means ± SE; n = 3. **P < 0.01.

Fig. 5. Mitochondrial size and complexity determined with fluorescence-activated cell sorting of sham-operated animals (white bars) and in heart failure (black bars) are shown. Data are means ± SE; n = 5 to 6. ***P < 0.01 for T, P, and I or compared with respective sham-operated group; †P < 0.05, ††P < 0.01, and †††P < 0.001 compared with IFM group of same treatment.
imposing significant diffusion obstacles (19) and that SSM are the preferentially assessed population. Thus, in the light of our findings, one may alternatively conclude from their results that respiratory capacity of SSM (main population assessed in permeabilized fibers) is not impaired but that IFM properties (majority of mitochondria and increased accessibility in isolated mitochondria) are changed. Based on this interpretation, the study is fully consistent with ours.

In rat heart, Garnier et al. (15) describe a slight reduction in respiratory capacity in heart failure for SSM (skinned fibers). Interpreting these findings with the eye of our results may suggest that the described slight reduction may have been due to a few impaired IFM reached by the skinned fiber technique and that mainly SSM assessed by this technique have been normal or only slightly affected. Thus the skinned fiber technique may not be the best methodology to characterize mitochondrial function under conditions when IFM and SSM are affected differentially.

One concern assessing mitochondria isolated through differential centrifugation has been mechanical damage induced by isolation procedures. The mechanical shear forces during the isolation procedure have been shown to disrupt SSM and IFM and to influence mitochondrial properties and viability. We demonstrated that differential centrifugation results in the selection of structurally intact mitochondria (8). While this selection is a shortcoming of the differential centrifugation procedure, the mitochondrial yield in our subpopulations was comparable, allowing a conclusion that the subpopulations were similarly affected by this selection. In any case, the results found would thereby only underestimate mitochondrial damage.

The reduction in citrate synthase activity in heart homogenates in our study suggests reduced mitochondrial mass. Reduction of mitochondrial mass may essentially be explained by reduced mitochondrial number or reduction in mitochondrial size. However, electron microscopic analysis revealed reduced mitochondrial volume density but not mitochondrial number. These results suggest that possibly reduced mitochondrial size may be causing the loss in citrate synthase activity.

Rosca et al. (38) found in a dog model of dilative cardiomyopathy similar respiratory capacities in SSM and IFM and with pacing induced heart failure reduced mitochondrial capacities of both IFM and SSM comparably (38). However, as animal model, heart failure model, and severity differ from our pressure overload rat model, it is not surprising that the findings are different. One may suggest that the mechanisms of mitochondrial respiratory impairment may be different.

To explain the differences in mitochondrial subpopulations, two models have been suggested. It has been suggested that SSM support membrane-related processes (17) and/or basic cell function and protein synthesis (2) and IFM support muscle contraction (36), relating the observed differences between both to the difference in function. Others have suggested a reticulum (1, 24) with a continuum of oxidative capacities with the less oxidative SSM at the exterior of a cell to establish a gradient for oxygen and substrate transport into the IFM region (4). Both models are supported by experimental evidence. Regarding our model of heart failure, oxidative capacities of both IFM and SSM are impaired, but IFM’s capacity is impaired to a higher extent. This results in a loss of the differences in respiratory capacities of IFM and SSM. In a setting of a suggested continuum of oxidative capacities, such continuum would get lost in heart failure. Irrespective of any continuum, which experimentally cannot be measured with the current techniques, it is striking to note that the decrease in state 3 respiration mainly observed in IFM was associated with the onset of systolic dysfunction. We previously demonstrated that mitochondrial function at earlier time points when systolic function was still normal (10 wk of pressure overload), remained normal, or was even elevated (2 wk of pressure overload) (12). These findings support the commonly accepted notion that failure to produce ATP results in contractile dysfunction. The impairment in mitochondrial function was much more pronounced in IFM and correlates well with impaired contractile function. This may be regarded as an indication supporting the notion that IFM are producing ATP primarily for contractile function.

In conclusion, we demonstrate in this study that differences in SSM and IFM in healthy hearts are differentially affected in pressure overload, with IFM showing a greater depression in respiratory capacity than SSM when subjected to pressure overload. The correlation of the strong effect of pressure overload on IFM together with the simultaneous appearance of mitochondrial and contractile dysfunction may indicate support for the hypothesis of IFM primarily producing ATP for contractile function.

ACKNOWLEDGMENTS
T. Doenst was Heisenberg professor of the Deutsche Forschungsgemeinschaft (German Research Foundation) at the University of Leipzig.

GRANTS
This study was supported by grants of the Deutsche Forschungsgemeinschaft to T. Doenst (DO 6024/1-6, 1-8).

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

AUTHOR CONTRIBUTIONS
M.S. conception and design of research; M.S., A.S., P.A.A., and M.O. performed experiments; M.S., A.S., P.A.A., and M.O. analyzed data; M.S., A.S., P.A.A., and T.D. interpreted results of experiments; M.S. prepared figures; M.S. drafted manuscript; M.S. and T.D. edited and revised manuscript; A.S., and T.D. interpreted results of experiments; M.S. prepared figures; M.S., A.S., P.A.A., and T.D. approved final version of manuscript.

REFERENCES


