Respiratory chain complexes in healthy and cardiomyopathic hearts

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Abstract

Dysfunction of mitochondrial oxidative phosphorylation (OXPHOS) in heart muscle is postulated to play a major role in heart failure development. Abnormalities in ATP production and mitochondrial ATP synthase (complex V) activity, increased respiratory stress (ROS) with free radicals overproduction as well as apoptotic mechanism stimulation were found in humans and animal models with non-mitochondrial cardiomyopathies. Different secondary changes of respiratory chain complexes I, II, III and IV were reported in heart failure but the way of their pathological or compensatory influence not established. Also frequency of the primary mitochondrial cardiomyopathies is not known, especially in adults.

The aim of the study was to assess the respiratory chain activity in 28 cardiomyopathic patients qualified for heart transplantation due to end-stage heart failure and 7 patients with hypertrophic cardiomyopathy undergone myomectomy. Own laboratory reference values were prepared using 9 healthy donors hearts.

The whole study group demonstrated a number of significant changes in respiratory chain activity. There were decrease in citric synthase activity and complex III activity, and increase in complex II activity. Activities of complex I and IV did not differ significantly from the reference values. In five out of 36 patients the results might indicate a probability of mitochondrial disease (complex I or complex IV deficit).

The further proteomic investigations did not reveal any primary pathological changes.

Conclusion. 1/ The study confirms the general presence of OXPHOS dysfunction in hearts of the patients with end-stage heart failure and severe cardiomyopathies. New research techniques should be applied to uncover its mechanism and role. 2/ The primary mitochondrial cytopathy should be carefully excluded in the patient with cardiomyopathy of unknown etiology.

Key words: heart, respiratory chain complexes, spectrophotometry, healthy, cardiomyopathy, heart transplantation, myomectomy, mitochondrial disease

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**Introduction**

Possible contribution of mitochondria in the development of various types of cardiomyopathies (CMPs) seems almost certain due to mitochondrial role in energy production, free radicals generation and apoptosis [1, 3, 8]. The significance of mitochondrial dysfunction in different types of CMP is still not fully recognized and elucidated [22, 27, 32, 34, 36]. Empirical data (usually individual case reports) indicate that CMP both in adults and children may be associated with pathogenic mutation of genes located in mitochondrial DNA (mtDNA) [19, 26, 30, 35] as well as primary deficit of respiratory chain complex I, III, IV, or V [9, 11, 29, 33]. Heart involvement may be part of multiorgan clinical dysfunction (MELAS, MERRF) or appear as an isolated finding. Mitochondrial cardiomyopathy as a salient clinical feature may be observed for example in mutations of mtDNA gene coding for leucine1 tRNA at the position 3302-3303 [4, 31]; in complex IV deficit associated with SCO2 gene mutation [14]; in Barth syndrome [37] and in several other mitochondrial disorders [6, 21]. Recognition of these primary cardiomyopathies may be accidental and their prevalence particularly in adults is unknown. On the other hand the role of mitochondrial oxidative phosphorylation (OXPHOS) dysfunction in non-mitochondrial CMPs has been suspected for a long time and extensively investigated [5, 10, 12].

The results frequently appear controversial and still must be considered inconclusive.

Heart muscle mitochondria may be studied using well established techniques applied to skeletal muscle investigation. Spectrophotometric assessment of respiratory chain complexes activity in tissue sample homogenate and histochemical assessment of oxidative enzymes including cytochrome c oxidase are among most widely used techniques. However the reliable data concerning the range of normal and pathological values have not been available for a long time. Before heart transplantation era OXPHOS assessment was possible only in small endomyocardial biopsy samples limiting the range of investigation methods. Development of heart transplantation changed the situation since explanted hearts as well as hearts of potential donors became available for investigation. Yet, in medical literature there are still scarce data concerning normal OXPHOS measurements [5, 20], its practical usefulness is anyway limited by the necessity of establishing own norms in each mitochondrial laboratory.

**Material**

The study group consisted of 28 heart explants and 7 myomectomized heart tissue.

End-stage heart failure being a basis of qualification for transplantation was the main inclusion criterion in the heart explant subgroup. All the patients suffered from cardiomyopathy (dilated – DCM, idiopathic or ischemic), they were in NYHA III/IV class and had EF < 40. The age of the patients ranged from 14 to 65, average 52 years. Only one child was included. There were 4 females and 24 males. In the heart explant subgroup the material was collected in the period of 2001–2005. The maximum storage period was 6 years.

The myomectomy study subgroup consisted of the heart tissues excised during surgical treatment from the left ventricle in 7 patients, two children and 5 adults (2 females, 4 males). All patients demonstrated severe hypertrophic cardiomyopathy (HCM) which fulfilled a standard clinical indications for myomectomy. These heart samples were obtained in the period of 2005–2007. Biochemical analysis was performed within 24 months from the surgery.

Hearts obtained from 10 healthy, adults organ donors not suitable for transplantation served as the reference group. Only donor patients with maintained ventilatory and hemodynamic requirements were included. From each heart three samples were obtained (left ventricle, right ventricle, intraventricular septum) and immediately snap frozen into liquid nitrogen until biochemical analysis.

The study protocol was approved by the Bioethical Committee of Institute of Cardiology in Warsaw.

**Methods**

**Histological, histochemical and immunohistochemical studies**

Histological, histochemical and immunohistochemical methods applied in the study were performed as described earlier for frozen skeletal muscle [23]. Stains and histochemical reactions included: hematoxylin and eosin, modified Gomori trichrome, oil red O, cytochrome c oxidase, succinate dehydrogenase, NADH dehydrogenase and acid phosphatase.

Immunohistochemistry was performed with antibodies against CD3, CD4, CD8, CD68, ICAM, CD15, CD45 and desmin.

**Spectrophotometric studies**

Spectrophotometric assays was performed for the measurement of complex I (NADH: ubiquinone oxidoreductase, rotenone sensitive), complex II (succinate: ubiquinone oxidoreductase, malonate sensitive), complex III (ubiquinone: cytochrome c oxidoreductase, antimycin sensitive), complex IV (cytochrome c oxidase-COX), and citrate synthase enzyme activities in left ventricle, right ventricle and interventricular septum heart homogenates as described previously for skeletal muscle [16, 17] with modification in volume of homogenization buffer to reach cardiac muscle aliquots protein in concentration in the range of 2–4 mg/ml. Protein was determined by the Lowry method. The ratio between the activity of individual respiratory chain complex and citric synthase (CS) was calculated to eliminate the possible effect of changes in number of mitochondria in patient cells.

**Western blot analysis**

Proteomic study was adopted from the methods described earlier for the skeletal muscle biopsy [18].

Protein concentrations of each sample was determined with a Bio-Rad Protein Assay. Western blotting was carried out as previously described [24]. Samples were loaded
onto SDS-PAGE gels and after electrophoresis transferred to PVDF. After blocking, blots were probed with anti-OXPHOS antibodies (1:1000), anti-complex I subunits: anti-NDUFS 3 antibody (1:1000), anti-NDUFA 6 antibody (1:700), anti-GRIM 19 antibody (1:500), and anti-COX I antibody (1:1000), anti-COX IV antibody (1:500) and anti-COX Va antibody (1:500) (MitoSciences). The signal was revealed using alkaline phosphatase (AP) Conjugate Substrate Kit (Bio-Rad) after hybridization with goat anti-mouse IgG (H+L) (Bio-Rad) AP conjugated secondary antibody.

Blue Native Gel Electrophoresis and in gel activity assay of the mitochondrial fraction

Human heart biopsies were washed twice with PBS, resuspended in the buffer containing 250 mM sucrose, 1 mM EGTA, 50 mM Tris-HCl, 1 mM DTT, and protease inhibitor cocktail at pH 7.4 and gently disrupted by 15 up-and-down strokes in tight glass-glass homogenizer. The homogenate was centrifuged at 600 g for 5 min twice. The resulting supernatants were centrifuged at 10,000 g at 10 min. The pellets containing mitochondria were washed with homogenization buffer and centrifuged again at 10,000 g for 10 min. Mitochondrial pellets were solubilized with 1.5 M aminocaproic acid, 50 mM Bis-Tris at pH 7.0 and 1% dodecylmaltoside. Samples were incubated on ice for 20 min and then centrifuged 20,000 g for 15 min to remove unsolubilized material. Protein concentration in the supernatants was determined by Bradford’s method using Bio-Rad Protein Assay. Supernatants containing protein complexes were combined with 5% w/v suspension of Coomassie brilliant blue G-250 in the 1.5 M aminocaproic acid, 50 mM Bis-Tris at pH 7.0 buffer (adding 0.5 ml of Coomassie suspension to each 10 ml of supernatant). To carry out Blue Native Electrophoresis (BN PAGE) samples were separated on a big dimensional (1 mm/16 cm/20 cm) 5%–12% gradient acrylamide gel. Mitochondria isolated from rat heart were used as an internal standard to see the quality of separation and to calibrate the BN PAGE gel (in kDa). To visualize activity of individual respiratory chain complexes the gel have to be incubated at room temperature with the suitable solutions as described [18].

Data analysis

All data are shown as mean ± SD of the mean. The Student’s t-test was used for comparison differences between control and patients groups. A p value < 0.05 was considered significant.

All tests were performed using Statistica Software Version 8.0 (StatSoft Poland).

Results

Ranges of the normal reference values of complex I, II, III and IV of enzymatic respiratory chain activities in healthy human heart left ventricle, right ventricle and interventricular septum are shown in Table 1 using 95% confidence interval (95% CI).

Heart explant subgroup

Significant changes in the activity of some respiratory chain complexes were found in the study group of 28 explanted cardiomyopathic hearts (Table 2). Mean value of citric synthase activity was markedly decreased (p<0.01) being below the lowest control value in 12 out of 28 left ventricle specimens studied (Fig. 1). Protein content did not differ significantly between the reference and the study groups. Activity of complex I did not differ significantly between the control and the whole studied group (Table 2, Fig. 2). However two transplanted left ventricle tissues (patient 14 and 15) showed abnormally low complex I activity.

![Fig. 1 Activity of citric synthase in the muscle samples from left ventricle of explanted hearts of the patients with cardiomyopathy and cardiac failure. Dashed lines show mean value and range of laboratory reference values (8 left ventricle control specimens)
Activity of complex II was significantly higher in the failing explants group in the comparison with the healthy controls (Table 2). The difference was significant if activity values were expressed as percentage of citric synthase activity ($p<0.05$). Nineteen affected hearts showed the complex II activity value at the highest borderline or above the control (Fig. 3).

Complex III activity in the failure heart explants was generally decreased. Differences of the values expressed by protein content and control values were of high significance (Table 2, $p<0.001$). In 18 out of 28 affected hearts the complex III activity was below or at the lowest control level (Fig. 4). Values of the complex II+III were similarly decreased if expressed by protein content ($p<0.02$; Table 2).

### Table 1

Activities of respiratory chain complexes in healthy donors hearts (reference values)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N</th>
<th>Mean value</th>
<th>Standard deviation (SD)</th>
<th>Confidence interval (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>–95%</td>
</tr>
<tr>
<td><strong>Left heart ventricle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS nmol/min/mg prot</td>
<td>8</td>
<td>504.21</td>
<td>145.19</td>
<td>382.83</td>
</tr>
<tr>
<td>Complex I %CS /nmol/min/mg prot</td>
<td>8</td>
<td>10.65</td>
<td>5.91</td>
<td>5.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>/46.53/</td>
<td>/18.87/</td>
<td>/30.75/</td>
</tr>
<tr>
<td>Complex II %CS /nmol/min/mg prot</td>
<td>8</td>
<td>9.08</td>
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<td>6.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>/43.28/</td>
<td>/10.45/</td>
<td>/34.54/</td>
</tr>
<tr>
<td>Complex II+III %CS /nmol/min/mg prot</td>
<td>8</td>
<td>4.36</td>
<td>1.33</td>
<td>3.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>/20.93/</td>
<td>/6.65/</td>
<td>/15.37/</td>
</tr>
<tr>
<td>Complex III %CS /nmol/min/mg prot</td>
<td>8</td>
<td>52.46</td>
<td>9.30</td>
<td>44.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>/263.48/</td>
<td>/94.11/</td>
<td>/184.80/</td>
</tr>
<tr>
<td>Complex IV %CS /nmol/min/mg prot</td>
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<td>6.10</td>
<td>3.13</td>
<td>2.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td>/27.81/</td>
<td>/21.05/</td>
<td>/10.22/</td>
</tr>
<tr>
<td><strong>Right heart ventricle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS nmol/min/mg prot</td>
<td>9</td>
<td>472.0</td>
<td>75.53</td>
<td>413.96</td>
</tr>
<tr>
<td>Complex I %CS /nmol/min/mg prot</td>
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<tr>
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<td>/14.98/</td>
<td>/43.17/</td>
</tr>
<tr>
<td>Complex II %CS /nmol/min/mg prot</td>
<td>9</td>
<td>7.83</td>
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<tr>
<td></td>
<td></td>
<td>/36.61/</td>
<td>/11.59/</td>
<td>/27.70/</td>
</tr>
<tr>
<td>Complex II+III %CS /nmol/min/mg prot</td>
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<td>5.62</td>
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<td>3.37</td>
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<tr>
<td></td>
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<td>/26.21/</td>
<td>/16.16/</td>
<td>/13.79/</td>
</tr>
<tr>
<td>Complex III %CS /nmol/min/mg prot</td>
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<td>56.66</td>
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<td>40.02</td>
</tr>
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<td></td>
<td></td>
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<td>/96.89/</td>
<td>/185.96/</td>
</tr>
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<td>Complex IV %CS /nmol/min/mg prot</td>
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<td>11.2</td>
<td>2.27</td>
<td>9.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>/51.97/</td>
<td>/9.84/</td>
<td>/44.41/</td>
</tr>
<tr>
<td><strong>Interventricular septum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS nmol/min/mg prot</td>
<td>9</td>
<td>483.83</td>
<td>62.57</td>
<td>435.7</td>
</tr>
<tr>
<td>Complex I %CS /nmol/min/mg prot</td>
<td>9</td>
<td>8.88</td>
<td>3.06</td>
<td>6.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>/42.71/</td>
<td>/15.64/</td>
<td>/30.69/</td>
</tr>
<tr>
<td>Complex II %CS /nmol/min/mg prot</td>
<td>9</td>
<td>8.46</td>
<td>2.43</td>
<td>6.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>/40.91/</td>
<td>/13.44/</td>
<td>/30.58/</td>
</tr>
<tr>
<td>Complex II+III %CS /nmol/min/mg prot</td>
<td>9</td>
<td>5.09</td>
<td>1.22</td>
<td>4.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>/24.46/</td>
<td>/6.56/</td>
<td>/19.41/</td>
</tr>
<tr>
<td>Complex III %CS /nmol/min/mg prot</td>
<td>9</td>
<td>39.02</td>
<td>12.11</td>
<td>29.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>/195.71/</td>
<td>/44.33/</td>
<td>/161.64/</td>
</tr>
<tr>
<td>Complex IV %CS /nmol/min/mg prot</td>
<td>9</td>
<td>6.88</td>
<td>3.61</td>
<td>4.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>/33.23/</td>
<td>/17.95/</td>
<td>/19.44/</td>
</tr>
</tbody>
</table>
Mean value of complex IV activity was comparable in both reference and study groups (Table 2). Markedly decreased activity was found in three patients with heart failure (Fig. 5, patients 1, 2 and 5).

Spectrophotometric activity assay was also performed in 10 right ventricle specimens (patients 3, 4, 10, 12, 14, 21, 22, 24, 26), including cases with very low left ventricle complex I or IV activity. Normal activity was found in the second tissue sample for four out of 5 complex I/IV deficient left ventricle, excluding generalized respiratory chain deficit. Only one patient (patient 5) had complex IV decrease in both ventricle samples.

Average values of respiratory chain parameters for left and right ventricle did not differ remarkably and were respectively: for citric synthase $426.5 \pm 57.0$ and $381.8 \pm 60.7$ nmol/min/mg protein, for complex I $11.3 \pm 4.1\%$ and $11.8 \pm 29$.
2.9%, for complex II 7.5 ± 5.5% and 7.1 ± 5.6%, for complex III 42.4 ± 17.1% and 44.7 ± 11.3%, and for complex IV 5.7 ± 4.2% and 4.3 ± 2.1%.

Expression of complex I and IV subunits assessed by Western blotting in 10 left ventricle explants showing low/high spectrophotometric activity values was at the normal range in all examined cases (Fig. 6, see the same patients in Fig. 2 and 5). The data indicated that a true complex I/IV deficit may be excluded at the protein level as a pathogenic basis of cardiomyopathy in these cases.

**Myomectomy subgroup**
Table 3 summarizes the results obtained in the myomectomy study subgroup. Histological features of cardiomyopathy (hypertrophic) was confirmed in all cases and the features of primary mitochondrial disorder did not found.

The activity of respiratory chain complexes measured in four left ventricle homogenates by spectrophotometric method was at the same range as in the left ventricle explants subgroup.

In comparison with the reference group of healthy donor hearts the citric synthase activity was similarly lower. BN PAGE and "in gel" activity assay also shown the normal expression and activity of complex IV (Fig. 7). Activity of mitochondrial ATP synthase (complex V) examined in three specimens was normally expressed (Fig. 7).

In summary, the study did not reveal a case with primary OXPHOS defect (a mitochondrial disorder) among the
examined myomectomised patients with hypertrophic cardiomyopathy.

Especially, the study enabled us to exclude the mitochondrial cardiomyopathy in one strongly suspected case (Table 3, patient B). The initial diagnosis of mitochondrial cardiomyopathy in this girl had been previously established indirectly depending on the low cytochrome oxidase activity in skeletal muscle [7]. In this study it was finally shown that the spectrophotometric activity of complex IV in the girl’s heart homogenate is normal (35.4 nmol/min/mg protein, control value 27.8 nmol/min/mg protein). Histochemistry, BN PAGE and „in gel” activity methods also confirmed normal activity and content of complex IV in the left ventricle tissue obtained during myomectomy in this patient (Fig. 7, patient B).

Discussion

The reference (own laboratory) spectrophotometric values for respiratory chain complexes and citric synthase activities were established for three regions of human heart: left ventricle, right ventricle and intraventricular septum. The spectrofotometric measurement of OXPHOS parameters in healthy donor heart showed very high value of citric synthase activity in
### Table 3

<table>
<thead>
<tr>
<th>Patient, gender, Age of onset</th>
<th>Histological and histochemical features</th>
<th>Immunohistochemistry</th>
<th>Activity of respiratory chain complexes I-IV expressed by percentage of CS*</th>
<th>Mitochondrial proteins/complex subunits expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Male (MZ) Adult</td>
<td>Cardiomyocyte hypertrophy, severe focal interstitial fibrosis. COX(+), No lipid accumulation</td>
<td>CD3(–); CD4(–); CD8(–); CD68(+); ICAM(+); CD15(+); CD45(+)</td>
<td>SC 388.5 nmol/min/mg prot, complex I 8.3 %, complex II 11.5 %, complex III 48.6 %, complex IV 8.8 %</td>
<td>Normal expression and activity of complex IV and mitochondrial ATP synthase (see Figure 7)</td>
</tr>
<tr>
<td>B. Female (WA) Child 16 y/</td>
<td>Severe cardiomyocyte hypertrophy and endocardial fibrosis. Mild interstitial fibrosis. Focal mononuclear inflammatory infiltrates. COX(+), No lipid accumulation</td>
<td>CD3(+); CD4(–); CD8(–); CD68(+); CD15(+); CD45(+)</td>
<td>SC 404.7 nmol/min/mg prot, complex I 5.5 %, complex II 15.3 %, complex III 55.7 %, complex IV 8.7 IV %</td>
<td>Normal expression and activity of complex IV and mitochondrial ATP synthase (see Figure 7)</td>
</tr>
<tr>
<td>C. Male (JJ) Adult</td>
<td>Mild cardiomyocyte hypertrophy and interstitial fibrosis. Endocardial fibrosis and thickening. COX(+), No lipid accumulation</td>
<td>CD3(–); CD4(–); CD8(–); CD68(+); CD45(–)</td>
<td>SC 344 nmol/min/mg prot, complex I 6.3 %, complex II 7.1 %, complex III 67.8 %, complex IV 11.1 %</td>
<td>Normal expression and activity of complex IV and mitochondrial ATP synthase (see Figure 7)</td>
</tr>
<tr>
<td>D. Female (HF) Adult</td>
<td>Severe interstitial fibrosis with focal scarring. Cardiomyocyte hypertrophy. COX(+), No lipid accumulation</td>
<td>CD3(+); CD4(–); CD8(–); CD68(+); CD15(+); CD45(+)</td>
<td>SC 390.8 nmol/min/mg prot, complex I 8.1 %, complex II 10.3 %, complex III 56.9 %, complex IV 4.9 %</td>
<td>ND</td>
</tr>
<tr>
<td>E. Male (BJ) Adult</td>
<td>Mild cardiomyocyte hypertrophy. Focal interstitial fibrosis. COX(+), No lipid accumulation</td>
<td>CD3(+); CD4(–); CD8(–); CD68(+); CD15(–); CD45(+)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>F. Male (MJ) Child 13 y/</td>
<td>Marked cardiomyocyte hypertrophy, altered myocardial architecture. COX(+), No lipid accumulation</td>
<td>CD3(–); CD4(–); CD8(–); CD68(+); CD15(–); CD45(–).</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>G. Female (ZK) Adult</td>
<td>Cardiomyocyte hypertrophy, marked interstitial fibrosis. COX and lipids not assessed</td>
<td>CD3(+) ;CD4(–); CD8(+) ;CD68(+); CD15(+); CD45(+)</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Reference values see table 1.*

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**Fig. 7** Blue native polyacrylamide gel electrophoresis and in gel activity assay.

Legend:
BN PAGE of human heart mitochondria (patients A, B and C) and rat heart mitochondria (M). In gel activity assay for the evaluation of complex IV (panel 1) and V (panel 2) activity.
so higher although the relative activity value (expressed by percentage of citric synthase activity) appeared comparable between the left ventricle and the skeletal muscles homogenates. Similar data were published earlier [15, 20], and may present relatively higher amount of mitochondrial mass in the heart. The reference values established for the other heart localizations (right ventricle, intraventricular septum) were in proportion to the left ventricle reference.

The histological, histochemical, biochemical and proteomic studies undertaken to search for a primary mitochondrial defect as the cause of cardiomyopathy were negative in all patients included in the study. Any patient with a primary OXPHOS defect or a mitochondrial cardiomyopathy was not identified among 29 transplanted and 7 myocardectomized patient were not confirmed in the study by furthemore assessment of the complex subunits content and/or activity in the heart tissue. The results seemed to confirm that the primary mitochondrial pathology contribution is rare in isolated cardiomyopathies, especially in adult patients.

Similar approach was undertaken earlier by a few authors and gave similar results.

Jarreta et al in 2000 [15] studied mitochondrial respiratory activities in explants of 17 adult patients with dilated cardiomyopathy and 17 healthy donor hearts. Lower citric synthase activity and complex III (and complexes I+III activity) depression were found by the spectrophotometric method. Due to a suspicion of the primary deficit of complex III cytochrome b gene was sequenced in two of the patients. The search for pathogenic mutation(s) was negative.

On the other hand, screening the whole mitochondrial genome in a group of 45 idiopathic DCM patients revealed increased number of novel mtDNA point mutations (encoding mitochondrial complex I and IV subunits or tRNA genes) in comparison with the group of 62 ischemic DCM subjects. Some mutations occurred in highly conserved aminoacids region and were heteroplasmic which strongly suggested their pathogenicity. The mutation in the D-loop region (c. 16189T>C) associated with susceptibility to DCM was present in 15.6% of patients and in 9.7% of controls [26]. The authors concluded that mtDNA mutations altering OXPHOS function can be remarkably relevant for pathogenesis of dilated cardiomyopathy.

In this study significant changes of OXPHOS function were found in the heart tissue of patients with end-stage heart failure. The marked decrease in citric synthase activity, and in complex III and I+III activities were observed. Oppositely, the activity of complex II was higher then in the control. Similar mitochondrial dysfunction was already described [15, 19] and is not fully understood.

Introducing a new methodological approach by assessment not single complex activities but the active supercomplexes of respiratory chain (so called respirosome, i.e. complex I/complex III dimer/complex IV) will possibly help to elucidate the importance and mechanism of OXPHOS activity decreasing in the end-stage heart failure [25].

Recently, a switch in energetic metabolism by activating the fetal gene expression program (with glycolytic pathways activation and beta-oxidation pathways suppression) was described in the end-stage heart failure experimental animals [12]. The switch leads to mtDNA depletion, to decline of respiratory chain enzyme activities and decreased ATP production. The lowering the mitochondrial biogenesis and function in severe heart failure (probably compensatory to avoid the consumption of large oxygen amount) may explain our findings of decrease in citric synthase activity and complex III activity. The phenomenon unlikely benefits the energy homeostasis and therefore likely aggravates the disease [12].

Finally, it must be emphasized that in each case of cardiomyopathy possible primary mitochondrial defect should be searched for by enzymatic and molecular studies, bearing in mind that secondary as well as compensatory mitochondrial dysfunction may be present. It is important that in a stage of severe heart failure mitochondrial pathogenesis is investigated due to specific problems concerned with indications for heart transplantation [2, 28], and possible maternal inheritance. It seems that the issue of mitochondrial cardiomyopathies is better known and understood in children than in adult patients [13, 29].

Tissue sample processing methods and reference values of respiratory chain activity presented in this study make it possible to implement mitochondrial diagnostics in each cardiomyopathic patient undergoing cardiobvascular intervention which allow to obtain myocardium for investigation.

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