Cortical Microvascular and Inflammatory Effects of High Fat Diet Consumption

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High Fat Diet does not cause a stroke but still a *silent infarction or microvascular stalling* ➔ cognitive decline

Major or Minor Stroke
- Most cases had silent infarction or microvascular stalling

Hypertension and Diet
- Risk factors for stroke

(Freeman et al., 2014)
High Fat Diet
Silent Infarction or Microvascular Stalling
Hypoxia & Neuronal Change
Neurodegeneration
Cognitive Decline
Experiment

• High fat diet (42% Fat)
• 5 Mice
  – 2 control
  – 3 HFD fed
• \textit{Imaged once a week for 3 weeks:} Speckle & Two Photon Excitation Microscopy
Objectives

Cortical Inflammation:
Microglia Number & Collagen Deposition

Microvascular Flow: Microvessel Stalling

Cerebral Perfusion: Speckle Contrast

Neuron Changes: Neuronal Morphology
Methods: Live Imaging

Mouse with Cranial Window

Anesthetized Mouse Setup for Imaging
Two Photon Excitation Microscopy

Fluorescent Imaging:
Uses genetically modified mice or fluorescent dyes
Vasculature

IV Injection; Texas Red-dextran; Plasma

100 µm
Microglia: first response from CNS, act as macrophages for brain and spinal cord
Vasculature & Microglia

Plasma
Microglia
Leukocytes: white blood cells, during inflammation may leave vasculature due to adherence to endothelial lining
Vasculature, Microglia & Leukocyte

Plasma
Microglia
Leukocytes

100 µm
Neurons

Genetically modified to express Yellow Fluorescent Protein (YFP) for Neurons
Vasculature, Microglia, Leukocyte & Neurons

Plasma
Microglia
Leukocytes
Neurons

100 µm
Collagen: protein that occurs in higher quantities during tissue regeneration, such as from inflammation
Vasculature, Microglia, Leukocyte, Neurons & Collagen

Plasma
Microglia
Leukocytes
Neurons & Collagen
Conclusion

• Idea: High Fat Diet ➔ cognitive decline due to *silent infarction or microvascular stalling*

• Mice imaged for a total of three weeks
  – 1 baseline & 2 HFD

• Used Two Photon Excitation Microscopy
  – Allows for viewing deep in living tissue
  – Split channels due to different wavelengths
    • Can put all together as a composite or view separately
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Reference:
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Nathaniel Pineda
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Objectives

Determining the effect of HFD by analyzing:

- Microglia Number
- Cortical inflammation: Noise
- Speckle Perfusion
Objectives

Determining the effect of HFD by analyzing:

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Cellular Components within Brain

neurons
astrocytes
microglia
vasculature
macrophages

Cellular Components within Brain
Microglia

- First line of **immune** defense within CNS
  - Brain and spinal chord
  - React to decrease inflammation

- ***Cranial surgery would also lead to an increase in microglia***
Microglia Results

109 Normal Diet

263 HFD Week 1

182 HFD Week 2
Microglia Results

109 Normal Diet

263 HFD Week 1

182 HFD Week 2
Collagen Build-Up

- Natural resource of the body
- Component of skin tissue that aids wounds

Normal Diet | HFD Week 1 | HFD Week 2
Objectives

Determining the effect of HFD by analyzing:

• Microglia Number

• Cortical inflammation: Noise

• Speckle Perfusion
Imaging of vasculature, shows that the HFD consumption increases noise.
Signal-to-Noise Ratio

Baseline Week 0

HFD Week 1

HFD Week 2

Count: 3500  
Mean: 7.637  
Max: 67  
StdDev: 8.755  
Mode: 2 (802)

Count: 3500  
Mean: 8.932  
Max: 107  
StdDev: 11.723  
Mode: 5 (1273)

Count: 3500  
Mean: 31.156  
Max: 255  
StdDev: 37.502  
Mode: 7 (542)
Objectives

Determining the effect of HFD by analyzing:

• Microglia Number

• Cortical inflammation: Noise

• Speckle Perfusion
Laser Speckle Contrast Imaging

Indirect measure of tissue perfusion

- Laser beam **illuminating** ROI with light scattering from the sample to the camera
- Short multi-exposures
- Do not appear uniform, appear as **speckles**
- Used to quantify blood flow

*ROI - Region of Interest

Speckle Contrast Results

- Greater shade of blue shows a reduction of blood flow
- Closer to red shows increased blood flow
- Reduction in capillary velocity

Week 1 After HFD

Week 2 After HFD
Conclusions/ Future Directions

- Surprising time scale: 1 week
- HFD may be pro-inflammatory
- The effect of changing HFD on/off
- Behavior & cognitive testing
- More animals and time
- Determine prevalence of vascular stalls
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