# Relevance of fatty acid oxidation in regulation of the outer mitochondrial membrane permeability for ADP

Adolfas Toleikis*, Julius Liobikas, Sonata Trumbeckaite, Daiva Majiene

*Institute for Biomedical Research, Kaunas University of Medicine, Eiveniu Str. 4, LT-3007 Kaunas, Lithuania*

Received 7 August 2001; revised 2 November 2001; accepted 6 November 2001

First published online 20 November 2001

Edited by Vladimir Skulachev

**Abstract The present study on saponin-treated rat heart muscle fibers has revealed a new function of the fatty acid oxidation system in the regulation of the outer mitochondrial membrane (OMM) permeability for ADP. It is found that oxidation of palmitoyl-CoA+carnitine, palmitoyl-L-carnitine and octanoyl-L-carnitine (alone or in combination with pyruvate+malate) dramatically decreased a very high value of apparent $K_m$ of oxidative phosphorylation for ADP. Octanoyl-D-carnitine, as well as palmitate, palmitoyl-CoA, and palmitoyl-L-carnitine were not effective in this respect, when their oxidation was prevented by the absence of necessary cofactors or blocked with rotenone. Our data suggest that oxidation, but not transport of fatty acids into mitochondria, induces an increase in the OMM permeability for ADP. © 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.**

*Key words:* Saponin-permeabilized heart muscle fiber; Michaelis–Menten constant; Outer mitochondrial membrane permeability; Oxidative phosphorylation; Fatty acid oxidation

## 1. Introduction

The last decade studies of permeabilized heart and skeletal muscle fibers have led to the observation that outer mitochondrial membrane (OMM) played a crucial role in the mechanism of regulation of mitochondrial respiration in vivo [1–3]. It was shown that, in contrast to isolated mitochondria, OMM possessed a low permeability for ADP (high apparent $K_m$ of oxidative phosphorylation for ADP (app. $K_m^{ADP}$)) in saponin-permeabilized cardiac and slow-twitch skeletal muscle fibers.

Permeability of OMM for ADP seems to be regulated by some specific cytoskeleton-related proteins bound to porin pores [2,3]. These proteins are lost during isolation of mitochondria [2,3]. Our studies [4,5] with saponin-permeabilized cardiac fibers confirmed a high value of app. $K_m^{ADP}$ in regulation of oxidative phosphorylation, showing also a temperature dependence of this parameter, namely: a decrease in app. $K_m^{ADP}$ value at the higher, physiological temperature 37°C if compared with 22°C [4]. The app. $K_m^{ADP}$ values in the above-mentioned studies were estimated by using glutamate+malate, pyruvate+malate or succinate as a respiratory substrate [1–7]. Meanwhile, it is well known that fatty acids are the major, if not the sole, respiratory substrate in the heart cell in many cases, such as fasting, high-fat diet, hibernation, diabetes, post-ischemic reperfusion. On the other hand, it was shown that fatty acids, and their CoA and carnitine esters, as surfactants, in an appropriate concentration, might increase permeability of inner and outer mitochondrial membranes and other biological membranes [8–10]. Moreover, it was shown [11] that fatty acids, palmitoyl-CoA and other surface-active agents altered the surface charge and the surface potential of mitochondria and other biological membranes, causing changes in app. $K_m$ values for substrates of several membrane-bound enzymes. The uncoupling effect of non-esterified fatty acids was known for a long time ([12], for review, see [8,13]). Recent investigations of the mechanism of their action have elucidated the role of such anion carriers as the ATP/ADP antiporter, the glutamate/aspartate antiporter and the dicarboxylate carrier in the fatty acid-induced uncoupling of oxidative phosphorylation [13,14]. Noteworthy, it affects the kinetics of oxidative phosphorylation in isolated mitochondria by increasing app. $K_m^{ADP}$ [15]. Under certain conditions, non-esterified long-chain fatty acids promote an increase in non-selective mitochondrial permeability by opening a cyclosporin A-sensitive [16] or cyclosporin A-insensitive [17] mitochondrial permeability transition pore. This process correlates with an increase in OMM permeability and a release of cytochrome *c* from mitochondria [18].

A recent finding [19] that a 22 kDa polyanion (a specific inhibitor of voltage-dependent anion channel, also called mitochondrial porin) inhibits palmitate and palmitoyl-CoA oxidation, but does not affect oxidation of palmitoylcarnitine, octanoate, glutamate and pyruvate in isolated mitochondria, is also of interest. This, together with other data obtained (for review, see [20]), suggests involvement of mitochondrial porin in the long-chain acyl-CoA transport. On the basis of these data, one may assume possibility of interaction between ADP and the long-chain acyl-CoA transport through the porin channel.

The aim of the present study was to investigate the influence of palmitoyl-CoA, palmitoyl-L-carnitine and other fatty acids on app. $K_m^{ADP}$ in regulation of oxidative phosphorylation in the heart mitochondria in situ. Below, we present experiments showing that a very high value of app. $K_m^{ADP}$, which is characteristic of saponin-treated rat cardiac fibers respiring on pyruvate+malate, drastically decreases in case of the fatty acid oxidation.

*Corresponding author. Fax: (370)-7-796 498.
*E-mail address:* toleikis@kmu.lt (A. Toleikis).

*Abbreviations:* OMM, outer mitochondrial membrane; app. $K_m^{ADP}$, apparent $K_m$ of oxidative phosphorylation for ADP

0014-5793/01/$20.00 © 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.
PII: S0014-5793(01)03171-4

## 2. Materials and methods

Hearts of male Wistar rats weighing 250–300 g were excised and rinsed in ice-cold 0.9% KCl solution. Bundles of the heart muscle fibers, approximately 0.2–0.3 mm in diameter, were prepared [5] and transferred to cooled solution A containing 20 mM imidazole, 20 mM taurine, 0.5 mM dithiothreitol, 7.1 mM $MgCl_2$, 50 mM 2-[*N*-morpholino]ethanesulfonic acid (MES), 5 mM ATP, 15 mM phosphocreatine, 2.6 mM $CaK_2EGTA$ and 7.4 mM $K_2EGTA$ (free $Ca^{2+}$ concentration 0.1 μM) (pH 7.0 adjusted with KOH at 2°C), supplemented with 50 μg/ml saponin (from Gypsophila; sapogenin content 17%; Sigma) and incubated for 30 min. Then the bundles were washed for 10 min in solution B, containing 20 mM imidazole, 20 mM taurine, 0.5 mM dithiothreitol, 1.6 mM $MgCl_2$, 100 mM MES, 3 mM $KH_2PO_4$, 3.0 mM $CaK_2EGTA$ and 7.1 mM $K_2EGTA$ (free $Ca^{2+}$ concentration 0.1 μM) (pH 7.1 adjusted with KOH at 37°C).

Oxygen uptake rates were recorded at 37°C by means of the Clark-type electrode system in solution B, supplemented with 2 mg/ml of bovine serum albumin (Fraction V; A4503, Sigma). Solubility of oxygen was taken to be 422 ngatoms/ml. Respiration rates were expressed as ngatoms O/min/mg of dry weight fibers (dry weight = wet weight before respiration measurement/4.85). The ADP regenerative system, consisting of 1.2 IU/ml lyophilized yeast hexokinase (Type V; EC 2.7.1.1; Sigma) and 24 mM glucose (Sigma), was added to the chamber before addition of heart muscle fibers. Titration was made by different ADP concentrations in each separate probe. The concentrations ranged from 5 to 2000 μM. $\Delta V$ was expressed as a difference between respiration rates in the presence and in the absence of added ADP. App. $K_m^{ADP}$ and $\Delta V_{max}$ were estimated from the least-squares fit to the Michaelis–Menten equation ($\Delta V$ vs. ADP concentration) by GraphPad Prism demo v3.0. The results are presented as means ± S.E.M. Statistical analysis was performed using Student's *t*-test, and $P < 0.05$ was taken as the level of significance. Octanoyl-L-carnitine and octanoyl-D-carnitine were obtained from Serva. Other biochemicals were obtained from Sigma. Palmitoyl-L-carnitine was dissolved in 40% ethanol solution, and palmitate in 96% ethanol. The final ethanol concentration in the fiber respiration measurements did not exceed 1% and did not affect respiratory parameters.

## 3. Results

Fig. 1 shows the results of an experiment on saponin-treated rat cardiac fibers when respiration rate with different substrates is measured at different ADP concentrations. It is seen (curve 1) that the maximal respiration rate with pyruvate+malate as a substrate is achieved at ADP concentration higher than 1 mM. The calculated mean app. $K_m^{ADP}$ value was 236 ± 24 μM (Fig. 2, column 1). Noteworthy, in each separate group of experiments (groups 1–6, see Table 1), estimation of app. $K_m^{ADP}$ with pyruvate+malate was performed, as a control, to exclude the influence of its possible variation on the estimation of app. $K_m^{ADP}$ differences between pyruvate+malate and fatty substrates. Since no statistical differences were observed in $K_m^{ADP}$ (not shown), they were combined to calculate the overall mean (Fig. 2, column 1).

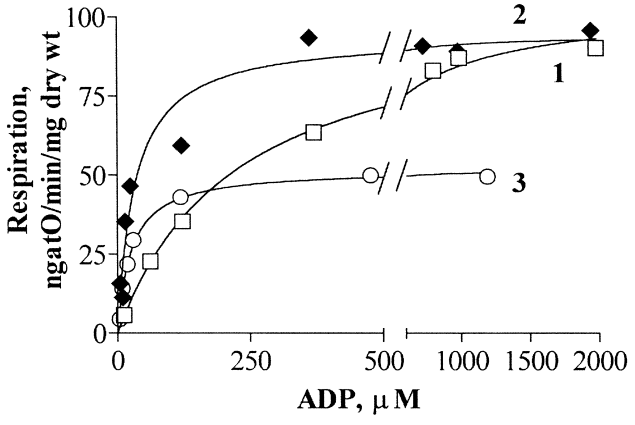


Fig. 1. Dependences of respiration rates of rat cardiac fibers on ADP concentration. 1: 6 mM pyruvate+6 mM malate, 2: 6 mM pyruvate+6 mM malate+0.1 mM octanoyl-L-carnitine, 3: 9 μM palmitoyl-L-carnitine+0.24 mM malate.

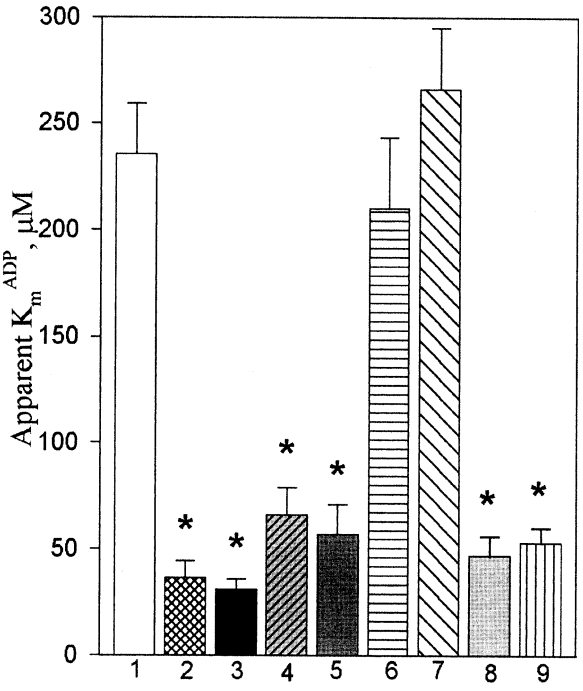


Fig. 2. Influence of respiratory substrates on the app. $K_m^{ADP}$ of saponin-treated rat cardiac fibers. 1: 6 mM pyruvate+6 mM malate ($n = 18$), 2: 9 μM palmitoyl-L-carnitine+0.24 mM malate ($n = 5$), 3: 12.2 μM palmitoyl-CoA+2.41 mM L-carnitine+0.24 mM malate ($n = 7$), 4: 0.36 mM octanoyl-L-carnitine+0.24 mM malate ($n = 5$), 5: 6 mM pyruvate+6 mM malate+0.1 mM octanoyl-L-carnitine ($n = 3$), 6: 6 mM pyruvate+6 mM malate+12.2 μM palmitoyl-CoA ($n = 5$), 7: 6 mM pyruvate+6 mM malate+2.5 μM palmitate ($n = 3$), 8: 6 mM pyruvate+6 mM malate, 9: 6 mM pyruvate+6 mM malate+9 μM palmitoyl-L-carnitine. *$P < 0.05$ vs. app. $K_m^{ADP}$ with pyruvate+malate (column 1). Note: in experiments 8 and 9, fibers were pretreated with 1.6 mg/ml of saponin for 2 min at 37°C, and the respiration medium was supplemented with 33 μM cytochrome *c*. For each measurement we used 3–5 mg (wet weight) of fibers.

Thus, results of experiments with pyruvate+malate as a respiratory substrate are in line with those obtained by other investigators [21], as well as with our own earlier findings [4,5], and, therefore, confirm the observation that, in saponin-treated cardiac fibers, the app. $K_m^{ADP}$ value is very high compared with that of isolated mitochondria (23 μM; [5]). This means that OMM permeability for ADP in situ is very low and significantly lower than that in isolated mitochondria [5,21]. Another situation was revealed when saponin-treated rat cardiac fiber respiration with palmitoyl-L-carnitine (+malate) was titrated with ADP (Fig. 1, curve 3 and Fig. 2, column 2). In this case, the maximal respiration rate was achieved at a much lower ADP concentration (of about 100 μM), and the mean app. $K_m^{ADP}$ value (36 ± 8 μM) was much lower if compared with that of pyruvate+malate oxidation. Similarly, low value of app. $K_m^{ADP}$ (31 ± 5 μM) was also obtained when palmitoyl-CoA+L-carnitine, instead of palmitoyl-L-carnitine, was used as a respiratory substrate (Fig. 2, column 3). Further

Table 1
Respiratory parameters of the saponin-permeabilized rat cardiac fibers with different substrates

| | Substrates | $n$ | $V_o$ | $V_{ADP}$ | $V_{ADP+CYTC}/V_{ADP}$ |
|---|---|---|---|---|---|
| 1 | Pyruvate+malate | 5 | 35 ± 3.0 | 126 ± 17 | 1.06 ± 0.03 |
| | Pyruvate+malate+palmitoyl-CoA | 5 | 36 ± 2.4 | 128 ± 14 | 1.01 ± 0.02 |
| 2 | Pyruvate+malate | 5 | 34 ± 3.5 | 105 ± 18 | 0.93 ± 0.01 |
| | Palmitoyl-L-carnitine+malate | 5 | 24 ± 2.2* | 75 ± 11 | 1.09 ± 0.1 |
| | Palmitoyl-CoA+malate+L-carnitine | 7 | 23 ± 2.6* | 75 ± 9 | 1.01 ± 0.04 |
| 3 | Pyruvate+malate | 4 | 42 ± 1.8 | 155 ± 8 | 1.02 ± 0.03 |
| | Pyruvate+malate+palmitate [2.5 μM] | 3 | 43 ± 2.1 | 151 ± 4 | 1.04 ± 0.03 |
| | Pyruvate+malate+palmitate [5 μM] | 4 | 48 ± 5.4 | 154 ± 17 | 1.03 ± 0.02 |
| 4 | Pyruvate+malate | 3 | 45 ± 2.0 | 153 ± 13 | 1.03 ± 0.02 |
| | Pyruvate+malate+palmitate [100 μM] | 3 | 60 ± 4.0* | 146 ± 15 | 1.02 ± 0.01 |
| 5 | Pyruvate+malate | 4 | 45 ± 0.5 | 161 ± 7 | 1.06 ± 0.1 |
| | Octanoyl-L-carnitine+malate | 5 | 44 ± 1.0 | 144 ± 7 | 1.07 ± 0.03 |
| | Pyruvate+malate+octanoyl-L-carnitine [0.1 mM] | 3 | 52 ± 5.0 | 136 ± 18 | 1.01 ± 0.1 |
| 6 | Pyruvate+malate | 3 | 44 ± 2 | 177 ± 17 | 1.04 ± 0.02 |
| | Pyruvate+malate+octanoyl-L-carnitine | 3 | 55 ± 1.8* | 169 ± 12 | 1.07 ± 0.03 |
| | Pyruvate+malate+octanoyl-D-carnitine | 3 | 43 ± 4.4 | 166 ± 2.5 | 1.03 ± 0.03 |
| 7 | Succinate (+rotenone) | 4 | 99 ± 4 | 189 ± 11 | 1.13 ± 0.03 |
| | Succinate(+rotenone)+palmitoyl-L-carnitine | 4 | 102 ± 2 | 184 ± 9 | 1.14 ± 0.04 |

$V_o$, respiration rate without ADP; $V_{ADP}$, respiration rate in the presence of 1 mM ADP; $V_{ADP+CYTC}$, respiration rate in the presence of 1 mM ADP and 33 μM cytochrome *c*. Concentration of succinate was 12 mM (+5 μM rotenone); of the other substrates, as indicated in the legend of Fig. 2. Concentration of octanoyl-D-carnitine was 0.36 mM. For each measurement we used 3–5 mg (wet weight) of fibers. *$P < 0.05$ vs. respiration rate with pyruvate+malate.

experiments showed that oxidation of the medium chain length fatty acid, octanoyl-L-carnitine (0.36 mM; Fig. 2, column 4), also caused a dramatic decrease in app. $K_m^{ADP}$ value in saponin-treated cardiac fibers. This phenomenon was observed with an even 3.6-fold lower concentration of octanoyl-L-carnitine (0.1 mM) used in combination with pyruvate+malate (Fig. 1, curve 2 and Fig. 2, column 5). However, this phenomenon disappeared when fibers were pre-treated with high concentration saponin (1.6 mg/ml) in order to destroy the integrity of OMM (confirmed by a considerable stimulation of respiration by cytochrome *c*). In this case, the app. $K_m^{ADP}$ value was low (Fig. 2, column 8, pyruvate+malate), and it was not affected by palmitoylcarnitine (Fig. 2, column 9).

It is noteworthy that under conditions excluding the possibility of oxidation of externally added fatty acid (palmitoyl-CoA added into the medium devoid of carnitine), app. $K_m^{ADP}$ value in fibers respiring on pyruvate+malate remained high, like in the case of pyruvate+malate alone (Fig. 2, column 6, compare with column 1). Analogically, exogenous palmitate (2.5 μM, see Fig. 2, column 7; identical data were obtained also with 5.0 μM and 100 μM palmitate, which is not shown), in the absence of CoA and L-carnitine, failed to affect the app. $K_m^{ADP}$ value in the case of pyruvate+malate oxidation, and palmitoyl-L-carnitine (9 μM) in the case of succinate+rotenone oxidation (129 ± 10 and 105 ± 15 μM ADP in the absence and in the presence of palmitoyl-L-carnitine, respectively; four paired experiments). Likewise, no changes of this parameter were also observed when non-oxidizable D-isomer of octanoylcarnitine was added to fibers respiring on pyruvate+malate (app. $K_m^{ADP}$ were 195 ± 27 and 170 ± 50 μM ADP in the absence and in the presence of octanoyl-D-carnitine, respectively; three paired experiments).

Table 1 shows statistical values of the main respiratory parameters (the basal respiration rate in the absence of external ADP, the state 3 respiration rate and the cytochrome *c* effect on the state 3 respiration) that are characteristic of all different experimental groups described above. It can be seen that the state 3 ($V_{ADP}$) and the state 4 ($V_o$) respiration rates with fatty substrates, palmitoyl-L-carnitine, palmitoyl-CoA+L-carnitine and octanoyl-L-carnitine, were similar (experiment 5) or slightly lower (experiment 2) than the respective rates of pyruvate oxidation. Besides, neither palmitoyl-CoA (minus L-carnitine) nor palmitoyl-L-carnitine (plus rotenone) nor palmitate (2.5 and 5 μM), under conditions preventing their oxidation, had any significant influence on the parameters of pyruvate or succinate oxidation. This is also true for a non-oxidizable fatty acid derivative, octanoyl-D-carnitine. However, with concentration of 100 μM, palmitate induced a slight uncoupling of oxidative phosphorylation (experiment 4). The integrity of OMM, as estimated by the cytochrome *c* effect on the state 3 respiration, was good and similar for all experimental groups.

## 4. Discussion

The above findings, for the first time, demonstrate that oxidation of fatty acids, palmitoyl-CoA, palmitoyl-L-carnitine and octanoyl-L-carnitine, in mitochondria located in the saponin-treated rat cardiac fibers causes a dramatic (up to 10-fold) decrease in app. $K_m^{ADP}$ value, if compared with that of the pyruvate+malate oxidation. A very high app. $K_m^{ADP}$, if compared with that of isolated mitochondria, was found earlier in several studies with respiratory substrates other than fatty acids, namely the glutamate+malate, pyruvate+malate and succinate [1–6].

This means that the fatty acid oxidation increases the OMM permeability for ADP. However, the mechanism of this phenomenon remains unclear. As demonstrated earlier by other investigators and by our laboratory, permeability of OMM for ADP increases when fibers are pre-treated with proteases [1,2,4,5,7], or when the integrity of OMM is injured by different factors, such as hypoosmotic shock [21] or ischemia [6]. Furthermore, it is known that at an appropriate concentration, palmitoyl-CoA and palmitoyl-L-carnitine, as detergents, may disturb intactness of outer and inner mitochondrial membranes [8,9].

However, as seen in Table 1, a stimulating effect of the exogenous cytochrome *c* on the state 3 mitochondrial respiration was negligible or absent. It should be stressed that it was similar with all respiratory substrates used in this study. This finding clearly shows that OMM remained intact in all experimental groups, and, therefore, its injury as the cause of increase in permeability for ADP in case of the fatty acid oxidation is denied. Concentrations of palmitoyl-CoA (12.2 μM) and palmitoyl-L-carnitine (9 μM) used in this study have clearly been too low to affect the integrity of OMM. When the OMM was injured by pre-treatment of fibers with high concentration saponin, the app. $K_m^{ADP}$ value considerably decreased (Fig. 2, column 8, when compared with column 1). In this case, it was not influenced by palmitoyl-L-carnitine oxidation (in the presence of pyruvate+malate; Fig. 2, column 9, when compared with column 8).

The both respiratory substrates, palmitoyl-L-carnitine and palmitoyl-CoA (+L-carnitine), were equally effective in decreasing app. $K_m^{ADP}$, but (as suggested in [19]) only the latter one is transported into mitochondria through the porin channel. Therefore, we may assume that the carnitine-dependent palmitoyl-CoA transfer through the porin channel is not responsible for facilitation of the ADP diffusion into mitochondria.

App. $K_m^{ADP}$ has remained high in experiments with pyruvate+malate+palmitoyl-CoA in the absence of carnitine (Fig. 2, column 6), i.e. in conditions preventing transfer of the exogenous palmitoyl moiety into mitochondria, and, thus, its oxidation by the enzyme system of β-oxidation localized in the matrix. Another experiment with the mixture of respiratory substrates – palmitoyl-L-carnitine and succinate (+rotenone) – was designed to reveal whether internal palmitoyl-CoA can affect this parameter. In these conditions, palmitoyl-L-carnitine can be transported across the inner membrane into the mitochondrial matrix, and can react with carnitine palmitoyltransferase II to be converted to palmitoyl-CoA. However, oxidation of the latter compound is prevented by rotenone. The app. $K_m^{ADP}$ values were not different during succinate (+rotenone) oxidation in the presence and in the absence of palmitoyl-L-carnitine. Taken together, these data show that neither the external nor the internal fatty acyl-CoA is active as a regulator of OMM permeability for ADP (does not affect the app. $K_m^{ADP}$ value). Increase in it becomes apparent exclusively in cases when fatty acid derivatives, i.e. palmitoyl-CoA, palmitoyl-L-carnitine and octanoyl-L-carnitine, are oxidized in mitochondria. In line with the above conclusion, non-oxidizable fatty acid derivative octanoyl-D-carnitine, when added to the mitochondria oxidizing pyruvate+malate, did not change the app. $K_m^{ADP}$ value.

It should be stressed that both L- and D-isomers of palmitoylcarnitine are transported as cations into mitochondria, and induce an energy-dependent swelling of the mitochondrial matrix in similar rate and to a similar extent [22]. Thus, it may be concluded that the swelling or any other fatty acid transport-related structural changes of mitochondria per se are not a sufficient factor to induce changes in the OMM permeability for ADP. Apparently, concerted action of both the fatty acid transfer-induced swelling of mitochondria and the fatty acid oxidation are necessary. Swelling of the matrix is known (i) to decrease the intermembrane space and (ii) to increase the number of contact sites between outer and inner membranes. As to the fatty acid oxidation, it seems to induce unknown changes in mitochondrial properties, which make porin more permeable for ADP.

Recently, a doubt was expressed concerning correctness of the high Michaelis constants of oxidative phosphorylation as determined in saponin-permeabilized cells and fibers [23]. Authors of this study assumed that app. $K_m^{ADP}$ values may be overestimated, i.e. erroneously too high, due to the complex structure of these biological systems in which at least two concentration gradients of ADP should exist – one across OMM, and the other between the cytosolic space and the bulk phase (incubation medium). This was suggested to hamper the diffusion of ADP into mitochondria. On the basis of our results, it seems unreasonable to assume a possibility of alteration of intracellular membrane structures beyond mitochondria. Therefore, we think that the above speculations on the unreliability of app. $K_m^{ADP}$ values determined with saponin-treated cardiac fibers are doubtful. This conclusion is in agreement with experimental and theoretical results of some other investigators [24], showing no ADP gradients between the bulk water phase inside the permeabilized cardiac fiber and the incubation medium.

Taken together, the data presented in this paper for the first time demonstrate the interaction of the fatty acid oxidation system with the porin channel, and its relevance in the regulation of the OMM permeability for ADP. The precise mechanism of this phenomenon, however, remains to be elucidated in further experiments.

*Acknowledgements:* This work was in part supported by the Lithuanian State Science and Studies Foundation.

## References

[1] Saks, V.A., Veksler, V.I., Kuznetsov, A.V., Kay, L., Sikk, P., Tiivel, T., Tranqui, L., Olivares, J., Winkler, K., Wiedemann, F. and Kunz, W.S. (1998) Mol. Cell. Biochem. 184, 81–100.
[2] Saks, V.A., Kuznetsov, A.V., Khuchua, Z.A., Vasilyeva, E.V., Belikova, J.O., Kesvatera, T. and Tiivel, T. (1995) J. Mol. Cell. Cardiol. 27, 625–645.
[3] Kay, L., Li, Z., Mericskay, M., Olivares, J., Tranqui, L., Fontaine, E., Tiivel, T., Sikk, P., Kaambre, T., Samuel, J.-L., Rappaport, L., Usson, Y., Leverve, X., Paulin, D. and Saks, V.A. (1997) Biochim. Biophys. Acta 1322, 41–59.
[4] Toleikis, A., Majiene, D., Trumbeckaite, S., Dagys, A. and Jasaitis, A. (1996) Biosci. Rep. 16, 513–519.
[5] Liobikas, J., Kopustinskiene, D.M. and Toleikis, A. (2001) Biochim. Biophys. Acta 1505, 220–225.
[6] Kay, L., Rossi, A. and Saks, V. (1997) Mol. Cell. Biochem. 174, 79–85.
[7] Kuznetsov, A.V., Tiivel, T., Sikk, P., Kaambre, T., Kay, L., Daneshrad, Z., Rossi, A., Kadaja, L., Peet, N., Seppet, E. and Saks, V.A. (1996) Eur. J. Biochem. 241, 909–915.
[8] Wojtczak, L. (1976) J. Bioenerg. Biomembr. 8, 293–311.
[9] Piper, M.H., Sezer, O., Schwartz, P., Hütter, J.F., Schweickhardt, C. and Spieckermann, P.G. (1984) Basic Res. Cardiol. 79, 186–198.
[10] Goni, F.M., Requero, M.A. and Alonso, A. (1996) FEBS Lett. 390, 1–5.
[11] Wojtczak, L. and Nalecz, M.J. (1979) Eur. J. Biochem. 94, 99–107.
[12] Pressman, B.C. and Lardy, H.A. (1956) Biochim. Biophys. Acta 21, 458–466.
[13] Skulachev, V.P. (1998) Biochim. Biophys. Acta 1363, 100–124.
[14] Skulachev, V.P. (1999) J. Bioenerg. Biomembr. 31, 431–445.
[15] Panov, A.V., Konstantinov, Y.M. and Lyakhovich, V.V. (1975) J. Bioenerg. Biomembr. 7, 75–85.
[16] Wieckowski, M.R., Brdiczka, D. and Wojtczak, L. (2000) FEBS Lett. 484, 61–64.

[17] Sultan, A. and Sokolove, P.M. (2001) Arch. Biochem. Biophys. 386, 52–61.
[18] Di Paola, M., Cocco, T. and Lorusso, M. (2000) Biochem. Biophys. Res. Commun. 277, 128–133.
[19] Turkaly, P., Kerner, J. and Hoppel, C. (1999) FEBS Lett. 460, 241–245.
[20] Kerner, J. and Hoppel, C. (2000) Biochim. Biophys. Acta 1486, 1–17.
[21] Saks, V.A., Vasilyeva, E.V., Belikova, J.O., Kuznetsov, A.V., Lyapina, S., Petrova, L. and Perov, N.A. (1993) Biochim. Biophys. Acta 1144, 134–148.
[22] Levitsky, D.O. and Skulachev, V.P. (1972) Biochim. Biophys. Acta 275, 33–50.
[23] Gellerich, F.N., Laterveer, F.D., Korzeniewski, B., Zierz, S. and Nicolay, K. (1998) Eur. J. Biochem. 254, 172–180.
[24] Saks, V.A., Kaambre, T., Sikk, P., Eimre, M., Orlova, E., Paju, K., Piirsoo, A., Appaix, F., Kay, L., Regitz-Zagrosek, V., Fleck, E. and Seppet, E.K. (2001) Biochem. J. 356, 643–657.