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 tissue
- 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 (Ruskin, Suter, Ross, & Masino, 2013)
- Replaces body fuel from metabolizing glucose to ketone bodies (b-hydroxybutyrate, acetoacetate, acetone)
- 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 analgesic

ADENOSINE AND A_1 RECEPTOR SUBTYPE (A_1R)

- Adenosine is an inhibitory neuromodulator
- A_1 receptor (A_1R) is the most abundant adenosine receptor in the brain
- A_1Rs are coupled to K^+ channel activation and inhibition of Ca^{2+} 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 A_1Rs (Masino et al., 2009)

Methods

Diet and Living

- Experiments evaluated male C57Bl/6 mice bred at the Psychobiology Laboratory
- C57BL/6 male mice were fed standard rodent chow (LabDiet 5001) 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 A_1 receptor on control diet (KO CD)
  4. Knockout of A_1 receptor on ketogenic diet (KO KD)

- Mice selected to be on the KD were fed the BIO-SERV F3666 diet (6.6:1) for 3 weeks prior to experimentation (76.7% fat 8.5% protein, 3.18% carbohydrates)

Behavioral Test

Electronic Plantar Von Frey

- Tests tactile sensitivity and nociceptive pain before (baseline) and after being injected with 20ul of 0.05 mg/mL of Complete Freund's Adjuvant (CFA) a heat-killed tuberculosis bacteria, in right-hind paw, causing persistent inflammation (apparent 4 hours after injection)
- Electronic von Frey measured the maximum applied pressure of hind paw, until withdrawal or raising of paw
- Probe was applied in the center of the four walking pads on hind paw
- The right hind paw was tested in each mouse and then the left hind paw, allowing at least 60 seconds before testing the same paw again
- Each hind paw (R/L) was recorded three times per day and averaged together
- Plantar von Frey was recorded 4 hours, 2 days, 4 days, and 7 days post-injection
- A lower amount of pressure applied suggests a higher tactile sensitivity indicating more pain

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

Behavioral Tests Results

Injected Paw (Right)

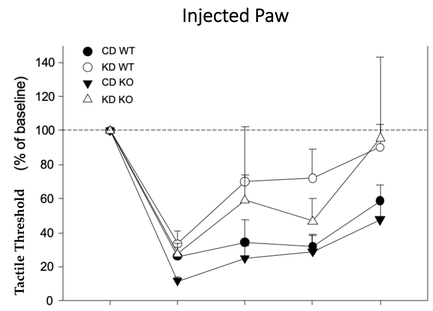

Figure 1 An ANOVA on baseline scores showed no significant difference in the tactile sensitivity prior to the CFA injection. There was a significant effect for time across all groups (P<.0001) and diet (p=.005). The effect of diet and genotype on tactile sensitivity was also statistically significant (p=.028).

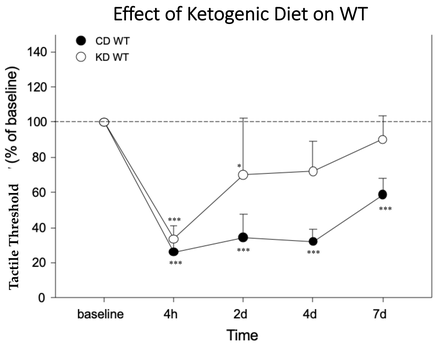

Figure 2 All post-injection points are significantly different from baseline for the WT mice on CD (P<0.001). At 4hrs and 2 days post injection there was a significant difference from baseline for the KD WT mice (P<0.001 and P<0.017). There is no significant difference from baseline on days 4 and 7 for the KD WT mice (P<0.07 and P<1.00). The increased sensitivity due to CFA had reversed by 4 days in the KD WT mice in contrast to the CD WT mice.

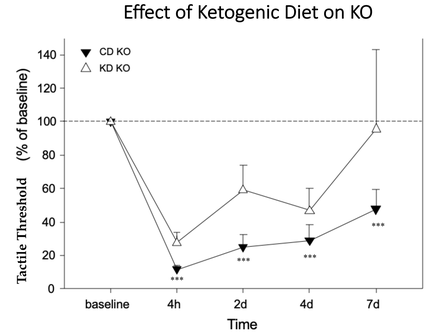

Figure 3 The CD KO animals were still significantly different from baseline at 7 days. ANOVA on tactile sensitivity for the KD KO mice was not significant.

Uninjected Paw (Left)

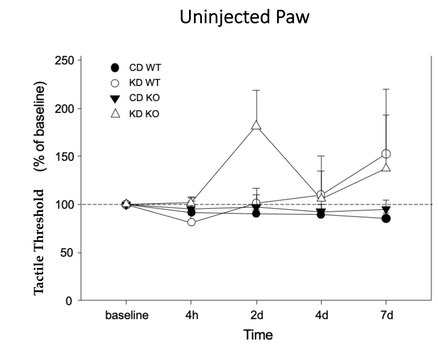

Figure 4 An ANOVA on the uninjected paw (left) showed no significant changes across time for all treatment groups, except for the KD KO mice, but no post hocs were significant for the KD KO group.

*P<0.05, **P<0.01, *P<0.001

Physiological Results

Genotype	Diet	Avg.Body Weight (g)	Avg. Blood Glucose (mg/dL)	Avg. Ketone B-hydroxybutyrate (mM)
WT	CD	24.08	170.5	0.425
WT	KD	20.25	99.25	2.78
KO	CD	24.25	151	0.075
KO	KD	23.28	133.75	1.28

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<.0.0001). 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.

Discussion

KD WT

- There was a significant effect towards reduced pain apparent four days after injection in the KD WT mice. The applied pressure at days 4 and 7 post injection were not significantly different from baseline, which suggests that the increased tactile sensitivity from the CFA injection had reversed in the KD WT mice compared to the CD WT mice. These results suggest that the KD could help reduce inflammatory pain and result in a faster recovery than mice on the CD.

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 curves of the KD graphs (KD WT and KD KO), so can infer that there could be an effect of the KD on the KO as there is on the WT mice.
- If the KD was affecting the KO mice, this could indicate that the diet did not have an effect on adenosine signaling.

Uninjected Paw

- Left paw showed no significant changes across time for all groups suggesting that the left paw scores were consistent with the baseline scores and were not affected by the CFA injection in the right paw, as expected.

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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