Fatty acid substrates minimally contribute to mitochondrial oxidative phosphorylation in glioma

Mahalaxmi Ganjoo, Ashok S, Srinivas G.
Department of Biochemistry, SCTIMST

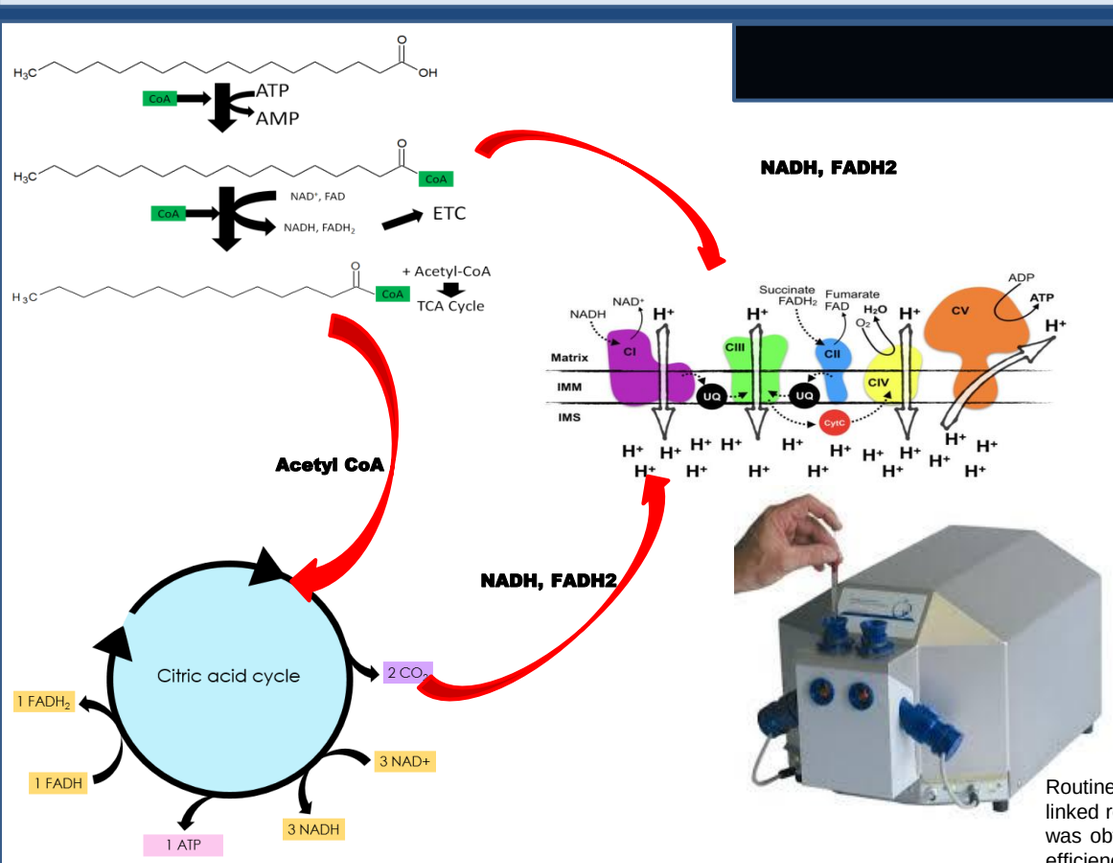
Introduction

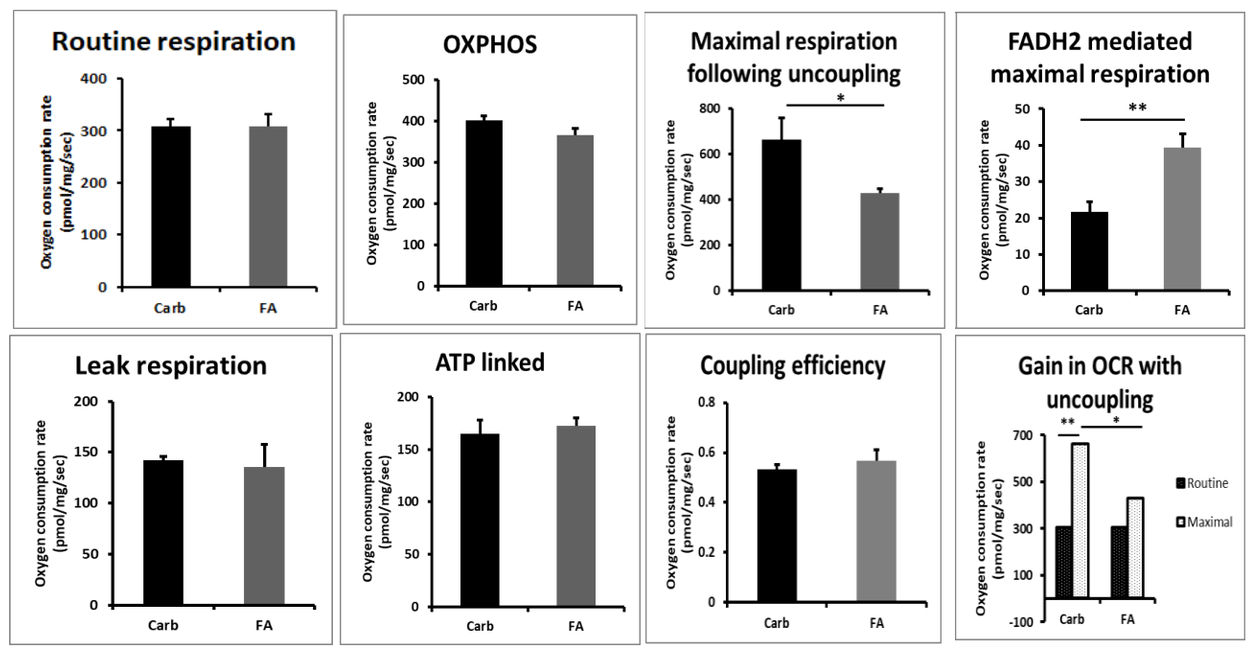
Glioma is the most aggressive of all brain tumors with a poor prognosis. Research in tumors have shown that the cancer cells show alteration in the metabolism that make them more metabolically plastic and capable of utilizing varied sources of fuel for energy production. Recent studies in glioma metabolism have pointed out the importance of fatty acids and lipids in glioma cell survival. Glioma cells have high fatty acid (FA) uptake and when the fatty acid uptake is blocked, the glioma aggressiveness is substantially reduced. In cancer cells, the fatty acids can be utilized for either mitochondrial energy production or biomass generation and it remains to be elucidated if FA are utilized for mitochondrial metabolism in glioma.

Methodology

- Mitochondrial measurements were done in U251 astrocytoma cell line with Oroboros O2K High resolution respirometry.
- Cells were harvested and re-suspended in MIRO5 respiration buffer.
- Fatty substrates included Palmitoyl carnitine and Octanoyl cartinine while carbohydrate substrates included Glutamate and Pyruvate.
- Following OCR measurements with carbohydrate or FA substrates, CCCP was used to uncouple the mitochondria to assess maximal respiration.
- Rotenone (inhibitor of NADH oxidoreductase/Complex1 of ETC) was employed to measure the contribution of FADH2 in OCR.
- Experiment was terminated with Antimycin A1 (Complex3 inhibitor) and values were normalized with total protein.

Results





Routine respiration was the OCR measurement after suspending the cells in MIRO5. Leak respiration was measured following permeabilization with digitonin. ATP linked respiration was measured by ADP supplementation. OXPHOS was the OCR measurement following carbohydrate or fatty acid substrates. Maximal respiration was obtained following CCCP uncoupling. FADH2 mediated maximal respiration was obtained after blocking NADH mediated respiration with Rotenone. Coupling efficiency was obtained with the formula (R-L)/R where R is routine respiration and L is leak respiration. Gain in OCR with uncoupling is depicted as a comparison of routine and maximal respiration for both carbohydrate and fatty acid substrates.

Results

- The FADH2 mediated respiration was shown to be higher in FA group compared with the carbohydrate group which could be contributed by the beta oxidation suggesting FA metabolism in glioma.
- The differences in the maximal respiration and gain in OCR with uncoupling it shows that the FA substrates were only minimally able to drive the electron transport chain (ETC) as compared to the carbohydrate substrates.

Conclusion

Although the higher FADH2 mediated respiration in the FA group shows the presence of beta oxidation in the mitochondria, with the maximal respiration and gain in OCR following uncoupling it can be concluded that in glioma cells the mitochondrial ability to utilize FA to drive the ETC is minimal compared with the same for carbohydrate substrates. We thus hypothesize that in glioma fatty acids may not contribute for energy production through beta oxidation and may be utilized for biomass generation or cellular signaling.

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