Comments to thesis "Energy metabolism of skeletal muscle" submitted by Moustafa Elkalaf.

Submitted thesis is designed to investigate energy metabolism of muscle cells during growth and differentiation. It contains two parts. The first one presents information about the role of glucose and galactose in the medium on the cell growth and proliferation and the metabolic activity of cultured cells. In the second part was investigated the inhibitory effect of the of the triphenyl phosphonium moieties with the different lengths of the alkyl side chain on mitochondrial respiration, because TPP^+ is now very often used for targeting of various pharmacologically effective compounds to mitochondria.

Both parts try to establish optimum conditions for clinical studies of pathogenesis of muscle disorders and try to eliminate controversies concerning incubation conditions and define optimum substrate composition for the growth and differentiation of muscle cells.

In both parts are presented a new important data, in fact each of them could be sufficient as a theme for dissertation. The thesis contains a good review of literature with 180 references. The presented data were obtained by a wide spectrum of methods: cultivation of cells, isolation of mitochondria, measurements of enzyme activities, cell and mitochondrial respiration, evaluation of mitochondrial functions by immunofluorescence and flow-cytometer.

The main results are summarized in conclusions as:

1. Galactose is not suitable fuel for skeletal muscle.
2. Oxidative respiration is enhanced by lowering glucose
3. Glucose level optimization is essential to reveal the variation between different bioenergetic profiles
4. High glucose decreases mitochondrial respiratory capacity.

5. The hydrophobicity of mitochondrial targeting molecules negatively affects respiratory efficiency.
6. Methyltriphenylphosphonium inhibits Krebs cycle.

These data will help to established optimum conditions for evaluation of defects in energy metabolism of skeletal muscle both for diagnostic purposes and for studies of their pathogenesis.

The candidate has shown that he is able to define aims of his experimental work used wide spectrum of methods and evaluate and present experimental data and prepare them for publication.

I can therefore recommend the presented thesis as a basis for nomination MUDr. Moustafa Elkalaf - Doctor of Philosophy.

I have some remarks to presented thesis that are related to formal presentation of his work and that could be help him in preparation of his results for publication and other students in preparation of their thesis.

My major comments is related to the fact that the manuscript is too heavy i.e. 1kg 200g and contains too many data. The reader or reviewer has to digest and evaluate sometimes the data of six parameters in four experimental groups in two biological models. Also discussion is divided in six discussions to particular parts of results and elimination of tables from the Results and their presentation as Supplement is not comfortable for the reader.

Minor comments that should be corrected:
- list of abbreviations is on p. XVII and not XVI.
- what means homogenate enriched in the mitochondrial fraction. According to methods homogenate of the frozen tissue was divided into two fractions 600g sediment and 600g supernatant. Mitochondira should be in 600g supernatant, but this cannot be called homogenate.
- why you call glucose normoglycemic concentration (1g/L) - low glucose, when it is close to physiological concentration and why for your data you use g/L whereas for data from literature mM?

- Fig 3.2 has above the picture galactose 1g/L in the text to figure 5g/L.
- Fig. 44 is too small, 3 curves presented are not well seen.
- p. 6 - in Introduction it should be mentioned that succinate dehydrogenase has a specific position in Krebs cycle because contrary to other members of the cycle localized in the matrix is integral component of the respiratory chain in the mitochondrial inner membrane. This could be related to other remark on p.61 that "low glucose cells have higher content of mitochondria then HG. CS did not show any difference which indicates no functional changes in mitochondrial matrix enzymes." But there are changes of mitochondrial membrane enzymes Complex I and III, however, complex II is not increased similarly as CS. May be its biogenesis is more related to Krebs cycle than to the respiratory chain.
- p. 26 –what means 10% FBS?

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Prague Nov. 3. 2015.

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