STRUCTURAL AND FUNCTIONAL PROPERTIES OF HUMAN HEART ATRIAL APPENDAGE MITOCHONDRIA: CHANGES DURING OPEN HEART SURGERY AND RELEVANCE TO THE WHOLE HEART ENERGY METABOLISM AND CELLULAR MEMBRANES IN THE EVALUATION AND PROGNOSIS OF INJURY

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Summary

Objectives: This study has been designed to evaluate disturbances of respiration of atrial appendage mitochondria occurring during heart surgery and their relation with the level of cardiac injury.

Design and Methods: The mitochondrial respiratory parameters were measured *in situ*, i.e. in atrial appendage fibers of patients with ischemic heart disease undergoing heart surgery under hypothermic the whole heart cardioplegia. Respiratory parameters were analysed together with troponin T (TnT), lactate dehydrogenase (LDH) and creatine kinase (CK) activity in blood.

Results: The study revealed a tight correlation between the maximally elevated activity of LDH in the blood (sampled after the cardiac operations) and two respiratory parameters of atrial appendage mitochondria (both measured before cardioplegia), – respiratory control index (negative, $r = -0.61$) and percentage of atractyloside-insensitive respiration (positive, $r = 0.80$).

Conclusions: The data obtained in this work allow us to conclude that investigation of respiration of atrial appendage mitochondria can be applied for the assessment of the disturbances in the whole heart mitochondria occurring during heart surgery and, that their studies before cardioplegia may provide useful information for the prognosis of cardiac cell energetics, function disturbances and recovery after cardioplegia.

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The degree of myocardial damage during open-heart surgery depends on multiple factors, such as the type and extent of the surgical procedure, the duration of aorto-pulmonary bypass and aortic cross-clamp, etc. We have observed, that postoperative cardiac insufficiency and myocardial ischemia, based on troponin T (TnT) values (vs. creatine kinase (CK), lactate dehydrogenase (LDH)), can be detected earlier and more easily [1,2]. On the ground of the available findings [3,4], one may assume that good prognosis/recovery of the cardiac function should depend on the energetic state of myocardium (before heart surgery), which, in its turn, depends on the structural and functional properties of mitochondria [3].

However, functional investigations of the human heart mitochondria, particularly those isolated from ventricles, in most cases are limited by the lack of tissue. The saponin-skinned fiber technique [5] partially overcomes this problem as it does not require isolation of mitochondria and, therefore, needs much less of cardiac tissue, particularly, when fibers are prepared with saponin+crude collagenase, causing a substantial increase in the maximal fiber respiration rate as compared to the fibers prepared only with saponin as permeabilizing agent [6,7]. In order to assess the respiratory capacity of cardiac mitochondria and its changes during heart surgery we used the saponin+collagenase treated human heart

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atrial appendage fibers [8]. However, the question whether the changes revealed in atrial appendage fibers properly reflect the situation in the heart ventricles remain obscure, as the leakage of cytosolic LDH, CK and TnT from the cardiac cells depend on the permeability of cellular membranes, which, in its turn, depend on the energy state of the cell [3]. We have assumed that at least some structural and functional parameters of atrial appendage mitochondria should reflect the disturbances in the whole heart cellular membranes integrity and, thus, should be related to the appearance of TnT, CK and/or LDH in the blood after cardioplegia/heart surgery.

The aim of present study was to investigate the problems mentioned above and the correlation between respiratory control index and other parameters of atrial appendage mitochondria and the level of LDH, CK and TnT in the blood after the cardiac operations. The data obtained mean that some of the atrial appendage mitochondria respiratory parameters measured before heart surgery have a prognostic/predictive value and reflect the degree of loss of ventricular cell integrity and energy metabolism produced by ischemia and reperfusion of the heart.

Design and Methods

The human heart atrial tissue (about 20 mg biopsies) was obtained from 20 patients with ischemic heart disease and from 3 patients with valvular defect before and after hypothermic (+15˚C) cardioplegia (the second sample was taken 10–15 min after reperfusion). The average age of the patients was 64.5 ± 10.7 years, the duration of cardioplegia during heart surgery – 51.6 ± 27.4 min. The severity of the left ventricular dysfunction was evaluated according to the level of the left ventricular ejection fraction from echocardiography.

Bundles of fibers, 0.2–0.3 mm in diameter, were prepared with saponin (50 μg/ml) to permeabilize sarcolemma [4] and collagenase (type IV, Sigma, USA; 3 mg per ml of skinning solution) and were used for the oxygraphic fiber respiration measurements at 37˚C [6–8].

Due to the lack of human tissue and seeking to evaluate similarity of ventricular and atrial appendage mitochondria's response to ischemia, mongrel male rabbits, weighing 2.5–3.5 kg, were used in five experiments. The experiments were carried out on normal and ischemia-damaged cardiac fibers prepared from ventricles and atrial appendage, exactly as described above. Total ischemia was induced *in vitro* by autolysis (37˚C, 1.0 h) [9].

Respiration rates (with 10 mM succinate as a respiratory substrate + 5 μM rotenone) were expressed as ngatomO/min/mg dry weight of fibers.

Cardiac markers were investigated in blood samples of 23 patients before and 8, 12 and 48 hours after heart surgery using immunoelectrochemiluminescence assay ("ELECSYS 1010", Roche) for quantitative TnT determination in serum and evaluated kinetics of the release TnT and cardiac enzymes, LDH and CK total activity. Total CK activity was investigated by standard fluorimetric and LDH – by spectrophotometric kinetic methods.

Data of clinical and biochemical variables of patients were expressed as mean ± SD, mitochondrial respiratory parameters – as mean ± SE. Regression analysis was performed with simple linear and multivariate stepwise models. Pearson's correlation coefficient was used, and $p < 0.05$ was considered statistically significant.

Results and Discussion

The saponin+collagenase-treated human heart atrial appendage fibers respiration measurements by oxygraphic method (see Figure 1) allows the assessment of the functional activity as well as integrity

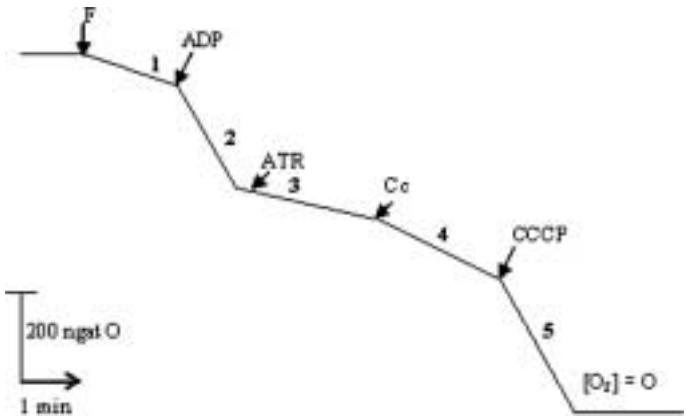


Figure 1. The scheme of oxygraphic recordings of the saponin + collagenase-treated human heart atrial appendage fibers respiration. Order and final concentrations of additions: 1 – fibers (F, 3–5 mg wet weight) respiration in the presence of 10 mM succinate+5.3 μM rotenone (V_o); 2 – maximal State 3 respiration rate after addition of 1 mM ADP (V_{ADP}); 3 – atractyloside, 105 μM (V_{ATR}); 4 – cytochrome c, 15.4 μM (V_{ATR+C}); 5 – carbonyl cyanide m-chlorophenylhydrazone, 2.1 μM (V_{CCCP+C})

Table 1. The effect of hypothermic cardioplegia on mitochondrial respiratory parameters in permeabilized human heart atrial appendage fibers

Parameter	*n*	*Before cardioplegia*	*After cardioplegia*	*P*
V_o	23	25.9 ± 10.7	27.6 ± 9.0	0.165
V_{ADP}	23	58.3 ± 22.9	51.8 ± 15.6	0.02
V_{ATR}	18	20.7 ± 7.9	21.6 ± 8.1	0.64
V_{ATR+C}	18	24.9 ± 8.2	28.0 ± 9.2	0.07
V_{CCCP+C}	17	55.9 ± 16.8	50.4 ± 14.7	0.104
RCI-1	23	2.43 ± 0.60	1.92 ± 0.32	0.0006
RCI-2	18	2.96 ± 0.89	2.47 ± 0.68	0.02
$V_{ADP} - V_o$	23	32.3 ± 14.8	24.2 ± 9.6	0.001
$V_{ATR}\%$	18	38.1 ± 13.0	43.9 ± 12.8	0.152
V_{ATR+C}/V_{ATR}	20	1.27 ± 0.19	1.35 ± 0.34	0.395

V_o – respiration rate with succinate+rotenone (15 mM + 5 μM) in the absence of adenosine diphosphate (ADP); V_{ADP} and $V_{ADP+ATR}$ – respiration rate in the presence of ADP (1.05 mM) and ADP+atractyloside (1.05 mM + 0.10 mM), respectively; V_{ATR+C} – in the presence of atractyloside+cytochrome c (0.10 mM + 30 μM); V_{CCCP+C} – in the presence of carbonyl cyanide-m-chlorophenylhydrazone+cytochrome c (2.1 μM + 30 μM); RCI-1 – respiratory control index (V_{ADP}/V_o); RCI-2 – respiratory control index (V_{ADP}/V_{ATR}); $V_{ATR}\%$ – percentage of atractyloside uninhibited respiration

of outer and inner membranes of mitochondria *in situ*, their respiration rates in different metabolic states, maximal respiratory capacity and the degree of coupling between oxidation and adenosine diphosphate (ADP) phosphorylation/adenosine triphosphate (ATP) synthesis [6–8]. The average values of mitochondrial respiratory parameters of patients with IHD studied before and after hypothermic cardioplegia of 51.6 ± 27.4 min duration followed by 10–15 min of normothermic reperfusion are presented in Table 1.

As can be seen from Table 1, the comparison of all respiratory rates measured in different metabolic conditions shows that only State 3 (V_{ADP}), i.e. maximal respiration rate in the presence of ADP, has been significantly changed due to cardioplegia – it decreased by 11 percent compared to the control value (before cardioplegia). Relative respiratory parameters, calculated from the absolute respiration rates of fibers, were influenced more markedly. In most cases, their changes after ischemia/reperfusion were statistically highly significant. The most sensitive to cardioplegia appeared to be the respiratory control index (RCI-1 and RCI-2; decreased by 21 and 17 percent, respectively) and the external ADP-dependent respiration rate ($V_{ADP} - V_o$; decreased by 25 percent). These data reflect an increase in the mitochondrial inner membrane permeability to protons and other ions causing an uncoupling of oxidative phosphorylation and the related decrease in mitochondrial capacity to synthesize ATP, and essentially coincide with our earlier findings [8].

Figure 2 shows that respiratory control index decreases with the increase in the duration of cardioplegia, which is different in each patient.

Noteworthy, that according to our results obtained on rabbits (Table 2), changes of the main respiratory parameters – maximal respiration rate (V_{ADP}) and respiration rate before addition of ADP (V_o) – observed during 1 h of total ischemia *in vitro* (37°C) are very similar in atrial appendage fibers and ventricular fibers. This indicates that mitochondria in these different tissues are equally sensitive to ischemia. Accordingly, one may suggest that investigation of human heart atrial appendage fibers respiration may be used to assess, at least semiquantitatively the cardioplegia-induced changes in the structural and functional properties of ventricular and whole heart mitochondria. In this regard, as the metabolic status of patient cardiomyocytes differs from that of rabbits investigation of patient tissues – atrial appendage and ventricular – would be of value.

Interestingly, the increase in the degree of stimulation of human atrial appendage fibers respiration by cytochrome c (V_{ATR+C}/V_{ATR}) is small (6.3 percent) and not statistically significant. Cytochrome c – the mitochondrial respiratory chain component – is loosely bounded to the outer surface of inner membrane. Its content in mitochondria decreases during ischemia [10] due to the injury of outer membrane and dissociation of cytochrome c from inner membrane. Thus, the data obtained mean that neither the outer membrane of mitochondria nor the content of cytochrome c was altered markedly under the conditions of myocardial

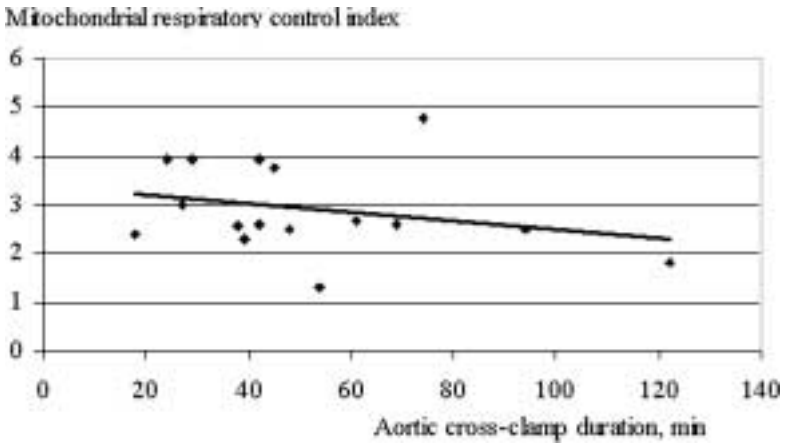


Figure 2. Dependence of respiratory control index of atrial appendage fiber's mitochondria on the duration of heart cardioplegia

Table 2. The effect of normothermic (37°C) ischemia on mitochondrial respiratory parameters in saponin-permeabilized rabbit heart ventricular and atrial appendage fibers (percentage changes from control)

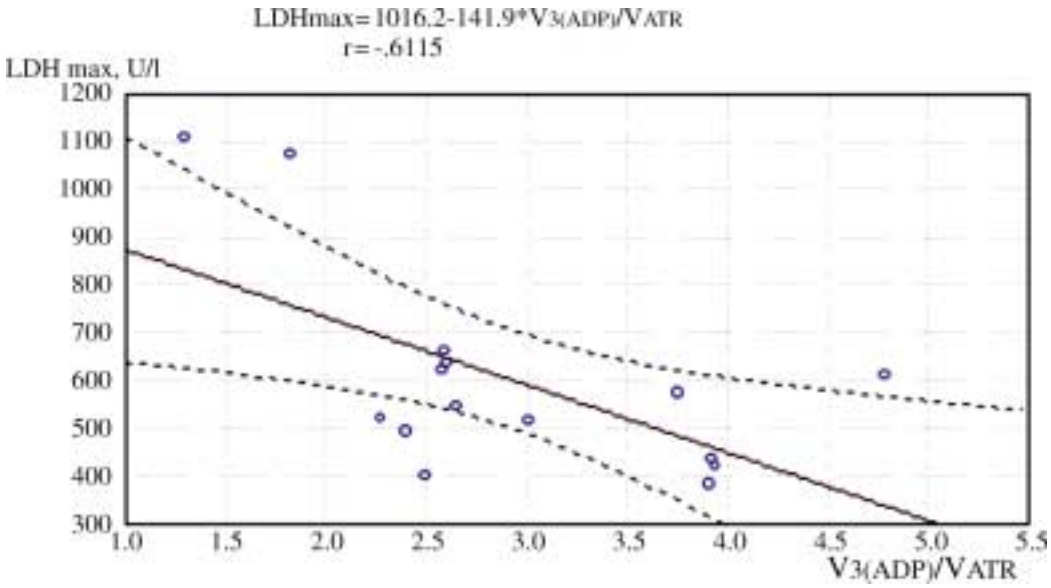
Parameter	*Atrial appendage*	*Ventricle*
	Substrate: glutamate 5 mM + malate 6.2 mM	
V_o	15 ± 10	17 ± 3
V_{ADP}	-41 ± 3	-46 ± 3
	Substrate: succinate 37 mM + amytal 2 mM	
V_{ADP}	-30 ± 3	-28 ± 4

Data of 5 paired experiments are presented as mean ± SE. Effects of ischemia on ventricular and atrial appendage fibers were not statistically different
V_o – respiration rate in the absence of adenosine diphosphate (ADP); V_{ADP} – respiration rate in the presence of ADP (1.0 mM)

ischemia/reperfusion in these patients. It is noteworthy, that a marked cytochrome c induced increase in atractyloside-insensitive respiration rate (1.27 fold) was found before cardioplegia indicating that the outer membrane of atrial appendage mitochondria of patients with IHD was slightly injured.

The levels of peak release of LDH and CK activity and the TnT content in the blood samples taken after cardioplegia revealed an increase by 2.8, 5.9 and 93 times, respectively, in comparison with the control (before cardioplegia) values of these parameters. It is known that most part of cellular LDH is localized in the cytosol. High specific activities of LDH were also found to be associated with mitochondria (intermembrane space and inner membrane+matrix) [11,12]. This enables mitochondria isolated from heart muscle to oxidize lactate with a high rate which is comparable to that of pyruvate [12,13]. Though in our experiments mitochondrial outer membrane injury was not detected after cardioplegia/reperfusion (see above), the possibility that mitochondrial LDH could release from the cardiac cells, at least in appreciable amounts, seemed unlikely. Thus, cardioplegia-induced elevation of LDH content in blood is, most probably, related with its release from/with cytosol. These data clearly demonstrate the ischemia/reperfusion-induced injury of cardiac cellular membranes, which is known being closely related with the worsening of cellular energy metabolism [3].

To elucidate the relationship between respiration of heart atrial appendage mitochondria and integrity of myocardial cellular membranes we have calculated coefficients of correlations between mitochondrial respiratory parameters (presented in Table 1) and LDH, CK activity as well as TnT content in the blood of patients with IHD. It should be noted that no significant correlation was found between mitochondrial parameters (before or after cardioplegia), CK and TnT. The cause of this observation is not clear and, therefore, should be elucidated in further experiments. However, tight correlations were observed between preischemic (before cardioplegia) values of mitochondrial respiratory control index (V_{ADP}/V_{ATR}) and atractyloside-insensitive respiration rate (V_{ATR}%), the parameters reflecting the mitochondrial inner membrane permeability to ions as well as the degree of coupling of oxidative phos-



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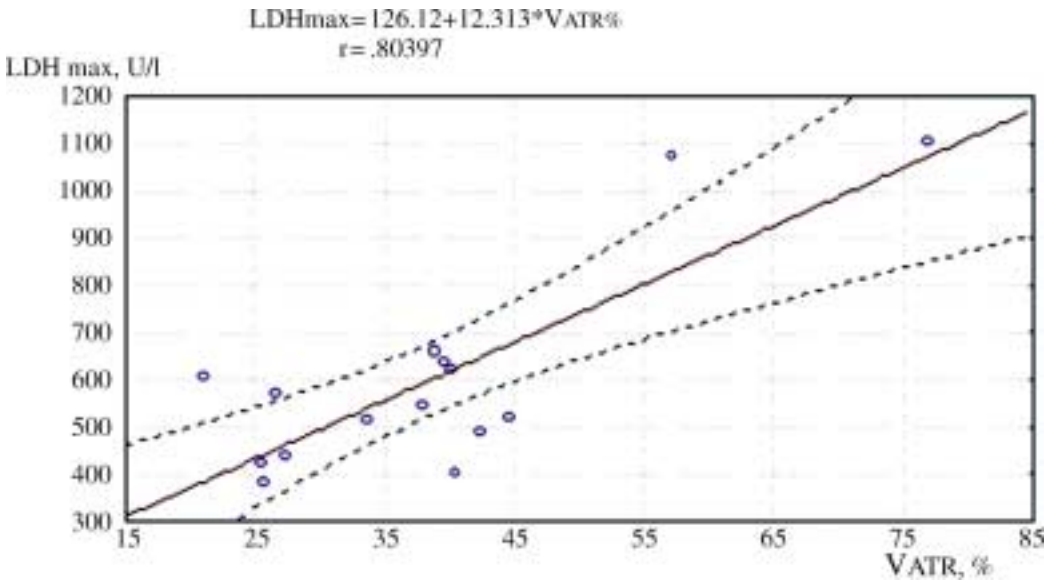

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Figure 3. Correlation between LDH_{max} in the blood and mitochondrial respiratory parameters – respiratory control index (RCI-1; i.e. $V3_{(ADP)}/V_{ATR}$, picture A) and percentage of atractyloside-uninhibitable respiration rate (V_{ATR}%; picture B) both measured before cardioplegia

phorylation and efficacy of ATP production, and the LDH activity in blood estimated postoperatively at the time of its maximal elevation (Figures 3A and 3B; $r = -0.61$ and $r = +0.80$, respectively). It should be noted that for correlation analysis all patient data were used because the number of patients with valvular disease was small (three of twenty three) and their data were not significantly different from those of ischemic patients. More extensive comparative study of different patients would be valuable as it would allow extrapolation of results to all groups of patients.

It should be noticed that the levels of LDH_{max} and TnT_{max} were in relation with aortic cross-clamp duration ($r = 0.69$; $r = 0.85$; respectively, $p < 0.05$), but the relation between the severity of the left ventricular dysfunction was significant only for TnT_{max} and CK_{max} ($r = 0.60$; $r = 0.53$; respectively, $p < 0.05$).

Conclusions

The data obtained show, that firstly, hearts of patients with IHD which have a lower respiratory control index RCI-1 and a higher atractyloside-insensitive respiration rate (V_{ATR}%) of atrial appendage fiber's mitochondria, i.e. a higher inner membrane permeability to ions and, thus, a lower ATP-producing capacity of mitochondria, are less re-

sistant to hypothermic ischemia/normothermic reperfusion. Secondly, that investigation of respiration of atrial appendage mitochondria, which appear to be equally sensitive to ischemia as ventricular organelles, can be applied for the assessment of the disturbances in the whole heart mitochondria occurring during heart surgery. Finally, the study of the mitochondrial function in the atrial appendage biopsy samples before ischemia (cardioplegia) and reperfusion may provide useful information for the prognosis of cardiac cell energetics, function disturbances and recovery after ischemia and reperfusion.

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