Altered Metabolism and DAM-signatures in Female Brains and Microglia with Aging

Nicholas R W Cleland[1], Garrett J Potter[1], Courtney Buck[1], Daphne Quang[1], Dean Oldham[1], Mikaela Neal[1], Anthony Saviola[2], Christy Niemeyer[3], Evgenia Dobrinskikh[4], Kimberley D Bruce[1]

Denver | Anschutz

[1] Division of Endocrinology, Metabolism and Diabetes, University of Colorado Anschutz Medical Campus, Aurora, CO; [2] Department of Biochemistry and Molecular Genetics, University of Colorado Anschutz Medical Campus, Aurora, CO USA.; [3] Department of Neurology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA.; [4] Section of Neonatology, Department of Pediatrics, University of Colorado Anschutz Medical Campus, Aurora, USA.

Introduction

- Alzheimer's Disease (AD) is a neurodegenerative disease characterized by neuroinflammation, Aβ plaque accumulation, and altered lipid and cholesterol processing.
- AD is more prevalent in women, and more severe in female ApoE4 carriers, highlighting a sex-specific susceptibility to perturbed lipid processing and AD[2].
- Onset of AD occurs most commonly in the later stages of life, after the onset of menopause, when sex hormones like estrogen and progesterone are lowest[3].
- Microglia, the brain resident macrophages, control neuroinflammation, Aβ phagocytosis, and lipid and cholesterol processing to affect AD risk.
- Microglia become activated in response to stimuli, leading to metabolic switching towards glucose utilization (glycolysis) and away from oxidative metabolism. Chronic activation can lead to metabolic reprogramming[4].
- Microglial metabolic reprogramming has also been observed in AD, as well as other neurodegenerative diseases, and contributes to microglial dysfunction and AD pathology[8].
- In aging and AD, microglia express the immunometabolic regulators; Lipoprotein Lipase (LPL), Triggering receptor on myeloid cells (TREM2), and Apolipoprotein E (ApoE), and their variants increase AD risk[5,6,7].
- Although microglia have been found to be sexually dimorphic in phenotype[9], and function, whether metabolic changes in aging female microglia drive mechanisms leading to increased risk of neurodegenerative disease is understudied[10].

Hypothesis

We hypothesize that aging and estrogen levels regulate microglial metabolism, and the metabolic reprgamming asscoiated with the increased AD risk in women.

Methods

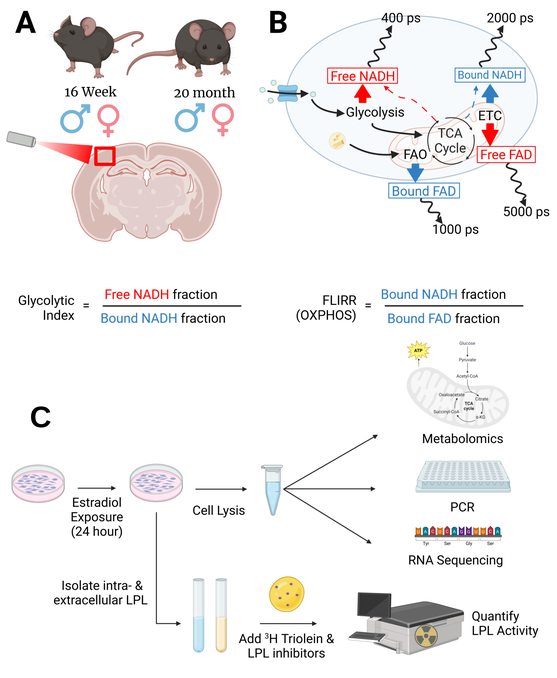

Figure 1. Data presented here used Fluorescence Lifetime Imaging Microscopy (FLIM) (**A, B**), metabolomics, qPCR, and bulk RNA sequencing to probe estradiol's (E2) effects on metabolic pathways and gene expression in BV-2 cells, monocyte derived microglia-like cells (MDMi's) and mouse brains. For all figures, (# p < 0.1, * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001).

2. Bulk RNAseq Reveals Increased Metabolic Dysregulation in Aged Female Mice

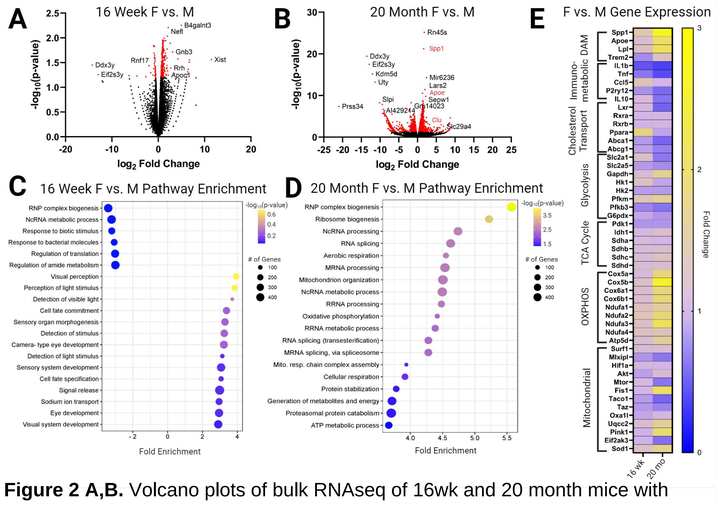

Figure 2 A,B. Volcano plots of bulk RNAseq of 16wk and 20 month mice with significant differences (predominantly in sex differences) in red. Genes known to be associated with AD (Apoe, Spp1, Clu) increased in females. **C.** Pathway analysis of 16wk mice RNAseq. **D.** Pathway analysis reveals differences in metabolic processes. **F.** Analysis of metabolic genes reveals sex differences with aging, suggesting potential metabolic derangement in aged females.

3. Female WT and 5XFAD Mice Exhibit Increased Lipoprotein Lipase Activity

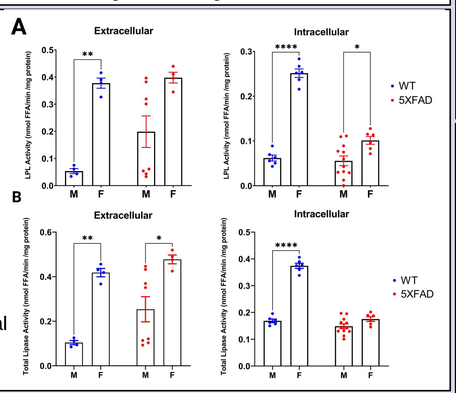

Figure 3 A. Extracellular and intracellular LPL activity in whole brain tissue of 9-month-old wild type (WT) and 5XFAD male and female mice. **B.** Total lipase activity in male and female, WT and 5XFAD mice

4. Estrogen Decreases DAM Gene Expression and Cholesterol Efflux in BV-2 cells

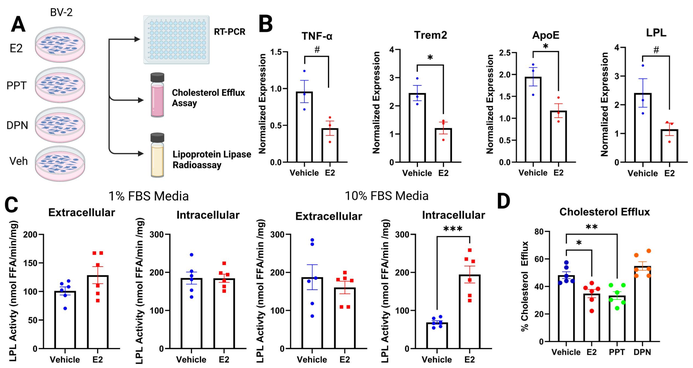

Figure 4 A. Schematic of experiments carried out in BV-2 microglia using ESR1 and ESR2-specific agonists PPT and DPN, respectively. **B.** qPCR gene expression data in BV-2 cells after 24 hours of drug exposure. **C.** LPL activity in BV-2 cells grown in either 1% FBS or 10% FBS exposed to E2 for 24 hours. **D.** Cholesterol efflux from BV-2 cells exposed to drug for 24 hours.

5. Estrogen Increases Oxidative Metabolism and Glycolysis in Low

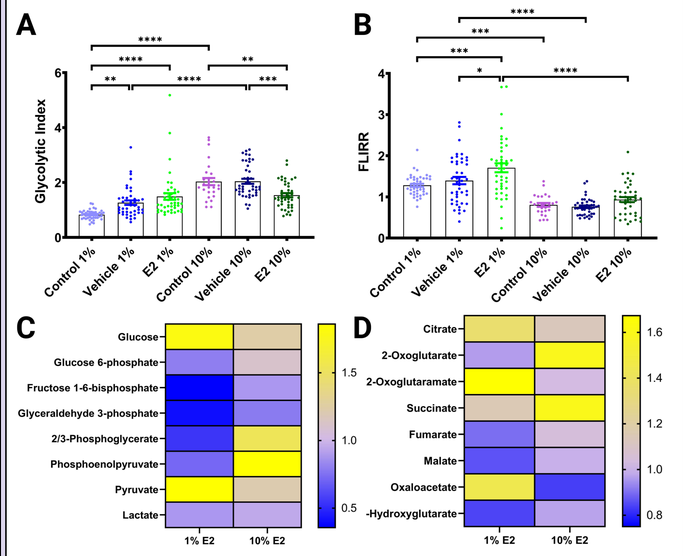

Figure 5 A. Glycolytic index of BV-2 microglia in 1% or 10% FBS containing media treated with equal volumes of media (control), ethanol (vehicle), or E2 dissolved in ethanol (E2). **B.** FLIRR of BV-2 microglia in 1% or 10% containing media treated with equal volumes of media (control), ethanol (vehicle), or E2 dissolved in ethanol (E2). **C.** Heatmap of glycolytic intermediates from metabolomics data. **D.** Heatmap of TCA cycle intermediates from metabolomics data.

6. Estrogen Decreases Age-asscociated Gene Expression and Protein Signatures in Human Microglia

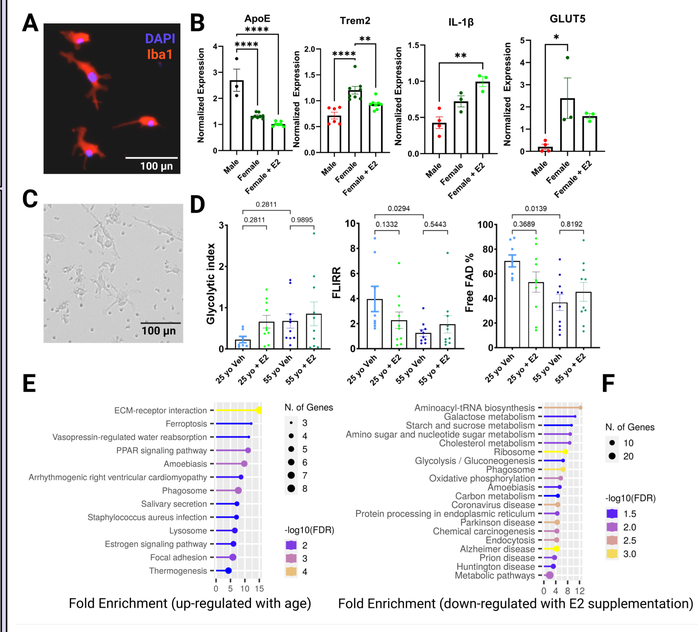

Figure 6 A. Representative image of monocyte-derived microglia-like (MDMi) cells. **B.** Normalized expression data from MDMi cells from an aged male and female donor, with female MDMi cells treated with E2 for 48 hours. **C.** Representative brightfield image of MDMi before FLIM analysis. **D.** FLIM analysis of MDMi derived from 25 yo female or 55 yo female donors after treatment with vehicle (0.000001% Ethanol) or E2 for 48 hours.

Summary & Conclusion

- With aging, female's brains and microglia exhibit exacerbated metabolic dysregulation.
- E2 inhibits expression of key genes involved in lipid and cholesterol processing as well as AD risk, suggesting a potential protective role for E2 that is lost with menopause.
- Human microglia from aged females exhibit higher DAM and GLUT5 gene expression compared to males, which may be responsive to E2 supplementation.
- Our data suggest hormone replacement therapy in menopausal women may reverse microglial metabolic reprogramming, which contributes to the neuropathogenesis of AD.

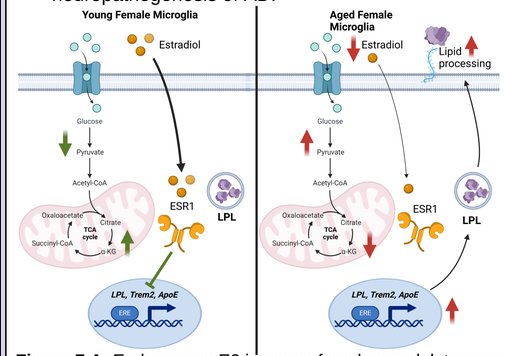

Figure 7 A. Endogenous E2 in young females modulates microglial metabolism and inhibits DAM gene expression. **B.** These protective affects are lost with aging and menopause, leading to metabolic reprogramming and DAM gene expression.

Future Directions

- Investigate the mechanisms underlying estrogen-mediated inhibition (e.g. EREs) of key microglial genes and the role of other hormones like testosterone and progesterone.
- Investigate therapeutics that modulate lipid processing in microglia and can rescue disease-associated microglial phenotypes.
- Investigate sex differences in glucose transporter expression and how fructose affects metabolism and dysregulation.
- Investigate effects of novel drugs that target tissue-specific estrogen receptors and how this can influence microglial metabolism and activation state.

This work has been published and can be found here:

References

1. Zhang et al. (2016) PMID: **26687838**
2. Verghese et al. (2011) PMID: **21349439**
3. Scheyer et al. (2018) PMID: **30298180**
4. Lynch (2020) PMID: **31704314**
5. Wang et al. (2020) PMID: **32579671**
6. Krasemann et al. (2017). PMID: **28930663**
7. Keren-Shaul (2017) PMID: **28602351**
8. Miao et al. (2023) PMID: **37341833**
9. O'Connor et al. (2023) PMID: **36902168**
10. Guillot-Sestier et al. (2021) PMID: **34112929**

Acknowledgments

Ludeman Family Center for Women's Health Research
UNIVERSITY OF COLORADO ANSCHUTZ MEDICAL CAMPUS
NIH National Institute on Aging