Biol. Stud. 2023; 17(3): 243–252 • DOI: https://doi.org/10.30970/sbi.1703.735
www.http://publications.lnu.edu.ua/journals/index.php/biology

UDC: 612.34:612.26:577.23

UNCOUPLED RESPIRATION STABILITY OF ISOLATED PANCREATIC ACINI AS A NOVEL FUNCTIONAL TEST FOR CELL VITALITY

Anastasiia Zub*, *Bohdan V. Manko*, *Bohdan O. Manko*, *Volodymyr Manko*, *Andriy Babsky

Ivan Franko National University of Lviv, 4 Hrushevsky St., Lviv 79005, Ukraine

Zub, A., Manko, B. V., Manko, B. O., Manko, V., & Babsky, A. (2023). Uncoupled respiration stability of isolated pancreatic acini as a novel functional test for cell vitality. *Studia Biologica*, 17(3), 243–252. doi:10.30970/sbi.1703.735

Background. Assessment of cell viability is crucial in cell studies. Testing plasma membrane integrity is a traditional approach of evaluating cell viability. Mitochondrial functional capacity closely correlates with plasma membrane integrity and overall cell health. This study aimed to investigate whether any aspect of mitochondrial adaptive capacity in isolated pancreatic acini is associated with the quality of said preparations, as determined by the dye exclusion method.

Materials and Methods. Experiments were carried out on male Wistar rats weighing 250–300 g. A suspension of isolated pancreatic acini was obtained using collagenase. The rate of oxygen consumption of rat isolated pancreatic acini was measured with Clark oxygen electrode. Basal respiration of isolated pancreatic acini was recorded for approximately 2 min. Afterwards, the mitochondrial adaptive capacity was examined using FCCP in concentrations from 0.5 to 2 μM. Uncoupled respiratory stability was calculated as a ratio of respiration rate at high and low FCCP concentrations. Plasma membrane integrity was assessed with trypan blue staining. A total of 74 preparations of isolated pancreatic acini were used in this study.

Results. In all experiments, 92–99 % of pancreatic acinar cells exhibited negative trypan blue staining, indicating intact plasma membranes. The basal and maximal uncoupled respiration rates were not affected by the fraction of trypan-negative cells. However, acini preparations with <less than 95 % plasma membrane integrity had significantly lower uncoupled respiration rates when exposed to a high concentration of FCCP (2 μM), indicating reduced stability of uncoupled respiration.

Conclusions. Results of the study suggest that the stability of uncoupled respiration can serve as a novel metabolic functional test to complement the existing methods for assessing cell vitality.

Keywords: pancreas, acinar cells, viability, uncoupled respiration

INTRODUCTION

In vitro experiments involving isolated live cells, whether cultured immortalized cell lines or primary cells obtained from living organisms, are extensively conducted in various biological sstudies. Cell viability is a critical aspect for conducting accurate examinations of biological processes. Impaired metabolism, disrupted ion transport, and compromised physiological responses to stimuli are observed in damaged or necrotic cells. Traditional approaches to evaluate cell viability include testing plasma membrane integrity using techniques like trypan blue staining or assessing metabolic activity with methods such as the MTT test.

Mitochondrial functional activity in cells is highly correlated with plasma membrane integrity. In renal cells, cold injury causes disruption of cell morphology, a 70% lactate dehydrogenase activity release, and nearly complete loss of both basal and uncoupled cell respiration (Cassim *et al.*, 2022). In cardiac myocytes, hypoxia-reoxygenation induces a reduction of maximal uncoupled respiration in parallel with an increase of cell necrosis measured with a combination of calcein-AM and plasma membrane impermeant ethidium homodimer-1 (Pfleger *et al.*, 2015). In pancreatic acinar cells, hydrogen peroxide causes a decrease in both basal and maximal respiration, while menadione intensifies basal respiration and reduces maximal uncoupled respiration; both compounds also cause an increase in lactate dehydrogenase release (Armstrong *et al.*, 2018). Interestingly, antioxidant mitoQ also causes a significant decrease in both uncoupled respiration and plasma membrane integrity of pancreatic acinar cells (Armstrong *et al.*, 2019). In many experimental settings, the mitochondrial damage precedes cell death with or without the signs of plasma membrane rupture (Armstrong *et al.*, 2019; Bock & Tait, 2020; Manko *et al.*, 2021). It is thus expedient to develop new highly sensitive cell viability assays based on mitochondrial function evaluation.

Recently, our research group characterized the mitochondrial adaptive capacity of pancreatic acinar cells using a comprehensive set of uncoupled respiration parameters (Manko *et al.*, 2019). The present study aimed to investigate whether any aspect of mitochondrial adaptive capacity in isolated pancreatic acini is associated with the quality of isolated pancreatic acini preparations, as assessed with the dye exclusion method.

MATERIALS AND METHODS

Materials. Reagents used in experiments were purchased from Sigma-Aldrich (St. Louis, Mo): sodium chloride, glucose, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), soybean trypsin inhibitor, bovine serum albumin (BSA), sodium pyruvate, glutamine, FCCP, collagenase type IV; Merck Chemicals (Burlington, Mass) – Calcium chloride dihydrate. All other reagents were of the purest available grade.

Experimental animals. All manipulations with animals are accomplished in accordance with the ’European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes’ (Council of Europe No 123, Strasbourg 1985). Experimental protocols were approved by the Animal Care and Use Committee

of Ivan Franko National University of Lviv (protocol No. 33-04-2023 of 03.04.2023). Experiments were carried out on male Wistar rats (250–300 g). Animals were kept under the standard conditions of a vivarium at a constant temperature with 12:12-h light-dark cycle and on a basic diet. *In vitro* experiments were performed on pancreatic acini isolated from animals, which were starved for 12 h prior to the experiment.

Isolation and viability assessment of pancreatic acini. A suspension of isolated pancreatic acini was obtained with collagenase (type IV, 0.2 mg/mL) as previously reported (Manko *et al.*, 2013) in basic incubation medium containing (mM): NaCl – 140.0, KCl – 4.7, $CaCl_2$ – 1.3, $MgCl_2$ – 1.0, HEPES – 10.0, glutamine – 2.0, sodium pyruvate – 2.0, glucose – 10.0; BSA – 2.5 mg/mL; soybean trypsin inhibitor – 0.1 mg/mL and essential MEM amino acid supplement; pH set at 7.4 with NaOH. Cell counting was performed with a hemocytometer with trypan blue staining as described previously (Manko *et al.*, 2021).

Oxygen consumption. The rate of oxygen consumption of isolated pancreatic acini was measured with Clark oxygen electrode at 37 °C using YSI 5300 Biological oxygen monitor (Yellow Springs Instruments) or SI929 6-channel Oxygen Meter (Strathkelvin). Extracellular-like respiration medium contained (mM): NaCl – 140.0, KCl – 4.7, $CaCl_2$ – 1.3, MgCl2 – 1.0, HEPES – 10.0, glucose – 10.0; BSA – 2.5 mg/mL and soybean trypsin inhibitor – 0.1 mg/mL; pH set at 7.4 with NaOH. Protonophore FCCP was added stepwise into the respiration chamber in 0.5 μM aliquots (2.0 μM total) to reach the maximal uncoupled respiration rate as described earlier (Manko *et al.*, 2019).

Statistical analysis. Results are presented as means ± S.E.M. Statistical analysis was performed using Origin Pro 2018 (Northampton, Mass) software. The significance of difference between the groups was determined with a one-way ANOVA followed by a Turkey corrected post-hoc *t*-tests in case of a significant difference according to ANOVA.

RESULTS and DISCUSSION

In the course of this study, to ensure a high statistical power, a total of 74 preparations of isolated pancreatic acini were examined. To reduce the effects of investigator bias, a total of 59 of these preparations (experiment 1) were performed by one scientist and other 15 were later reproduced by another scientist (experiment 2). The viability of pancreatic acini was assessed with trypan blue staining immediately after the isolation procedure. In all experiments, cell viability exceeded 92 %, while in experiment 2 it was significantly higher than in experiment 1 (**Fig. 1**, **Fig. 2*B***). This may indicate the difference in cell preparation technique or in viable cell number estimation between the two scientists. Cells with intact plasma membrane are not stained with trypan blue and thus are difficult to count in large three-dimensional acini under the microscope. We have also divided experiment 1 into two clusters based on cell viability with a 95 % cutoff as a typical indicator of a high-quality cell preparation (**Fig. 1*C***).

Basal respiration of freshly isolated pancreatic acini was recorded for approximately 2 min. Afterwards, titration with FCCP with 0.5 μM aliquots (final concentrations 0.5, 1.0, 1.5 and 2.0 μM, ~2 min for each concentration) was performed to reach the maximal uncoupled respiration rate. We have noted that the kinetics of respiration response to FCCP varied greatly among preparations. In part of experiments, the maximal respiration was already reached at 0.5–1 μM FCCP; further increase in the protonophore concentration resulted in a marked inhibition of oxygen consumption rate (**Fig. 1*A***). In other cases, the respiration rate steadily increased up to 1.5–2 μM FCCP (**Fig. 1*B***). There were also all kinds of intermediate kinetic responses. In order to describe such

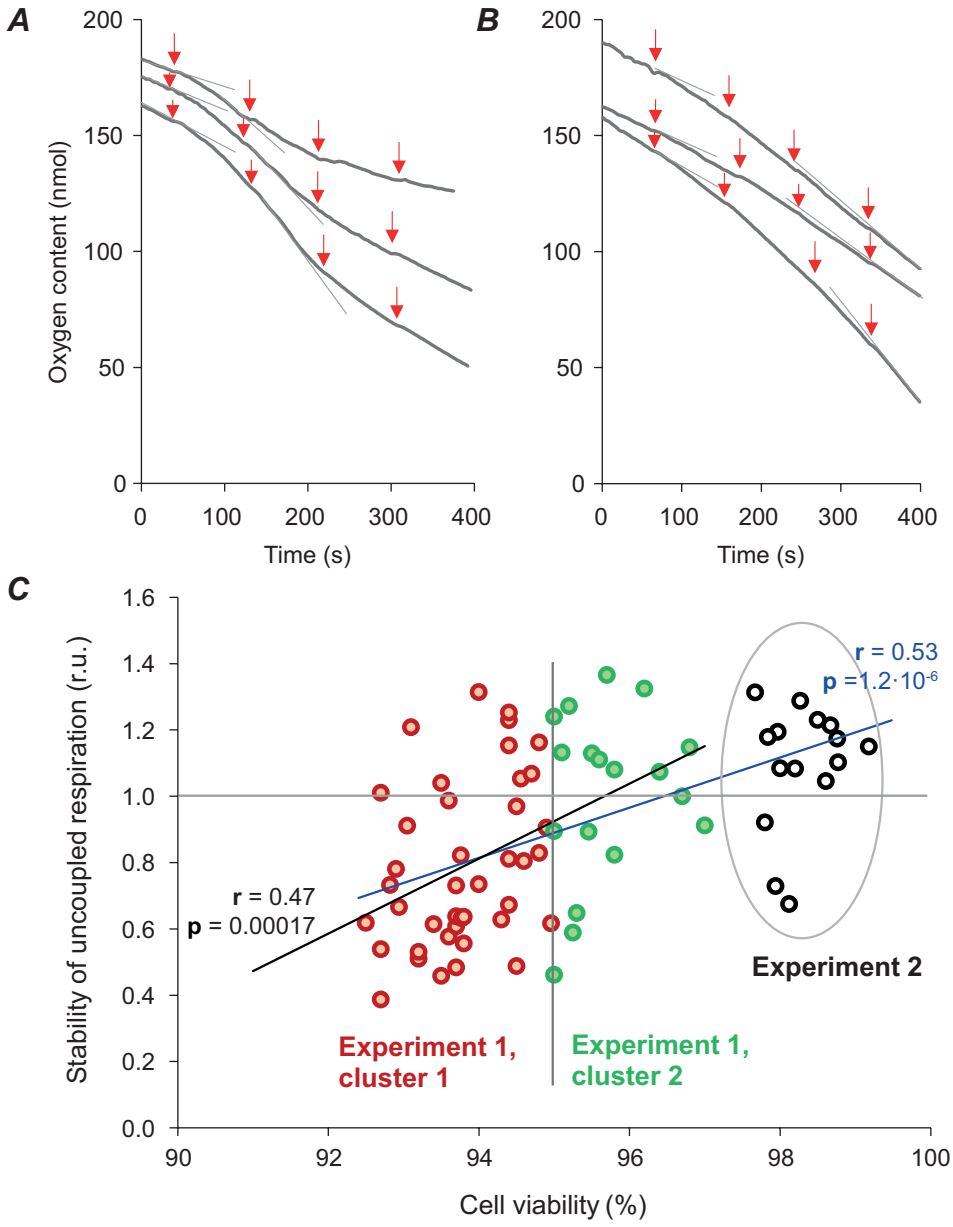

Fig. 1. Dependence of uncoupled respiration stability of isolated pancreatic acini on cell viability: ***A***, ***B*** – different examples of original respiration recordings, arrows indicate addition of 0.5 μM FCCP aliquot; ***C*** – data from experiment 1 were split into cluster 1 – cell viability below 95 % (red points) and cluster 2 – cell viability over 95 % (green points); experiment 2 data form cluster 3 with cell viability over 95 % (black points). Black and blue lines are the results of linear regression of experiment 1 data (black) and both experiments (blue) with corresponding Pearson's r and p values. Each experiment is presented by a separate point. Experiment 1 was performed by one scientist with a YSI 5300 biological oxygen monitor, while experiment 2 was performed by another scientist with a SI929 6-channel oxygen meter

discrepancies in FCCP effects, formerly we used $[FCCP]_{max}$ – the optimal FCCP concentration when the maximal respiration rate is reached (Manko *et al.*, 2019). However, the discrete nature of this parameter renders statistical analysis problematic. In addition, it is difficult to establish optimal concentration when neighboring values of respiration are very close to each other. Thus, we calculated the uncoupled respiratory "stability" as a ratio of respiration rate at high and low FCCP concentrations according to the equation:

$$Stability = (V_{1.5}+V_{2.0})/(V_{0.5+}V_{1.0}),$$

where V is an uncoupled respiration rate with index as FCCP concentration in μM.

To test if the uncoupled respiratory stability depended on the plasma membrane integrity, we have plotted data of the two parameters at **Fig. 1**. Above a horizontal dashed line (Stability = 1.0) are experiments in which the respiration rate continued to increase when high concentrations (1.5–2 μM) of FCCP were added. Conversely, when high FCCP concentrations inhibited respiration, the stability was lower than 1. Clearly, most of the datapoints (31 of 41) in cluster 1 of experiment 1 have stability values under 1, which is not the case for cluster 2 (7 of 18) and experiment 2 (3 of 15) (**Fig. 1**). Furthermore, there was a significant moderate positive correlation between the stability of uncoupled respiration and the number of trypan-negative cells with Pearson's $r = 0.53$ for the whole sample ($n = 74$, $p = 0.0000012$) and 0.47 for experiment 1 data ($n = 59$, $p = 0.0001728$).

The statistical analysis of the differences between three experimental clusters was performed with ANOVA followed by a post-hoc Turkey test. As expected, the fraction of trypan-positive dead cells was significantly lower in experiment 1 cluster 2 compared to cluster 1, and even lower in experiment 2 (**Fig. 2*B***). The basal (no FCCP added) and maximal respiration rates remained the same between all groups (**Fig. 2*A***, ***C***). It was previously shown that basal and maximal respiration rates are proportional to cell viability (Armstrong *et al.*, 2018; Cassim *et al.*, 2022). Apparently, small cell viability differences do not significantly affect respiration parameters. However, the uncoupled respiration upon 2 μM FCCP was significantly higher in experiment 2 compared to experiment 1 cluster 1, indicating higher uncoupled respiration stability (**Fig. 2*A***). There was no statistical difference between the respiration rates in Experiment 1 cluster 1 and experiment 2, even though the experiments were performed with different instruments and by different scientists (see Methods section) (**Fig. 2*C***).

Our study revealed a notable association between slight differences in cell viability, as determined by the trypan blue exclusion test, and significant effects on uncoupled respiration stability. Basal respiration of trypan-positive cells with either digitonin-permeabilized (Horbay *et al.*, 2012; Manko *et al.*, 2013; Pesta & Gnaiger, 2012; Rose *et al.*, 2019) or otherwise damaged (Cassim *et al.*, 2022) plasma membrane is usually more than 5-fold lower compared to intact cells due to metabolite efflux from cytoplasm. The respiration of permeabilized pancreatic acinar cells can be restored by the addition of exogenous oxidative substrates and ADP (Manko *et al.*, 2013; Manko & Manko, 2013). In our study, we investigated the respiration of pancreatic acini in extracellular-like respiration medium without ADP or Tricarboxylic acid cycle oxidative substrates. In addition, high Ca^{2+} and NaCl concentration in extracellular-like respiration medium is toxic to mitochondria of pancreatic acinar cells (Maléth & Hegyi, 2016; Manko *et al.*, 2013). Because of this, we assume that a small fraction of trypan-positive necrotic cells has a minimal contribution to oxygen consumption. Thus, uncoupled respiration stability is the aspect of the viable trypan-negative cell population metabolism.

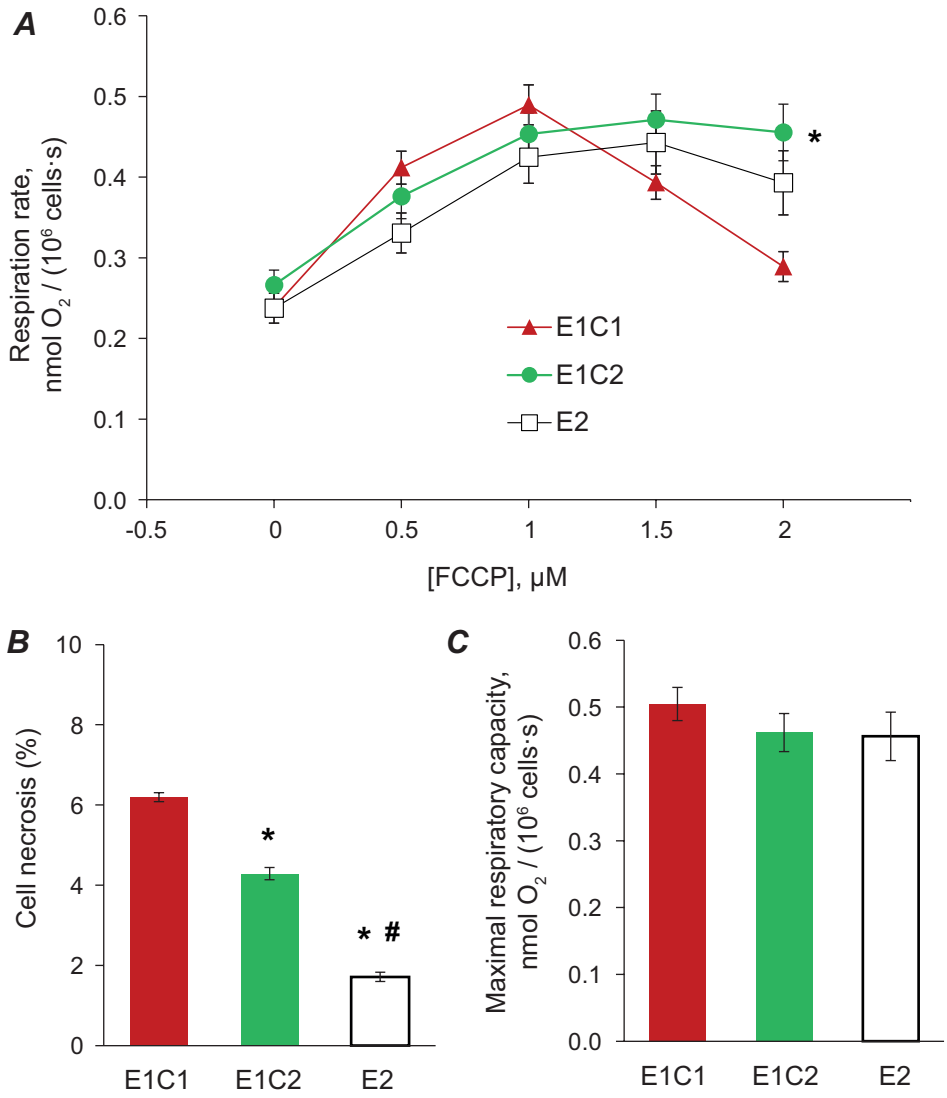

Fig. 2. Effect of isolated pancreatic acini viability on their basal and uncoupled respiration: ***A*** – respiration rates of pancreatic acini in cluster 1 (E1C1) and 2 (E1C2) of experiment 1, and experiment 2 (E2) upon uncoupling with FCCP in increasing concentrations (x axis shows the resulting FCCP concentration in the respiration chamber with 0.5 μM stepwise titration); ***B*** – the difference in pancreatic acinar cell necrosis in three groups; ***C*** – the maximal uncoupled respiration of pancreatic acinar cells; * – significant difference vs E1C1, $P \leq 0.05$, # – significant difference vs E1C2, $P \leq 0.05$, n = 41 (E1C1), 18 (E1C2) and 15 (E2), data are shown as M ± S.E.M.

We have previously established that the uncoupled respiration stability in isolated pancreatic acini depends on the type of oxidative substrate added to the medium (Manko *et al.*, 2019). We observed that the rapid decline in uncoupled respiration can be attributed to the limited accumulation of certain oxidative substrates (such as succinate, α-ketoglutarate, and malate) due to the low plasma membrane permeability of

intact pancreatic acinar cells (Manko *et al.*, 2013, 2019), Interestingly, we found that the ester forms of these substrates (such as monomethyl-succinate and dimethyl-α-ketoglutarate), which can penetrate the cell membrane, supported higher stability of uncoupled respiration. Therefore, the decrease in uncoupled respiration stability in less viable acini preparations is most likely a result of depletion of intracellular oxidative substrates, which cannot be rapidly replenished by glycolysis alone.

MTT assay is a conceptually similar approach to studying cell metabolic activity, as it relies on the activity of NADPH-dependent oxidoreductases. In human trophoblast-like cells, the decrease in maximal uncoupled respiration upon action of linoleic acid correlates with MTT test results (Shrestha *et al.*, 2019). However, in astrocytes, glutamate treatment caused a decrease in maximal uncoupled respiration, but did not affect the MTT assay (Yan *et al.*, 2017). The direct comparison of sensitivity and reliability of the uncoupled respiration and MTT assay is a subject for further studies. An important issue of interpretation of results of the uncoupled respiration and other cell metabolism tests is that the changes of metabolic parameters are often not related to cell viability. The pitfalls of MTT assay related to the use of metabolic inhibitors in experiments have been discussed recently (Stepanenko & Dmitrenko, 2015). Similarly, we reported that the decrease of maximal uncoupled respiration in pancreatic acinar cells oxidizing glutamine was not accompanied by any changes in cell viability (Manko *et al.*, 2021).

CONCLUSION

The findings of this study lead to several important conclusions. Firstly, it is advisable to use only preparations of pancreatic acini with high viability (> 95 % trypan-negative) in experiments to avoid the effects of negative metabolic shift associated with lower quality preparations. However, further investigations are required to determine whether this conclusion holds true for other cell types and to establish appropriate viability thresholds. Secondly, the conventional assessment of maximal uncoupled respiration rate proves to be insufficiently sensitive in detecting mitochondrial and cellular damage. We have established that the stability of uncoupled respiration, i.e., the ability of mitochondria to sustain a high respiration rate with an increasing uncoupler concentration, is more sensitive to cell damage caused by isolation procedures. It is still unknown if early mitochondrial and cell damage induced by toxins or other factors can be detected similarly. Thirdly, relying solely on a single approach (plasma membrane integrity or cell metabolic activity) is inadequate for comprehensive cell viability and functional integrity evaluation. We propose using the stability of uncoupled respiration as a novel metabolic functional test to complement the existing methods for assessing cell viability.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: The authors have no competing interests to declare that are relevant to the content of this article.

Human Rights: This article does not contain any studies with human subjects performed by any of the authors.

Animal Rights: All experiments were carried out in accordance with the „European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes” (Council of Europe No. 123, Strasbourg 1985). All animal procedures were approved by the Committee for the Care and Use of Animals of the Ivan Franko National University of Lviv under the protocol No. 33-04-2023 of 03.04.2023.

AUTHOR CONTRIBUTIONS

Conceptualization, [M.B.O.; M.V.]; methodology, [M.B.O.]; investigation, [Z.A.; M.B.V.]; resources, [M.V.; B.A.]; data curation, [M.B.O.]; writing – original draft preparation, [Z.A.; M.B.V., M.B.O.]; writing – review and editing, [M.V.; B.A.]; visualization, [M.B.O.] supervision, [M.B.O., M.V.; B.A.]; project administration, [M.V.; B.A.]; funding acquisition, [–].

All authors have read and agreed to the published version of the manuscript.

REFERENCES

Armstrong, J. A., Cash, N. J., Ouyang, Y., Morton, J. C., Chvanov, M., Latawiec, D., Awais, M., Tepikin, A. V., Sutton, R., & Criddle, D. N. (2018). Oxidative stress alters mitochondrial bioenergetics and modifies pancreatic cell death independently of cyclophilin D, resulting in an apoptosis-to-necrosis shift. *Journal of Biological Chemistry*, 293(21), 8032–8047. doi:10.1074/jbc.ra118.003200
Crossref ● PubMed ● PMC ● Google Scholar

Armstrong, J., Cash, N., Morton, J., Tepikin, A., Sutton, R., & Criddle, D. (2019). Mitochondrial targeting of antioxidants alters pancreatic acinar cell bioenergetics and determines cell fate. *International Journal of Molecular Sciences*, 20(7), 1700. doi:10.3390/ijms20071700
Crossref ● PubMed ● PMC ● Google Scholar

Bock, F. J., & Tait, S. W. G. (2020). Mitochondria as multifaceted regulators of cell death. *Nature Reviews Molecular Cell Biology*, 21(2), 85–100. doi:10.1038/s41580-019-0173-8
Crossref ● PubMed ● Google Scholar

Cassim, S., Martin, P.-Y., & Pascolo-Rebouillat, E. (2022). ADD10 protects renal cells from cold injuries by improving energy metabolism. *Biochemical and Biophysical Research Communications*, 634, 62–69. doi:10.1016/j.bbrc.2022.10.009
Crossref ● PubMed ● Google Scholar

Horbay, R. O., Manko, B. O., Manko, V. V., Lootsik, M. D., & Stoika, R. S. (2012). Respiration characteristics of mitochondria in parental and giant transformed cells of the murine Nemeth–Kellner lymphoma. *Cell Biology International*, 36(1), 71–77. doi:10.1042/cbi20110017
Crossref ● PubMed ● Google Scholar

Maléth, J., & Hegyi, P. (2016). Ca^{2+} toxicity and mitochondrial damage in acute pancreatitis: translational overview. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 371(1700), 20150425. doi:10.1098/rstb.2015.0425
Crossref ● PubMed ● PMC ● Google Scholar

Manko, B. O., Bilonoha, O. O., & Manko, V. V. (2019). Adaptive respiratory response of rat pancreatic acinar cells to mitochondrial membrane depolarization. *The Ukrainian Biochemical Journal*, 91(3). doi:10.15407/ubj91.03.034
Crossref ● Google Scholar

Manko, B. O., Bilonoha, O. O., Voloshyn, D. M., Zub, A. M., Ivasechko, I. I., & Manko, V. V. (2021). Pyruvate and glutamine define the effects of cholecystokinin and ethanol on mitochondrial oxidation, necrosis, and morphology of rat pancreatic acini. *Pancreas*, 50(7). doi:10.1097/mpa.0000000000001864
Crossref ● PubMed ● Google Scholar

Manko, B. O., Klevets, M. Yu., & Manko, V. V. (2013). An implication of novel methodology to study pancreatic acinar mitochondria under *in situ* conditions. *Cell Biochemistry and Function*, 31(2), 115–121. doi:10.1002/cbf.2864
Crossref ● PubMed ● Google Scholar

Manko, B. O., & Manko, V. V. (2013). Influence of Ca^{2+} on kinetic parameters of pancreatic acinar mitochondria *in situ* respiration. *The Ukrainian Biochemical Journal*, 85(4), 48–60. doi:10.15407/ubj85.04.048 (In Ukrainian)
Crossref ● PubMed ● Google Scholar

Pesta, D., & Gnaiger, E. (2012). High-resolution respirometry: OXPHOS protocols for human cells and permeabilized fibers from small biopsies of human muscle. In: C. Palmeira & A. Moreno (Eds.), *Mitochondrial bioenergetics. Methods in molecular biology* (Vol 810, pp. 25–58). Humana Press. doi:10.1007/978-1-61779-382-0_3
Crossref ● PubMed ● Google Scholar

Pfleger, J., He, M., & Abdellatif, M. (2015). Mitochondrial complex II is a source of the reserve respiratory capacity that is regulated by metabolic sensors and promotes cell survival. *Cell Death & Disease*, 6(7), e1835–e1835. doi:10.1038/cddis.2015.202
Crossref ● PubMed ● PMC ● Google Scholar

Rose, S., Carvalho, E., Diaz, E. C., Cotter, M., Bennuri, S. C., Azhar, G., Frye, R. E., Adams, S. H., & Børsheim, E. (2019). A comparative study of mitochondrial respiration in circulating blood cells and skeletal muscle fibers in women. *American Journal of Physiology-Endocrinology and Metabolism*, 317(3), E503–E512. doi:10.1152/ajpendo.00084.2019
Crossref ● PubMed ● Google Scholar

Shrestha, N., Cuffe, J. S. M., Holland, O. J., & Perkins, A. V. (2019). Linoleic acid increases prostaglandin E2 release and reduces mitochondrial respiration and cell viability in human trophoblast-like cells. *Cellular Physiology and Biochemistry*, 52(1), 94–108. doi:10.33594/000000007
Crossref ● PubMed ● Google Scholar

Stepanenko, A. A., & Dmitrenko, V. V. (2015). Pitfalls of the MTT assay: direct and off-target effects of inhibitors can result in over/underestimation of cell viability. *Gene*, 574(2), 193–203. doi:10.1016/j.gene.2015.08.009
Crossref ● PubMed ● Google Scholar

Yan, X., Shi, Z. F., Xu, L. X., Li, J. X., Wu, M., Wang, X. X., Jia, M., Dong, L. P., Yang, S. H., & Yuan, F. (2017). Glutamate impairs mitochondria aerobic respiration capacity and enhances glycolysis in cultured rat astrocytes. *Biomedical and Environmental Sciences*, 30(1), 44–51. doi:10.3967/bes2017.005
Crossref ● PubMed ● Google Scholar

СТАБІЛЬНІСТЬ РОЗ'ЄДНАНОГО ДИХАННЯ ІЗОЛЬОВАНИХ АЦИНУСІВ ПІДШЛУНКОВОЇ ЗАЛОЗИ ЯК НОВИЙ ФУНКЦІОНАЛЬНИЙ ТЕСТ ЖИТТЄЗДАТНОСТІ КЛІТИН

Анастасія Зуб, Богдан В. Манько, Богдан О. Манько, Володимир Манько, Андрій Бабський

Львівський національний університет імені Івана Франка
вул. Грушевського, 4, Львів 79005, Україна

Вступ. Аналіз життєздатності має вирішальне значення у клітинних дослідженнях. Перевірка цілісності плазматичної мембрани є традиційним підходом для визначення життєздатності клітин. Функціональна здатність мітохондрій тісно корелює з цілісністю плазматичної мембрани та загальною інтактністю клітини. Мета цього дослідження полягала в тому, щоби перевірити, чи пов'язані параметри мітохондріальної адаптивної здатності ізольованих панкреатичних ацинусів підшлункової залози із якістю препаратів ізольованих ацинарних клітин, визначеної за допомогою фарбування трипановим синім.

Матеріали та методи. Досліди проводили на щурах-самцях лінії Wistar масою 250–300 г. Суспензію ізольованих панкреатичних ацинусів отримували з використанням колагенази. Швидкість споживання кисню ізольованими ацинусами

підшлункової залози щурів вимірювали за допомогою кисневого електрода Кларка. Базальне дихання ізольованих ацинусів підшлункової залози реєстрували приблизно протягом 2 хв. Після цього досліджували адаптивну здатність мітохондрій за допомогою FCCP у концентраціях від 0,5 до 2 мкмоль/л. Незв'язану респіраторну стабільність розраховували як співвідношення частоти дихання за високих і низьких концентрацій FCCP. Цілісність плазматичної мембрани оцінювали за допомогою фарбування трипановим синім. У цьому дослідженні використано 74 різних препарати ізольованих ацинарних клітин підшлункової залози.

Результати. У всіх експериментах 92–99 % ацинарних клітин підшлункової залози були трипан-негативними. Базальне та максимальне роз'єднане дихання не залежали від фракції трипан-негативних клітин. Проте клітинні препарати з <95 % цілісністю плазматичної мембрани мали значно нижчу швидкість роз'єднаного дихання за високої (2 мкМ) концентрації FCCP, таким чином демонструючи низьку стабільність роз'єднаного дихання.

Висновки. Ми пропонуємо стабільність роз'єднаного дихання як новий тест метаболічної функції, що доповнює наявні методи оцінки життєздатності клітин.

Ключові слова: підшлункова залоза, ацинарні клітини, життєздатність, роз'єднане дихання

Received / Одержано 19 May, 2023 | Revision / Доопрацьовано 03 July, 2023 | Accepted / Прийнято 31 August, 2023 | Published / Опубліковано 27 September, 2023