The Influence of Free Fatty acids on Metabolism of Endothelial Progenitor Cells (HUVECs)

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Introduction: Free fatty acids are involved in various mitochondrial processes including gene expression, respiratory function, ROS production and mitochondrial apoptosis. Mitochondria play a central role in the mechanisms switching the protective effects of autophagy toward the unwanted proapoptotic effect. In this study we compare the effect of the dietary exogenous FFAs and toxic TNFα on metabolism and mitochondrial function of the primary human endothelial (HUVEC) cells.

Methods: HUVECs were incubated separately with arachidonic acid (AA), palmitic acid (PA), eicosapentaenoic acid (EPA) or oleic acid (OA) (30μM) for 24 hours. TNFα (5 ng/ml) was added to the cell media for the last 4 hours of incubation. The changes in mitochondrial inner membrane potential (ΔΨ) were followed by the fluorescence microscopy imaging (Bioimager BD) or flow cytometry using TMRM. Metabolic activity of mitochondria was analyzed by ATP production (ROCHE) and oxygen requirement using Oxygraph 2-K (Oroboros). Changes in gene expression were analyzed by microarray (Affymetrix), confirmed by qRT-PCR (Opticon Research).

Results*:* Incubation with PA and OA decreased ΔΨ, while it was significantly increased in the presence of EPA and AA. Addition of TNFα resulted in reduction of the ΔΨ; and potentiation of the negative effect of PA and OA was found. On the contrary EPA reversed the negative effect of TNFα. Incubation with TNFα and EPA as well as PA resulted in significantly increased oxygen consumption by mitochondria. Incubation with EPA significantly increased the ATP content. Microarray analysis revealed an induction of intracellular substrate transporters involved in lipid/glucose uptake, metabolism, as well as the induction of the ER-shock chaperones proteins. All FFA augmented the TNFα-induced autophagy-related genes (ie chaperones, *LAMP2*) expression.

Conclusion: EPA protected the glycolytic HUVECs against the FFA-induced lipotoxicity (potentiated by the presence of TNFα) by accelerating glucose uptake and metabolism, and thus protected against mitochondrial dysfunction, ER stress and consequences such as stress-induced LD formation (phospholipidosis), cellular dysfunction and death.

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