

ALTERED METABOLISM AND DAM-SIGNATURES IN FEMALE BRAINS AND MICROGLIA WITH AGING

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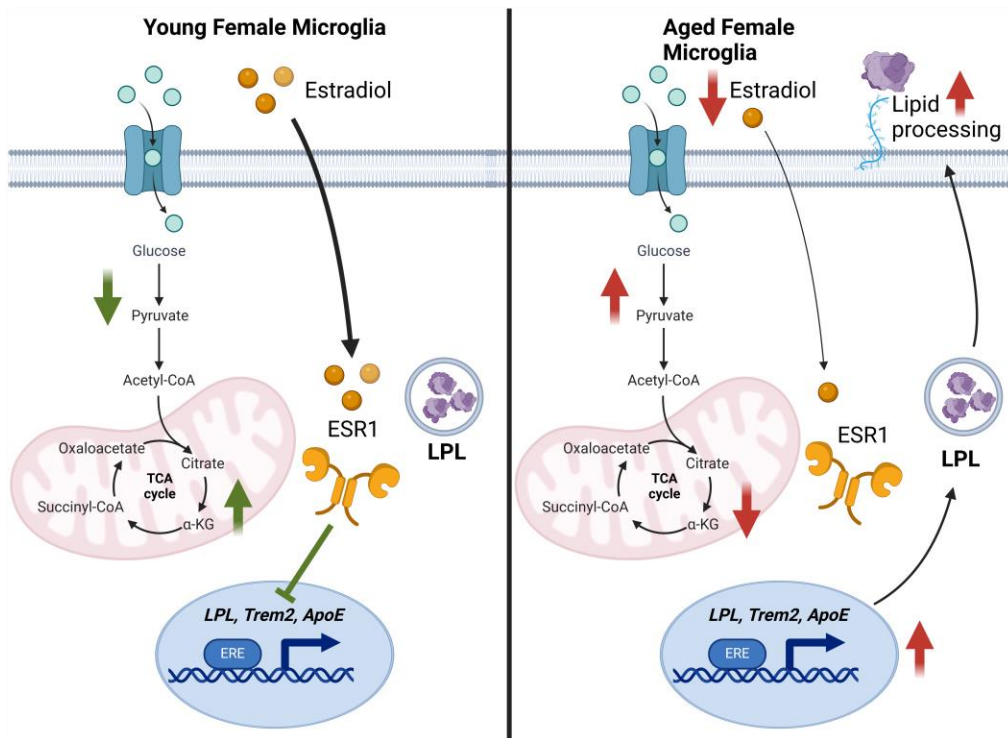
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Abstract

Despite Alzheimer's disease (AD) disproportionately affecting women, the mechanisms remain elusive. In AD, microglia undergo 'metabolic reprogramming', which contributes to microglial dysfunction and AD pathology. However, how sex and age contribute to metabolic reprogramming in microglia is understudied. Here, we use metabolic imaging, transcriptomics, and metabolic assays to probe age- and sex-associated changes in brain and microglial metabolism. Glycolytic and oxidative metabolism in the whole brain was determined using Fluorescence Lifetime Imaging Microscopy (FLIM). Young female brains appeared less glycolytic than male brains, but with aging, the female brain became 'male-like.' Transcriptomic analysis revealed increased expression of disease-associated microglia (DAM) genes (e.g., *ApoE*, *Trem2*, *LPL*), and genes involved in glycolysis and oxidative metabolism in microglia from aged females compared to males. To determine whether estrogen can alter the expression of these genes, BV-2 microglia-like cell lines, which abundantly express DAM genes, were supplemented with 17 β -estradiol (E2). E2 supplementation resulted in reduced expression of DAM genes, reduced lipid and cholesterol transport, and substrate-dependent changes in glycolysis and

oxidative metabolism. Consistent with the notion that E2 may suppress DAM-associated factors, LPL activity was elevated in the brains of aged female mice. Similarly, DAM gene and protein expression was higher in monocyte-derived microglia-like (MDMi) cells derived from middle-aged females compared to age-matched males and was responsive to E2 supplementation. FLIM analysis of MDMi from young and middle-aged females revealed reduced oxidative metabolism and FAD+ with age. Overall, our findings show that altered metabolism defines age-associated changes in female microglia and suggest that estrogen may inhibit the expression and activity of DAM-associated factors, which may contribute to increased AD risk, especially in post-menopausal women.

Keywords: Microglia, Alzheimer’s disease, aging, sex-differences, metabolism, lipids, lipoprotein lipase



Graphical Abstract A. Endogenous E2 in young females modulates microglial metabolism and inhibits DAM gene expression. **B.** These protective affects are lost with aging and menopause, leading to metabolic reprogramming and DAM gene expression.