Aquaporin-4 distribution correlates to increase in tau accumulation in Sprague Dawley rats fed a high fructose diet

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Research at BW

➢ BW Summer Scholars 2018
  ➢ Dr. Morris and Brandon Garrison
  ➢ Continued into Fall 2018

➢ Summer and Fall 2019
  ➢ Data are analyzed and complete
Alzheimer’s disease (AD)

- $230 billion will be spent on AD patient care during 2019 (AIM, 2019)
- 5.7 million individuals over 65 years of age are diagnosed with AD
- Post-diagnosis lifespan is 4-8 years
Mechanism of AD

- Memory impairment, irrational thinking, unreasonable mood changes, etc.
- Hippocampus (HPC) size decreases with AD
Leading model of AD

➢ Accumulation of Amyloid Beta and Neurofibrillary Tau tangles (NFTs)
Xie et al (2014). *Sleep Drives Metabolite Clearance from the Adult Brain*
Aquaporin-4 (AQP4)

- The predominant water channel protein in the brain
- Primarily localized on Astrocytic Endfeet within the CNS
- Occupies 50% of the surface area
- Regulates water homeostasis and waste products via exchanges of CSF-ISF interactions
- High levels of Glucose is seen to decrease the flow of CSF throughout the Glymphatic System (Vandal, 2014)
Aquaporin-4 (AQ4)

- Double knockout mice exhibit memory deficits and impaired long-term potentiation (Hubbard, 2018)
Alternative models for AD

- Insulin deficiency causes neurons to have impaired energy and eventually death.

- Vascular system fails to deliver blood/nutrients to the brain.
Alternative models for AD

➢ Insulin deficiency causes neurons to have impaired energy and eventually death

➢ Vascular system fails to deliver blood/nutrients to the brain
Prevalence of diabetes

➢ 9.4% (30.3 million) of Americans have a form of diabetes

➢ Another 84 million Americans are estimated to have prediabetes (CDC, 2017)

➢ Common symptoms include increased thirst/hunger and often urination
  ▶ If not managed properly, then increased risk of heart disease and stroke

➢ Estimated that diabetes is the 7th leading cause of death in the United States
  ▶ AD is the 6th leading cause of death (CDC, 2017)
Type 2 and Type 1 diabetes

➢ Type 2 diabetes is insulin resistance in peripheral tissues
  ➢ Aging, family history, and decreased muscle activity

➢ Type 1 diabetes is the destruction (typically autoimmune) of islet beta cells
  ➢ Genetic or viral causes
Understanding diabetes

- Insulin is a hormone that is necessary for glucose to enter cells.

- Type 1 and 2 diabetes leads to excessive glucose levels in the blood.
Type 3 diabetes correlated with AD

- Neurons are unable to respond to insulin, no glucose influx

*Projected Number of People Aged 65 or Older With Alzheimer’s Disease, by Age Group, United States, 2010–2050*

Hypothesis

➢ Aquaporin-4 distribution correlates to increase in tau accumulation in Sprague Dawley rats fed a high fructose diet
Streptozotocin (STZ)

➢ Toxic to pancreas beta islet cells that produce insulin

➢ Dose of 200 mg/kg

➢ Diabetic if blood glucose levels exceeded 300 mg/dL

➢ Nondiabetic is considered ~100 mg/dL
1 Week
STZ Injection (I.P. 200 mg/kg)

STZ timeline of Experiment

Blood glucose levels were measured and verified

Diabetes is induced

Brandon Garrison performed cognitive tests

3 Weeks

Collected the brain, then sliced, stained, and gathered results

3 Weeks

Analysis and Quantification
Working Model: High fructose (HF) diet

- 10-13 weeklong diet consisting of 60% fructose
- Levels can be similar to the typical American diet

- Two summers were used for the diet
Fructose inducing diabetes

➢ Fructose is commonly found in fruits and vegetables

➢ High fructose corn syrup

➢ Higher fructose diet overwhelms liver metabolism
Fructose vs Glucose (Stanhope, 2009)

- Participants were fed diet consisting of either 25% Glucose or Fructose sugar for 9 weeks
Introduction of 60% HF diet

10 Weeks
Type 2 diabetes is induced

Blood glucose levels were measured and verified

Collected the brain, then sliced, stained, and gathered results

3 Weeks
Brandon Garrison performed cognitive tests

3 Weeks
Analysis and Quantification
The weight of rats in each Summer group did not differ from the other groups (P>0.05)

Average Rat Weight for Summer 2018 and 2019

- 2018 CTRL Average
- 2018 HF Average
- 2019 CTRL Average
- 2019 HF Average

The Blood Glucose of STZ rats was significantly higher than CTRL and HF (P<0.05)
The Blood Glucose of HF rats was significantly higher than CTRL (P<0.05)
HF diet leads to prediabetic levels without increasing weight gain

- Not all Type 2 diabetic patients are overweight
  - Diet and age are the primary factors for onset Type 2 diabetes

- Next, I used Immunohistochemistry and Cresyl Violet stains to examine AQ4 distribution, NFT accumulation, and HPC size
Antibody Stain

- Desired Protein
  - AQ4
  - Tau
- Primary Antibodies
  - AQ4 primary
  - Tau primary
- Secondary Antibodies
  - AQ4 555
  - Tau 488
- Fluorescent Tag
<table>
<thead>
<tr>
<th>Condition</th>
<th>AQ4 in Astrocyte End-feet</th>
<th>AQ4 in Astrocyte Body</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>STZ Injection</td>
<td>Decreased Amount</td>
<td>Increased Amount</td>
</tr>
<tr>
<td>HF Diet Induced</td>
<td>Decreased Amount</td>
<td>Increased Amount</td>
</tr>
</tbody>
</table>

**Predictions**
A significant increase of AQ4 on astrocytic end-feet in control rats than diabetic rats (P<0.05)
Number of NFTs in the HPC

Control
Normal

STZ Injection
Increased Amount

HF Diet Induced
Increased Amount

Predictions
20x Tau (Fluorescent Microscope)
There is a higher number of NFTs in diabetic rat models ($P<0.05$)
Cresyl Violet Stain

➢ Stains Nissl substance throughout neuron cytoplasm

➢ Neuron location can be identified throughout the brain

➢ Size of HPC was measured using MBF Bioscience microscope program
Hippocampus Size

Control
- Normal

STZ Injection
- Reduced

HF Diet Induced
- Reduced

Predictions
Hippocampus size was not altered between control and diabetic rats (P>0.05).
Conclusion

Insulin receptors lose their sensitivity

Glucose cannot penetrate the cell

Type 2 Diabetes
Conclusion
Future Directions

➢ Use blood vessel colocalization stain with AQ4 and examine AQ4 reaction to diabetes

➢ Insulin receptor substrate 2 deficiency to evaluate any neuroinflammatory effects on insulin receptors (Grote, 2011)

➢ https://sugarscience.ucsf.edu/
➢ No more than 6 teaspoons (25 grams) of sugar per day for women and 9 teaspoons (38 grams) for men (AHA)
Acknowledgements

➢ Dr. Jacqueline Morris for being a great mentor for this project

➢ Dr Clare Mathes, Dr. Jeffery Zahratka, and Raisa Echols for helping me with any obscure issues in the lab

➢ MacKenzie Chappell and Maddie Lovejoy for data collection

➢ Chris Jones for taking care of the summer 2019 rat fructose diet

➢ OSGC, The Neuroscience department, Summer Scholars, the Kenneth and Lucy McCauliff scholarship for providing the necessary funding to perform this research
  ➢ UCRS and INS for funding the transportation to present this research

➢ My family for supporting me all through these years
THANK YOU!