Effect of a Ketogenic Diet on Acute Inflammatory Pain Induced by Complete Freund’s Adjuvant (CFA) in Male Mice

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Introduction

PAIN

• Around 20% of U.S. adults suffer from chronic pain and 8% have high-impact chronic pain limiting major life activities (NIH).
• Many different types of pain and pain progression disorders are due to chronic inflammation which can be hard to treat and manage.
• Inflammation is a normal and healthy response, however if it is not resolved and becomes chronic it can damage healthy tissues.
• Dietary strategies could be an effective method to manage pain symptoms and inflammation.

Methods

Diet and Living

• Experiments evaluated male C57BL/6 mice bred at the Psychology Laboratory.
• C57BL/6 male mice were fed standard rodent chow (LabDiet 5013) until they were 5–6 weeks of age.
• 4–5 mice were housed together in cages and had ad libitum access to food and water and kept on a 12-hour light-dark cycle.

Four Treatment Groups of Mice:
1. Wild type on control diet (WT CD)
2. Wild type on ketogenic diet (WT KD)
3. Knockout of A1 receptor on control diet (KO CD)
4. Knockout of A1 receptor on ketogenic diet (KO KD)

Mice selected to be on the KD were fed the BIO diet ad libitum.

Physiological Tests

• Electronic Plantar Frey Test
• Tactile sensitivity and nociceptive pain before (baseline) and after being injected with 20 μl of 10 μg/ml of Complete Freund’s Adjuvant (CFA) a heat-killed tuberculous bacteria, in right hind paw, causing persistent inflammation (apparent 4 hours after injection)

Future work

• Future work will focus on increasing the sample sizes, specifically in the KD KO group.
• Look at specific circulating markers of inflammation such as proinflammatory cytokines.
• Look at local signs of inflammation in the injected paw, such as white blood cells that are involved in inflammatory processes.
• Marble burying behavioral test will be used to further determine the effects of the KD on pain.
• Look at other adenosine receptors or other aspects of adenosine signaling.

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References


Figure 1 An ANOVA on baseline scores showed no significant difference in the tactile sensitivity prior to the CFA injection. There was a significant effect for all treatment groups (P<0.001) and diet (P<0.05). The effect of diet and genotype on tactile sensitivity was also statistically significant (P<0.02).

Figure 2 All post-injection points are significantly different from baseline for the WT mice on CD (P<0.001). At 3 and 2 days post-injection there was a significant difference from baseline for the KD WT mice (P<0.001 and P<0.017). There is no significant difference from baseline for the KD WT mice on CD (P<0.001). At 4hrs and 2.5 days post injection there was a significant effect size, so we would expect a faster recovery than mice on the KD.

KD KO
• ANOVA on tactile sensitivity for the KD KO mice was not significant due to high variability and low sample size, so we cannot make a definite comparison to CD KO mice. There are similar means and trends of the KD graphs (KD WT and KD KO), so we can infer that there could be an effect of the KD on the KO as there is on the WT mice.

Figure 3 The CD KO animals were still significantly different from baseline at 7 days. ANOVA on tactile sensitivity for the KD KO mice was not significant.

Discussion

• Around 20% of U.S. adults suffer from chronic pain and 8% have high-impact chronic pain limiting major life activities (NIH).
• Many different types of pain and pain progression disorders are due to chronic inflammation which can be hard to treat and manage.
• Inflammation is a normal and healthy response, however if it is not resolved and becomes chronic it can damage healthy tissues.
• Dietary strategies could be an effective method to manage pain symptoms and inflammation.

KETOGENIC DIET (KD)
• High fat, low carbohydrate, moderate protein diet
• Characterized by moderate hypoglycemia, reduced neuronal excitation, and increased adenosine levels
• Replaces body fuel from metabolizing glucose to ketone bodies (β-hydroxybutyrate, acetocacete, and acetoacetate)
• Ketones can make ATP more efficiently and increased levels of ATP lead to an increase in extracellular adenosine

KD MECHANISMS OF HYPOALGESIA AND ANTI-INFLAMMATION (Masino & Ruskin, 2013)
• Reduces excitatory and/or increases inhibitory mechanisms
• Fewer reactive oxygen species have anti-inflammatory effects
• Adenosine has anti-inflammatory effects
• Reducing glycolytic metabolism is analogous

ADENOSINE AND A1 RECEPTOR SUBTYPE (A1R)
• Adenosine is an inhibitory neurotransmitter
• A1 receptor (A1R) is the most abundant adenosine receptor in the brain
• A1R is coupled to K+ channel activation and inhibition of Ca+ channels which inhibits neuronal activity/excitability (Dunwiddie and Masino, 2001)
• Effect of adenosine activation is to reduce excitability in the brain by inhibiting neuronal excitability through A1R (Masino et al., 2009)

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Behavioral Tests

Statistical Analysis

• Data was analyzed using 3-factor ANOVA followed by multiple one-way ANOVAs and post hoc tests
• Data was considered significant when P<0.05

Table 1 Weight and blood chemistry at euthanization. KD mice had significantly higher β-hydroxybutyrate ketone levels compared to the mice on the CD (P<0.001). KD mice had significantly lower glucose levels (P<0.002) and body weights than CD mice (P<0.05). Mice on a KD were in ketosis.