Comments to the Ph. D. theses titled: Energy metabolism of skeletal muscle submitted by Moustafa Elkalf

The dissertation thesis is written in the classical form consisting of four main chapters: Introduction, Methodological procedures, Results and discussion and Conclusions. Two published manuscripts are not part of the thesis. The author aimed to assess: i) "all aspects of the relationship between cell culture conditions and the metabolic activity", ii) "the changes and if possible the deterioration in mitochondrial respiratory parameters of cultured cells in a high glucose concentration imitating uncontrolled hyperglycidemia, and to compare the mitochondrial performance to cells cultured in moderate glucose concentration" and iii) "metabolism, using different probes to study mitochondrial function and the subject of adverse reactions of mitochondrial targeting molecules".

The results belonging to the first and second aim of the thesis have brought valuable information for culturing and assay conditions of mouse myoblasts C2C12 with respect to using galactose in media, which affect mitochondrial metabolism, and its effect may differ in other cell lines as well as in human primary cell lines. There were obtained following findings: i) replacing glucose by galactose is not suitable for C2C12 cells and does not allow differentiation in this cell line, ii) maximal respiration is obtained under moderate concertation of glucose (1g/l) compare to high glucose (5g/l) and glucose free medium, iii) moderate concertation of glucose in the assay medium is the best condition to reveal differences in respiratory parameters in different metabolic phenotypes and iv) high glucose concentration (5g/l) during differentiation of C2C12 cells decreased maximal respiratory capacity and enzyme activity of CI and CIII complexes of respiratory chain compare to normoglycemic conditions.

The results belonging to the third aim of the thesis have brought important information about the adverse side effect of widely used mitochondrial membrane potential probes triphenylphosphonium (TPP^+) and methyltriphenylphosphonium salt (TPMP). There were revealed the two main findings in C2C12 cells as well as in skeletal muscle: first, increasing hydrophobicity of alkyl chain of TPP^+ negatively affected functional parameters of respiratory chain and second, TPMP significantly attenuates oxygen consumption rate via inhibiting Krebs cycle enzyme.

These findings may be important for setting of optimal experimental conditions and diagnostics of muscle pathophysiology.

Comments to the text of the thesis.

According to my opinion the text possesses the following serious weaknesses:

- Results and discussion are highly speculative mainly in *Chapter 3.*
- Numbering of the thesis chapters is not consistent.

- Despite of a sufficient number of references (180), there are 4 pages in the *Introduction* (pg. 4-8, chapters 1.1.1 – 1.1.3) without any reference. Contrary to that, there are some pages in the *Results* with numerous citations (e.g. pg. 64). There should be clearly presented obtained data in the *Results* section.

- Organization of the *Results and Discussion* sections is rather confusing. Information are often doubled. Moreover, it is not possible to find any short consistent comments to the obtained data containing the real value of the found changes.

General comment

The present work contains valuable results that have been published in two papers in international journals with IF. One manuscript is under review process. In addition, the author is co-author of other publications. The author has demonstrated his ability to handle a large number of methods, to evaluate data and clearly present the current state of knowledge in the thesis. However, in view of quite serious critical comments stated above I would recommend to include appropriate errata of the text before saving it to the library. After that the thesis will meet the requirements of the Charles University in Prague for granting the Ph.D. degree and I can recommend it for defence.

Questions to be discussed:

1) The first part of the study focused on glucose and galactose influence to mitochondrial metabolism in C2C12 cells and differentiation. It was shown that galactose is not metabolised. Are there expected some differences in the regulation of glucose uptake under four metabolic conditions in C2C12 cells (LG, HG, GAL, CF) and also in differentiated cells (LG, HG)?
2) In chapter 3.1 author showed that absence of glucose avoided differentiation of C2C12 in the presence of glutamin (4mM) and pyruvate (1mM). Is that due to lack of energy source (e.g. glutamin is not sufficient substrate) or due to other possible mechanisms?
3) In chapter 3.3.2 author showed decreased maximal respiratory capacity in C2C12 cells with high glucose phenotype, however there are not any changes in respiratory enzyme activity (pg.54). Is there possible explanation?
4) In chapter 6.3 is shown that TPMP (1μM) inhibits oxygen consumption rate (OCR) by about 60% and activates glycolysis (pg. 86). This effect is explained by Krebs cycle inhibition by TPMP (1mM) due to decrease of oxoglutarate dehydrogenase (OGDH) activity about 20%. Is the 20% decrease of OGDH sufficient condition for attenuation of OCR by about 60%?

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