

Title: Low glycaemic index diet reduces seizure susceptibility in a syndrome-specific mouse model of generalized epilepsy

Running title: Low-GI diet reduces absence seizures

Tae Hwan Kim^{1,3}, Bsci, PhD, Steven Petrou, PhD^{1,2,3}& Christopher, A. Reid, PhD¹

1. Florey Institute of Neuroscience and Mental Health, the University of Melbourne, Parkville, Australia.
2. Centre for Neural Engineering, The University of Melbourne, Parkville, Melbourne, Australia
3. Department of Anatomy and Neuroscience. The University of Melbourne, Parkville, Melbourne, Australia

Corresponding author

Dr Christopher Alan Reid

Florey Neuroscience Institutes, the University of Melbourne, Parkville, Victoria 3010, Australia

Tel: 61-3-8344 1957

Fax: 61-3-9347 0446

E-mail: careid@unimelb.edu.au

Summary: 204; **Number of pages:** 12; **Word total:** 1495 **Figures:** 2 **Tables:** 1

Key words: absence seizures, animal models, spike and wave discharge, diet, ketogenic, low-GI, high-GI

Summary

Purpose: Clinical evidence suggests that low glycaemic index diets are effective at reducing seizure frequency potentially through the stabilization of blood glucose levels. Here we investigate if diets containing carbohydrates with varying glycemic index (GI) can modulate seizure susceptibility in a mouse model of generalized epilepsy.

Methods: Electrocortical recordings were made from mice harboring the GABA_Aγ2 (R43Q) epilepsy mutation after three weeks on a low-or high-GI diet. Standard rodent diet was used as a control. Occurrence and durations of spike-wave-discharges (SWDs) were measured. An insulin injection was used to reduce blood glucose to levels known to precipitate SWDs in the GABA_Aγ2 (R43Q) mouse on the low and high-GI diets.

Key findings: SWD occurrence was reduced by approximately 35% in mice on the low-GI compared to high-GI diet. SWD occurrence was not different between high-GI diet and a standard diet suggesting that low-GI diet is protective. Weight gain of mice for all diet groups was identical suggesting that they were equally well tolerated. Under low blood glucose conditions SWD occurrence increased in the low and high-GI diets. Importantly, under low glucose conditions the low-GI diet no longer conferred protection against SWDs.

Significance: SWDs were reduced in mice on a low GI-diet suggesting it may be an effective and well tolerated therapy for generalized epilepsy. The lack of effect of low-GI diet when glucose levels are reduced suggests that seizure protection in the GABA_Aγ2 (R43Q) mouse model may be due to the diets ability to stabilize blood glucose levels.

Introduction

Dietary therapy is now commonly considered a useful treatment option in refractory epilepsy (Levy, Cooper et al. 2012). Most studies have investigated the high-fat and low-carbohydrate ketogenic diet with varying reports of success (Bailey, Pfeifer et al. 2005). Tolerability remains a significant issue, with patients often unable to adhere to the strict dietary regime. The precise mechanism of action of the ketogenic diet is not fully understood, but most likely multi-factorial (Bough and Rho 2007) potentially including the stabilization of brain glucose levels. Consistent with this, recent evidence indicates efficacy of a low glycaemic index (GI) diet in patients with refractory epilepsy (Pfeifer and Thiele 2005; Pfeifer, Lyczkowski et al. 2008; Muzykewicz, Lyczkowski et al. 2009; Coppola, D'Aniello et al. 2011), epilepsy in tuberous sclerosis complex (Larson, Pfeifer et al. 2012) and Angelman syndrome-related seizures (Thibert, Pfeifer et al. 2012). Small clinical trials in patient populations with severe epilepsy syndromes are feasible because of the large number of seizure events in these patients. These trials become difficult when more heterogeneous epilepsy populations are investigated. Mouse models provide a mechanism for testing varying therapeutic manipulations under conditions in which environmental and genetic factors can be more tightly controlled. Recent genetic discoveries have isolated a number of epilepsy causing genes enabling the engineering of 'syndrome-specific' mouse models based on human mutations. The GABA_A γ 2 (R43Q) mouse model of generalized epilepsy is remarkable in that it recapitulates the two primary seizure phenotypes seen in humans with the disease, including absence epilepsy and febrile seizures (Reid, Kim et al. 2013). Further, the GABA_A γ 2 (R43Q) mouse is sensitive to the first-line anti-absence drug, ethosuximide (Tan, Reid et al. 2007). We have also recently shown that reducing blood

glucose levels, either by overnight fasting or injecting insulin, results in a doubling of spike-and-wave discharge (SWD) events in the GABA_A γ 2 (R43Q) mouse (Reid, Kim et al. 2011). This is consistent with the idea that fluctuating glucose levels may also be an important determinant of seizure susceptibility in generalized epilepsy. Here we test the impact of low- and high-GI diets on SWD expression in the GABA_A γ 2 (R43Q) mouse model.

Accepted Manuscript

Materials and Methods

All experiments were approved by the Animal Ethics Committee at the Florey Institute for Neuroscience and Mental Health in accordance with The Code of Ethics of the World Medical Association for experiments involving animals. The GABA_Aγ2 (R43Q) mutation bred into the DBA/2J background strain mice (>N20 generations) was used between the ages of P42-45. Genotyping was done at P12 using a PCR-based method (Tan, Reid et al. 2007). All mice were housed under a 12h light dark cycle with free access to food and water.

Diet protocols: Pregnant and lactating mothers were kept on a standard rodent diet (Barastoc 8720310, Ridley Corporation Ltd, Australia). At weaning (P21), mice were placed on either a high-or low-GI carbohydrate diet (Specialty Feeds, Glen Forrest, WA, Australia) for the duration of the experiment. These two diets are derived from a standard rodent diet differing only in their carbohydrate type (Table 1). The standard rodent diet was used as a control.

Electrocorticogram (ECoG) recordings: Electrode implantation surgeries were performed as previously described (Tan, Reid et al. 2007; Reid, Kim et al. 2013). Mice were anesthetized with 1-3% isoflurane and two epidural silver 'ball' electrodes were implanted on each hemisphere of the skull. Electrodes were placed 3mm lateral of the midline and 0.5mm caudal from bregma. A ground electrode that was not touching the brain surface was placed 2.5mm rostral from bregma and 0.5mm lateral from the midline. A differential signal was recorded relative to this common ground. Mice were allowed to recover for at

least 48 hours after surgery. ECoGs were continuously recorded in freely moving mice for 12h epochs during the light cycle. For acute modulation of blood glucose experiments, EcoG recordings were made for 2h prior to the i.p. injection of 1.5 units of insulin (Sigma, Australia) and 2h following. Data from male and female mice were pooled in this study. Approximately equal female/male ratio were used in this study (5 males and 4 females for low-GI diet and 4 each for high-GI diet) ruling out any potential gender bias. Signals were filtered at 0.1 to 200Hz and sampled at 1 kHz using Powerlab 16/30 (ADInstruments Pty. Ltd., Sydney, NSW, Australia). EEG recordings were acquired during daylight hours. A full spike and wave discharge (SWD) was defined as an individual seizure event (Fig 1). Events were detected by eye with the observer blind to treatment group. Event duration was defined as time from the first to the final peak of the SWD.

Glucose measurements: Glucose measurements were made using the method previously described (Reid, Kim et al. 2011). Briefly, a small area of the tail was cut with a surgical blade allowing the collection of mixed peripheral blood. Blood glucose concentrations were measured using a human glucose meter (ACCU-CHEK Integra, Roche, Australia). Tails were gently massaged at defined intervals to re-establish blood flow and glucose measurements made. We took measures (low noise, gentle handling) to reduce the impact of stress on animals when taking blood for glucose measurements.

Statistics: All values are expressed in mean \pm SEM. All statistical comparisons were made using unpaired two-tailed student's t-Test unless indicated (Graphpad, Prism, CA).

Results

Low-GI diet reduces SWD occurrence

ECoGs recorded from GABA_Aγ2 (R43Q) mice on all diets display clear SWD events arising from a quiet background (Fig 1A). Mice fed a high-GI diet for three weeks showed almost identical SWD occurrence when compared to those fed a standard mouse diet (34.6 ± 3.1 Vs. 36.2 ± 4.4 SWD /h, n=8 and 8, P=0.76) suggesting that high-GI diets were not exacerbating seizures. Next we more specifically address the issue of the role of GI index in modulating SWD susceptibility by comparing diets that only differ in their carbohydrate type (Table 1). A low-GI diet significantly reduced SWD events when compared to mice fed a high-GI diet (Fig 1B). SWD durations were not significantly different between these two diets (Fig 1C). GABA_Aγ2(R43Q) mice fed low-GI diet for three weeks were physically indistinguishable from mice on a high-GI diet with similar increases in body weight over 21 days (Fig. 1D). Basal blood glucose levels at P44 were within the normal range and not different between the high- and low-GI diet groups (Fig 1E) consistent with the idea that low GI diets stabilize blood glucose level fluctuations rather than altering average levels.

A low-GI diet does not protect against the seizure precipitation effects of low blood glucose

1.5 IU kg⁻¹ insulin was injected IP into both the low-GI and high-GI diet animal cohorts. In both cohorts blood glucose levels were significantly reduced (from 7.7 ± 0.1 to 4.5 ± 0.4 mM, P<0.001, n=11 (baseline) and 9 (post-insulin treatment) in low-GI, and from 7.4 ± 0.2 to

4.3±0.6 mM, $P < 0.001$, $n = 10$ (baseline) and 9 (post-insulin treatment) in high GI). This reduction was comparable to previous published data on mice fed a control diet (Kim, Borges et al. 2013). Following the injection of insulin, blood glucose levels were identical in both the high- and low-GI diet cohorts (Fig 2B). There was a significant increase in the number of SWDs recorded in both low (20 ± 5 to 67 ± 10 SWDs/h, $n = 9$, $P = 0.002$, paired t-test) and high GI (35 ± 6 to 68 ± 12 SWDs/h, $n = 8$, $P = 0.046$, paired t-test) groups similar to that observed in previous studies (Reid, Kim et al. 2011; Kim, Borges et al. 2013). Interestingly, SWD occurrence was no longer significantly different for the mice on the low-GI diet when compared to those on a high-GI diet (Fig 2C).

Discussion

Dietary treatments that modulate energy substrates, such as the ketogenic diet, are now an important therapeutic option in epilepsy. Three broad mechanisms have been proposed for the ketogenic diet; stabilization of blood (and hence brain) glucose levels, the provision of alternative energy substrates and/or the antiepileptic action of metabolic products (Bough and Rho 2007). Triheptanoin is a triglyceride containing heptanoate that is metabolized to produce intermediates of the citric acid cycle capable of anaplerosis (i.e triheptanoin has the ability to provide an alternative substrate to the citric acid cycle that is independent of glucose). A diet supplemented with triheptanoin reduces SWDs in the GABA_Aγ2 (R43Q) mouse by ~40% suggesting that anaplerosis could be an important underlying mechanism for the ketogenic diet. The low GI diet examined here has a similar impact on SWDs. Interestingly, this seizure protective effect is not seen when blood glucose levels are lowered by insulin which is in contrast with triheptanoin supplementation (Kim, Borges et al. 2013). The contrasting effects of lowering blood glucose on SWD expression in the low-GI and triheptanoin supplementation diets suggest that each acts via a distinct mechanism. Further, our data also implies that standard diets used to feed mice are equivalent to high-GI, at least in the context of SWD susceptibility.

The cellular basis of the effectiveness of the low GI diet has not been established although glucose stabilization is an attractive candidate. High GI diets can cause large swings in levels including rapid falls in blood glucose that may precipitate seizures (Reid, Kim et al. 2011). Several cellular mechanisms could underlie SWD precipitation due to glucose level reductions. Inwardly rectifying potassium ATP-controlled channels (K_{ATP}) are known to be

sensitive to metabolic states and may change network excitability (Velisek, Veliskova et al. 2008). For example, opening of K_{ATP} channels in response to dropping glucose levels may reduce the threshold at which SWDs occur through the hyperpolarization of thalamocortical neurons (Crunelli and Leresche 2002). Alternatively, levels of glycolysis-derived metabolites may explain an increase brain excitability when glucose levels drop. For example, deprivation of citric acid cycle intermediates increase aspartate and decrease GABA (Lewis, Ljunggren et al. 1974), both major synaptic neurotransmitters implicated in epilepsy. Glucose is transported and metabolized to a higher extent in astrocytes compared to neurons. Given the role astrocytes play in the buffering of neurotransmitters (Glutamate and GABA) and ions (eg. K^+ ions) they are also well positioned to influence seizure susceptibility (Benarroch 2009). Other potential mechanisms including anaplerosis and/or antiepileptic action of metabolic products could also underlie the effectiveness of the low GI diet with further *in vitro* and *in vivo* investigations required to determine the cellular and network mechanisms underlying seizure protection.

Our data strongly supports the clinical studies in which the benefit of the low-GI diet has been reported for severe epilepsy (Pfeifer, Lyczkowski et al. 2008; Muzykewicz, Lyczkowski et al. 2009; Coppola, D'Aniello et al. 2011). It is also consistent with recent genetic studies that have highlighted the central role glucose plays in setting seizure susceptibility. GLUT1 deficiency syndrome that leads to reduced brain glucose results in epilepsy, including a high incidence of absence epilepsy (Arsov, Mullen et al. 2012) associated with SWDs which is modeled by the $GABA_{A\gamma 2}(R43Q)$ mouse (Reid, Kim et al. 2011). Importantly, the ketogenic diet is now considered a first-line treatment for patients with GLUT1 deficiency (Klepper 2008). Moreover, cortical excitability, as assayed by

transcranial magnetic stimulation , fluctuates with blood glucose suggesting that it may be an important contributor to seizure susceptibility (Badawy, Vogrin et al. 2013).

In conclusion, we have provided additional support for the use of low-GI diet as an alternative to the ketogenic diet in epilepsy. Our data also provides evidence that blood glucose stabilization can be a useful strategy. Given the relative ease of implementing a low-GI diet it should be considered in cases where adherence to the well-validated ketogenic diet has failed.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgments

This study was supported by the National Health and Medical Research Council of Australia (Grant numbers 400121, 628520& 631541).CAR was also supported by an Australian Future Fellowship, Australian Research Council (#FT0990628). SP was supported by a NHMRC fellowship. Florey Institute of Neuroscience and Mental Health is supported by Victorian State government infrastructure funds.

Figure legends

Figure 1. Low-GI diet reduces the expression of SWDs in the GABA_Aγ2 (R43Q) mouse model of generalized epilepsy. **A** Example ECoG traces from mice fed low GI diet (above) and high-GI diet (bottom) with SWD activity. **B** Average SWD occurrence and **C** SWD durations in animals fed low GI and high GI diet (n=9 and 8). **D** Body weights over 21 days of feeding the diets. **E** Blood glucose levels of mice fed low GI and high GI diet measured at P44 (n=11 and 10). All values are expressed as mean ± SEM, *p<0.05.

Figure 2. Insulin-mediated reductions in blood glucose similarly precipitate SWDs in GABA_Aγ2 (R43Q) mice fed low GI and high GI diets. **A** Example of ECoG traces displaying increased SWD occurrences post insulin-injection from mice fed low GI diet (above) and high GI diet (bottom). **B** Blood glucose levels post-insulin injection in mice fed low GI and high GI diets (n=9 and 9). **C** Average SWD occurrence post-insulin injection in mice fed low GI and high GI diets (n=9 and 8). All values are expressed as mean ± SEM.

Table 1

Diet composition of low GI and high GI diets.

References

- Arsov, T., S. A. Mullen, et al. (2012). "Early onset absence epilepsy: 1 in 10 cases is caused by GLUT1 deficiency." *Epilepsia* **53**(12): e204-207.
- Badawy, R. A., S. J. Vogrin, et al. (2013). "Cortical excitability changes correlate with fluctuations in glucose levels in patients with epilepsy." *Epilepsy Behav* **27**(3): 455-460.
- Bailey, E. E., H. H. Pfeifer, et al. (2005). "The use of diet in the treatment of epilepsy." *Epilepsy Behav* **6**(1): 4-8.
- Benarroch, E. E. (2009). "Astrocyte-neuron interactions: implications for epilepsy." *Neurology* **73**(16): 1323-1327.
- Bough, K. J. and J. M. Rho (2007). "Anticonvulsant mechanisms of the ketogenic diet." *Epilepsia* **48**(1): 43-58.
- Coppola, G., A. D'Aniello, et al. (2011). "Low glycemic index diet in children and young adults with refractory epilepsy: first Italian experience." *Seizure* **20**(7): 526-528.
- Crunelli, V. and N. Leresche (2002). "Childhood absence epilepsy: genes, channels, neurons and networks." *Nat Rev Neurosci* **3**(5): 371-382.
- Kim, T. H., K. Borges, et al. (2013). "Triheptanoin reduces seizure susceptibility in a syndrome-specific mouse model of generalized epilepsy." *Epilepsy Res* **103**(1): 101-105.
- Klepper, J. (2008). "Glucose transporter deficiency syndrome (GLUT1DS) and the ketogenic diet." *Epilepsia* **49 Suppl 8**: 46-49.
- Larson, A. M., H. H. Pfeifer, et al. (2012). "Low glycemic index treatment for epilepsy in tuberous sclerosis complex." *Epilepsy Res* **99**(1-2): 180-182.
- Levy, R. G., P. N. Cooper, et al. (2012). "Ketogenic diet and other dietary treatments for epilepsy." *Cochrane Database Syst Rev* **3**: CD001903.
- Lewis, L. D., B. Ljunggren, et al. (1974). "Changes in carbohydrate substrates, amino acids and ammonia in the brain during insulin-induced hypoglycemia." *J Neurochem* **23**(4): 659-671.
- Muzykewicz, D. A., D. A. Lyczkowski, et al. (2009). "Efficacy, safety, and tolerability of the low glycemic index treatment in pediatric epilepsy." *Epilepsia* **50**(5): 1118-1126.
- Pfeifer, H. H., D. A. Lyczkowski, et al. (2008). "Low glycemic index treatment: implementation and new insights into efficacy." *Epilepsia* **49 Suppl 8**: 42-45.
- Pfeifer, H. H. and E. A. Thiele (2005). "Low-glycemic-index treatment: a liberalized ketogenic diet for treatment of intractable epilepsy." *Neurology* **65**(11): 1810-1812.
- Reid, C. A., T. Kim, et al. (2013). "Multiple molecular mechanisms for a single GABAA mutation in epilepsy." *Neurology* **80**(11): 1003-1008.
- Reid, C. A., T. H. Kim, et al. (2011). "Low blood glucose precipitates spike-and-wave activity in genetically predisposed animals." *Epilepsia* **52**(1): 115-120.
- Tan, H. O., C. A. Reid, et al. (2007). "Reduced cortical inhibition in a mouse model of familial childhood absence epilepsy." *Proc Natl Acad Sci U S A* **104**(44): 17536-17541.
- Thibert, R. L., H. H. Pfeifer, et al. (2012). "Low glycemic index treatment for seizures in Angelman syndrome." *Epilepsia* **53**(9): 1498-1502.

Velisek, L., J. Veliskova, et al. (2008). "Metabolic environment in substantia nigra reticulata is critical for the expression and control of hypoglycemia-induced seizures." *J Neurosci* **28**(38): 9349-9362.

Accepted Manuscript

Ingredients (g/Kg)	Low GI	High GI
Casein	200.0	200.0
Starch Waxy Maize: 90% amylopectin + 1% amylose	-	568.0
Starch GelCrisp: 30% amylopectin + 70% amylose	568.0	-
Sucrose	85.0	85.0
Gelatine	20.0	20.0
Soybean oil	50.0	50.0
Cellulose	33.0	33.0
DL methionine	3.0	3.0
Calcium carbonate	13.1	13.1
Sodium chloride	2.6	2.6
AIN93 trace minerals	1.4	1.4
Potassium citrate	2.5	2.5
Potassium dihydrogen phosphate	6.9	6.9
Potassium sulphate	1.6	1.6
Choline chloride	2.5	2.5
AIN93 vitamin	10.0	10.0
Digestible energy, KJ/g	16.1	16.1

A

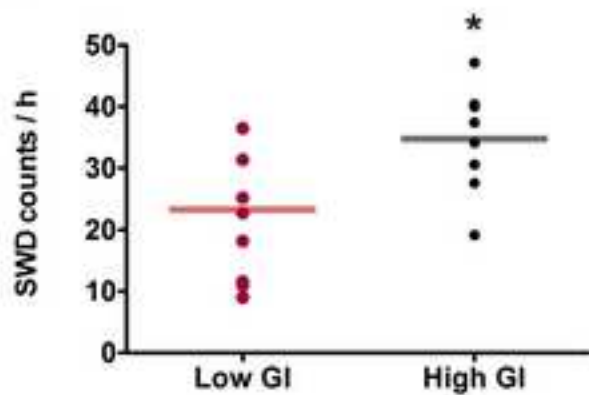
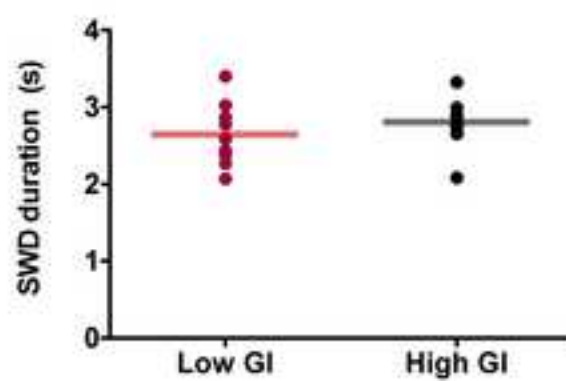
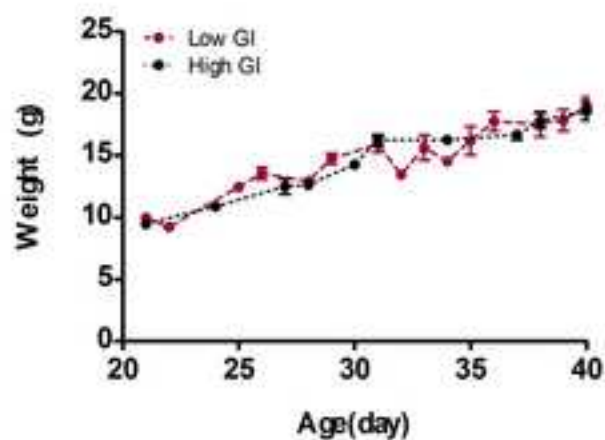
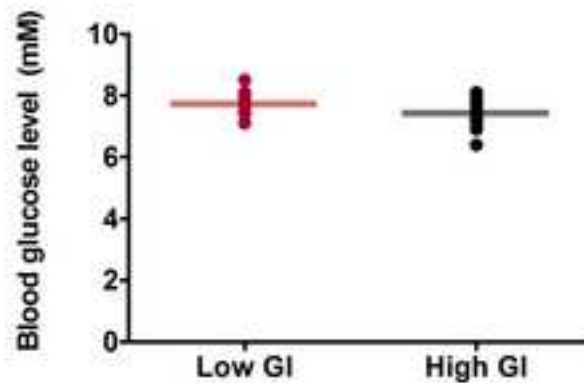
Low-GI



High-GI



1.5s
0.5mV

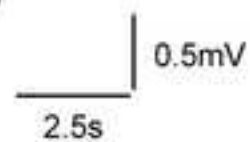
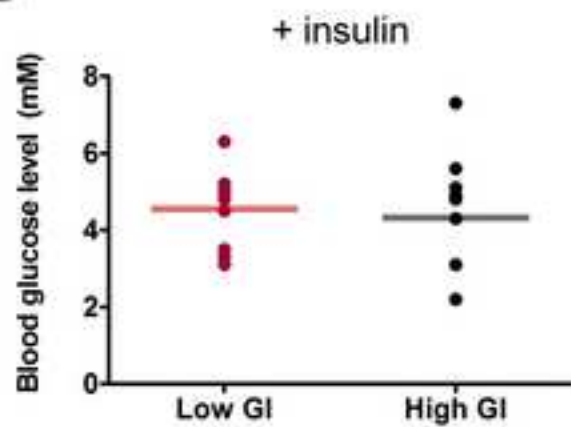
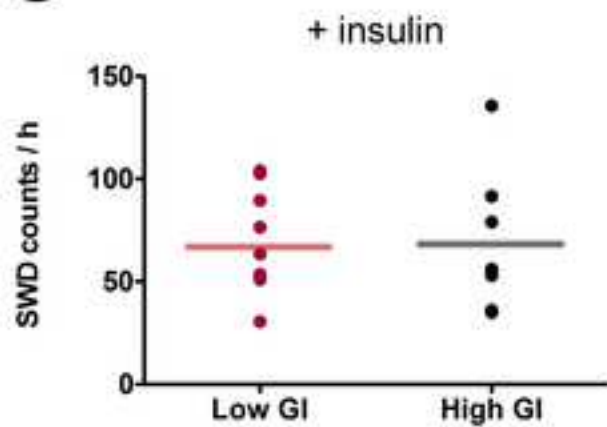
B**C****D****E**

A

Low-GI (+ insulin)



High-GI (+ insulin)

**B****C**

Highlights

- The Low GI diet reduce the expression of generalized seizures by ~30% in a mouse model
- Under hypoglycemic conditions the low GI diet was no longer effective
- Fluctuating blood glucose levels may act as a seizure precipitant
- A low GI diet may be an effective alternative dietary manipulation when more conventional diet options have failed

Accepted Manuscript



Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Kim, TH; Petrou, S; Reid, CA

Title:

Low glycaemic index diet reduces seizure susceptibility in a syndrome-specific mouse model of generalized epilepsy

Date:

2014-01-01

Citation:

Kim, T. H., Petrou, S. & Reid, C. A. (2014). Low glycaemic index diet reduces seizure susceptibility in a syndrome-specific mouse model of generalized epilepsy. *EPILEPSY RESEARCH*, 108 (1), pp.139-143. <https://doi.org/10.1016/j.eplepsyres.2013.10.014>.

Publication Status:

Accepted manuscript

Persistent Link:

<http://hdl.handle.net/11343/41857>