The Effect of Saturated Fatty Acid on Immune Metabolism in Microglia

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Introduction

Diet high in saturated fatty acids such as palmitic acid (PA) can increase the propensity for obesity, microglial activation, and central inflammation (neuroinflammation). Cognitive decline is associated with increased neuroinflammation and the progressive loss of neurons, leading to cognitive disorders such as Alzheimer’s Disease (AD). The dietary metabolite PA can activate microglia via toll-like receptor-4 (TLR-4) dependent pathways promoting inflammation. We have previously shown this is in part due to the interaction of the lipid binding protein FABP4 with PA and altered mitochondrial uncoupling protein 2 (UCP2) function.

Hypothesis: We hypothesized that PA alters microglial metabolism to directly trigger brain immune responses and impair cognition.

Objective: Characterize microglial metabolic changes that promote neuroinflammation by measuring intracellular metabolic activity following exposure to PA.

Methods

Cell culture and Reagents: A total of 2.0 x 10⁶ BV2 murine microglial cells were cultured in DMEM supplemented with 10% FBS and 1% PSN (Invitrogen). Cells were maintained at 37°C with 5% CO₂. A pan-FABP inhibitor (HTS01037) was reconstituted in dimethyl sulfoxide (DMSO) and diluted to a final concentration of 30μM, concentration was determined via previous studies. Lipopolysaccharide (LPS) was diluted to a final concentration of 100 ng/ml, PA was complexed with fatty acid free bovine serum albumin (BSA) and diluted to a final concentration of 0.1 mM in DMEM. Cells were incubated with treatments at times points of 4h and 18h.

Mitochondrial Stress Assay: Mitochondrial stress assay (Seahorse XF, Agilent) was performed on BV2 cells following treatment with LPS, HTS and PA. Rates of oxygen consumption (OCR) was used in determining ATP turnover, proton leak respiration and rates of glycolysis in microglia. Seahorse wave software was used to graph OCR.

Statistical Analysis: Statistical differences were determined using ANOVA and Holm-Sidak’s post-hoc test using Graph Pad Prism 7.

Results

Figure 2. Seahorse XF Mitochondrial Stress Test Parameters (A-D). A, Seahorse XF Cell Mito Stress Test profile of key parameters of mitochondrial respiration. Seahorse XF analyzers simultaneously measure this proton flux as well as mitochondrial O2 consumption rate (OCR). B, Seahorse XF Glycolysis Stress Test profile of key parameters of glycolytic function including Glycolysis Capacity, reserve, and Non-glycolytic Acidification. C, Agilent Mito Stress Test modulators of the ETC D, Representation of glycolysis-derived lactate, and respiration. Extracellular acidification rate (ECAR) is thus an indicator of glycolysis.

Figure 3. 18-Hour LPS Mitochondrial Stress Test (A-E). Immortalized BV2 cells were incubated for 18-hours with a vehicle control (PBS) and challenged with LPS. Glycolytic rates quantified included basal and maximum respiration with p < 0.0001 (A, B) and ATP turnover and proton leak, with p < 0.0001 (C, D). OCAR was graphed using wave software (E).

Figure 4. 4-Hour and 18-Hour PA Mitochondrial Stress Test (A-J). Immortalized BV2 cells were incubated for 4-hours with a vehicle control (BDA) and challenged with PA. Glycolytic rates at 4h quantified included basal and maximum respiration with p < 0.0001 and p < 0.0001 (A, B) and ATP turnover and proton leak, with p < 0.0023 and p = 0.0033 (C, D). OCAR was graphed using Seahorse Wave software (E). Glycolytic rates at 18h included basal and maximum respiration with p = 0.0009 and p = 0.0032 (F, G, H) and ATP turnover and proton leak, with p = 0.0065 and p = 0.0001 (I, J). OCAR was graphed using Seahorse Wave software (A).

Figure 5. 4h and 18h Mitochondrial Stress Test PA+/- HTS (A-J). Immortalized BV2 cells were incubated for 4-hours with a vehicle control (PBS) and challenged with PA +/- HTS. HTS inhibitor was added 3h prior to incubation. Glycolytic rates quantified included basal and maximum respiration with p < 0.0001 (A, B) and ATP turnover and proton leak, with p = 0.0032 and p < 0.0001 (C, D). OCAR was graphed using Seahorse Wave software (E). Glycolytic rates at 18h included basal and maximum respiration with p < 0.0001 (F, G, H) and ATP turnover and proton leak, with p = 0.0001 (I, J). OCAR was graphed using Seahorse Wave software (A).

Conclusions

- PA and LPS lower ATP turnover, proton leak, basal and max respiration, corresponding to shifts towards the M1 phenotype and onset of neuroinflammation (Figs 3-4).
- Inhibition of FABP4 stabilizes basal respiration and OCR in the presence of PA (4h and 18h) indicating preservation of the M2 phenotype (Figs 4-6).
- This evidence supports the involvement of an FABP4-UCP2 axis in mitochondrial metabolic reprogramming in microglia.

Future Studies

- Profile glycolytic flux in response to altered FABP4-UCP2 axis.
- Profile neuroinflammation and the FABP4-UCP2 axis using microglia-specific UCP2 null mice.
- Determine if metabolic reprogramming contributes to diet-induced cognitive decline using a rodent model lacking FABP4 (PA2 knockout/AKO mice).
- Using a traditional model of AD such as APP/Fswe/Pswe chimeric mice carrying genes linked to familial AD to profile microglial metabolic response during high fat feeding.

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References