8-2013

Ketogenic Diets and Pain [post-print]

Susan A. Masino
Trinity College, susan.masino@trincoll.edu

David N. Ruskin
Trinity College, david.ruskin@trincoll.edu

Ketogenic Diets and Pain

Susan A. Masino,* Ph.D.and David N. Ruskin, Ph.D.

Affiliations:

Neuroscience Program and Department of Psychology, Trinity College, 300 Summit St., Hartford, Connecticut 06106, USA

*Correspondence to:

Susan A. Masino

Department of Psychology / Neuroscience Program

Trinity College

300 Summit Street, Hartford, CT, USA 06106

Phone (860) 297-2557; Fax (806) 297-2538

susan.masino@trincoll.edu

Total word count: 5363

Abstract

Ketogenic diets are well-established as a successful anticonvulsant therapy. Based on overlap between mechanisms postulated to underlie pain and inflammation, and mechanisms postulated to underlie therapeutic effects of ketogenic diets, recent studies have explored the ability for ketogenic diets to reduce pain. Here we review clinical and basic research thus far exploring the impact of a ketogenic diet on thermal pain, inflammation, and neuropathic pain.

Keywords: chronic constriction injury; hypoalgesia; inflammation; neuropathic pain; Taxol

Introduction

Pain is one of the most commonly indicated health-related factors leading to poor quality of life.[1-3] Not surprisingly, persons suffering from pain are more likely to also suffer from anxiety or depression compared to the normal population.[2, 3] Pain can be difficult to assess and treat, and it often requires long-term management with a variety of pharmacological and non-pharmacological approaches.

Here we review correlative and direct evidence that ketogenic diets could offer a non-pharmacological option for reducing pain and inflammation. High-fat ketogenic diets have long been known to be effective against seizures,[4, 5] and metabolically the high fat, very low carbohydrate and restricted protein content limits available glucose and forces utilization of ketones for cell energy. Similar to epilepsy, pain is a condition that encompasses diverse underlying conditions.

Why would one propose that ketogenic diets might also be useful in treating pain, a seemingly disparate condition? There are several lines of evidence, outlined below, that strongly suggest that metabolic approaches to pain could provide new clinical opportunities. Some of these mechanisms have been tested directly and additional research is ongoing.

Ketogenic Diet, Pain and Inflammation: Postulated Mechanisms

Multiple hypotheses undergird postulated hypoalgesic and anti-inflammatory effects of a ketogenic diet. Here we highlight four main postulated mechanisms:

1) Like seizures, chronic pain is thought to involve increased excitability of neurons; for pain, this can involve peripheral and/or central neurons.[6, 7] Thus, there is some similarity of the underlying biology.

2) Anticonvulsant drugs are often prescribed for neuropathic pain, which is poorly responsive to typical analgesic drugs (see below). Logically, then, if there is any commonality in the actions of anticonvulsant drugs and the ketogenic diet, the latter should have some effect on neuropathic pain.

3) Reducing glycolytic metabolism appears to be anticonvulsant, whether it is accomplished by fasting, caloric restriction, high fat plus carbohydrate restriction (the ketogenic diet, the modified Atkins diet),[4, 5] or specifically blocking glycolysis with 2-deoxyglucose.[8] In a parallel fashion, fasting and 2-deoxyglucose are both analgesic,[9, 10] so the ketogenic diet is also likely to be.

4) The neuromodulator adenosine can be analgesic[11, 12] and is involved in acupuncture's effects;[13, 14] evidence has been accumulating that metabolic changes such as fasting, the ketogenic diet, and 2-deoxyglucose all boost adenosine signaling.[15-19] Recent evidence suggests that exercise can treat neuropathic pain via an adenosine-based mechanism.[20]

Despite these overlaps and clinical potential, there has been little work characterizing the relationship between ketogenic diets and pain directly. Recently pain was highlighted in an overview of neurological disorders that should be considered for metabolic therapy;[21] clearly, treatment of pain and inflammation is a prevalent and unmet need. Here we present evidence from clinical and basic research, including positive and negative findings from our laboratory, identifying ketogenic diets as a platform deserving further optimization and exploration.

The Ketogenic Diet and Thermal Pain

As an initial direct test of the relationship between a ketogenic diet and pain we chose an acute, simple model (the hotplate test) of momentary thermal pain in rats. Briefly, rats are placed on a warm plate and their latency to display discomfort (licking or raising their hindpaws) is recorded and the animal is removed immediately. We customized a temperature range such that the lower temperatures approached a 60-second ceiling (the maximum time period; all rats were removed after 60 seconds), and the highest temperature elicited a response in 10-15 seconds (thus, not uncomfortable immediately, but within a dynamic range that could be measured easily).

In rats fed a control versus a ketogenic diet for 2-3 weeks we tested a series of six temperatures ascending through our customized range using a once-per-day test (Fig. 1A) to generate temperature-response curves. Feeding with a ketogenic diet increased the latency to show behavioral indications of pain, i.e. it produced hypoalgesia to thermal pain. We found that this increased latency to thermal pain was present in juvenile and adult rats,[22] and in other studies determined that the ketogenic diet does not impact motor function.[23, 24] Therefore, motor deficits did not underlie this increased behavioral latency.

Recent basic research has delved into the mechanisms and variables controlling these ketogenic diet effects by several means. First, because of the wide array of genetic modifications available, we wished to test for these effects in mice. Disappointingly, after three weeks of a 6.6:1 ketogenic diet (diet ratio indicates fat : (carbohydrate + protein), by weight), there was no effect on the temperature-response curve in the common inbred mouse strain C57Bl/6 (data not shown). This outcome was in clear contrast to rats, where the same diet

produces these effects reliably.[22, 23] Other mouse strains might produce different results with the hotplate test. Similarly, other tests of thermal pain might be useful to explore diet-induced changes[25] and could further illuminate species differences.

Second, we were interested in whether other ketogenic diet formulations would also produce similar thermal hypoalgesia in rats. Ketogenic diets used clinically are typically in the 1:1 to 4:1 range, considerably milder than a 6.6:1 diet. A lower ratio (and thus a more liberal diet) can be more palatable and increase compliance, thus increasing clinical applicability. Therefore, we also tried a 3.0:1 ketogenic diet.[23] Compared at identical lengths of feeding (Fig. 1A,B), both ketogenic diets produced significant hypoalgesia at multiple temperatures. The milder diet, however, had no significant effect at the highest tested temperature (51°C). This pattern seems to indicate that hypoalgesia relates somewhat to ketogenic diet stringency, and also possibly that ketogenic diets are more effective against mild pain.

We also pursued the relationship between metabolic and behavioral effects of the ketogenic diet. Ketogenic diets produce long-recognized characteristic changes in blood chemistry, namely elevated ketones and lowered glucose. The comparative evolution of ketones, glucose, and hypoalgesia might provide insight into the mechanisms underlying hypoalgesia. We found partial dissociations at both short (up to 3 weeks) and long (10-11 weeks) time points during ketogenic diet treatment. After just two days of feeding with a 6.6:1 ketogenic diet, rats had elevated ketones and mildly reduced glucose (as expected; Fig 2B,C), but hotplate testing over days one through six of feeding revealed that there was no significant effect of diet on thermal pain sensitivity (Fig. 2A).[23] Therefore, simply raising blood ketones and lowering blood glucose is not sufficient to produce hypoalgesia. This result seems to rule out a

mechanism involving, for instance, direct acute effects of ketones on molecular targets underlying thermal pain sensitivity.

At the longest time point of the investigated time course, rats kept on a 3.0:1 ketogenic diet for at least ten weeks were significantly hypoalgesic (Fig. 3A), arguably more so than at two weeks (compare Fig. 1B to Fig. 3A). At ten weeks, ketones were still elevated, yet glucose was no longer reduced compared to levels in control diet-fed rats (Fig. 3B,C). Therefore, the presence of hypoalgesia does not depend on low glucose. Elevated ketones are present at all time points at which hypoalgesia is present, and so might be necessary – but are not sufficient (Fig. 2,3).

Together these studies demonstrate consistent effects on thermal pain with a higher and a lower ratio ketogenic diet, the latter increasing clinical utility in terms of compliance and palatability. In addition, these data show that thermal hypoalgesia is maintained after chronic ketogenic diet feeding (also increasing clinical utility). Finally, these experiments demonstrate that rat physiology adapts over time to re-establish blood glucose levels similar to those found on a control diet (as found in other studies[26]), and thus a significantly reduced blood glucose is not necessary for long-term effects in this model. Chronic physiological adaptations to low carbohydrate intake might relate to the mechanisms underlying thermal hypoalgesia.

The Ketogenic Diet and Inflammation

Many types of pain and painful or progressive conditions involve chronic inflammation. As noted above, several mechanistic threads support the hypothesis that a ketogenic diet will reduce inflammation. Compared to glucose metabolism, ketone metabolism produces fewer

reactive oxygen species - which are known to contribute to inflammation. A ketolytic metabolism produces fewer free radicals and reactive oxygen species through affecting the mitochondrial co-enzyme Q couple and the cytoplasmic glutathione couple.[27] Exogenously applied ketones reduce reactive oxygen species *in vitro*[28-32] and *in vivo*.[33] Therefore the ketone-based metabolism of a ketogenic diet should guard against inflammation.

Data suggesting positive effects of a ketogenic diet itself on inflammation or associated inflammatory processes have been accumulating recently. In rodents, ketogenic diets reduce reactive oxygen species in the brain[34] and reduce central inflammation and reactive oxygen species in a model of multiple sclerosis.[35] Two clinical papers have found that ketogenic diet feeding of 12 weeks to 6 months reduced signs of liver inflammation in obese patients with nonalcoholic fatty liver disease (in addition to improving various other physiological and biochemical variables).[36, 37] Unfortunately, basic research into non-alcoholic fatty liver disease has been hampered by species differences between mice and humans in their hepatic reaction to ketogenic diets.[38]

Beyond the effects of ketones and ketone metabolism, the high fat content of ketogenic diets will elevate fatty acid levels in the body, including long-chain polyunsaturated fatty acids that bind to peroxisome proliferator-activated receptors. When activated, these nuclear receptors induce transcriptional changes that culminate in enhanced lipid metabolism.[39] Synthetic peroxisome proliferator-activated receptor agonists reduce experimentally-induced inflammation,[40-42] and genetic knockout of a major peroxisome proliferator-activated receptor augments inflammatory reactions.[43] This effect appears to involve inhibition of pro-

inflammatory pathways involving Nuclear Factor κB, Signal Transduction And Transcription-1, and Nuclear Factor of Activated T-cells.[44]

An additional mechanism with anti-inflammatory potential is adenosine. As noted above evidence is mounting that a ketolytic metabolism elevates levels of the neuromodulator adenosine,[17, 19] and adenosine has long been known to be anti-inflammatory,[45, 46] acting via receptors at multiple sites including leukocytes,[47] endothelia,[48] and neurons.[49] Thus, the ketogenic diet might have anti-inflammatory effects through increased adenosine production.

In our own research, we tested the relationship between a ketogenic diet and experimentally-induced inflammation. We induced local inflammation in juvenile and adult rats that had been fed a 6.6:1 ketogenic diet for three weeks.[22] Inflammation was produced by subcutaneous injection of complete Freund's adjuvant into one hindpaw. Two days later, injected and uninjected contralateral hindpaws were examined for swelling and for plasma extravasation (i.e. fluid movement from blood vessels into the interstitial space). We found that ketogenic diet treatment significantly reduced both of these measures of inflammation in juvenile animals (Fig. 4). Further extending clinical applicability, the ketogenic diet also produced significant, albeit smaller, effects in adult rats.[22] Thus, evidence indicates that ketogenic diets can reduce inflammation, and thus may be helpful for inflammation-associated pain.

The Ketogenic Diet and Neuropathic Pain

Neuropathic pain is caused by lesion or dysfunction of nervous tissue; the most common forms are associated with diabetes, shingles, multiple sclerosis, anticancer drugs, and both HIV

infection and antiretroviral drugs.[50] The most potent painkillers, the opiates, have been viewed historically as poorly effective against this type of pain. Only a subset of patients with neuropathies may respond well to opiates;[51] alternatively, higher-than-typical opiate dosing may be necessary for effective pain control in neuropathies,[52] thus raising serious concerns about sedation and drug dependence. As noted above, anticonvulsant drugs are more commonly prescribed for neuropathic pain, but, as with opiates, these drugs can have sedative and cognitive side effects. Therefore, we examined the effects of the ketogenic diet in two models of neuropathic pain.

We first explored a chronic constriction injury of the sciatic nerve, a surgical procedure in which this nerve is ligated unilaterally. The chronic constriction injury involves placing multiple sutures around the sciatic nerve proximal to the point at which it branches into the tibial, common peroneal, and sural nerves. Each suture was tightened just enough to evoke a twitch in the innervated muscles. This experimental model of neuropathic pain produces allodynia (the painful sensation of normally innocuous stimuli) of the ipsilateral hindpaw lasting several weeks.[53]

To maximize potential for clinical translation, we started ketogenic diet treatment immediately after the chronic constriction injury or sham surgery (all procedures performed except for ligation). Hindpaw sensitivity to tactile stimulation with von Frey probes was assessed weekly after surgery. As expected, allodynia was robust ipsilateral to the affected nerve in mice on control diet at 1-3 weeks post-surgery (Fig. 5). In mice on the ketogenic diet, allodynia did not reach significance until 3 weeks. This pattern could be interpreted as a delayed onset to allodynia, yet scores from ketogenic diet-fed and control diet-fed nerve-

damaged mice never differed significantly, and a small change in ketogenic diet-fed sham-surgery mice at weeks 1 and 2 probably contributed to a loss of statistical significance when comparing sham versus treatment animal within each diet group. Also, thresholds were slightly reduced contralateral to the surgery in ketogenic diet-fed mice (data not shown).

Despite these caveats, there is little suggestion of a substantial effect of diet in this experimental paradigm. Because a sudden acute nerve injury typically cannot be predicted, we focused on initiating therapy after the injury in these initial experiments. However, initiating diet treatment after surgery, rather than prior, may have reduced its potential efficacy; prior dietary treatment may yield a different outcome for neuropathic pain due to injury and deserves further exploration. However, pretreatment with a ketogenic diet still holds clinical relevance for situations of planned surgeries, especially those with a significant risk for neuropathic sequelae.

We further explored the ketogenic diet and neuropathic pain in a more generalized neuropathic model: chemotherapy-induced neuropathy. Because chemotherapy is a scheduled intervention, we employed a protocol with dietary pretreatment. In addition we used rats, an animal model with positive results regarding ketogenic diets and pain.[22, 23] Finally, in this study we tested two ketogenic diets of differing strength.

After two weeks of feeding with control or ketogenic diets, juvenile rats were injected intraperitoneally with the antimitotic cancer drug paclitaxel (2 mg/kg, or 5% dimethylsulfoxide vehicle) every other day for four injections. Rats were tested with von Frey probes weekly for three weeks (from day 8 through day 29) for tactile allodynia of the hindpaws. Ketogenic diets appeared to have little effect on thresholds in vehicle-injected animals (Fig. 6B,C). In animals

receiving drug, robust allodynia developed in all diet treatment groups. In addition, drug-injected rats on the stricter 6.6:1 ketogenic diet - much stronger than diets used clinically - already had a slightly but significantly reduced mechanical threshold at the eight-day time point compared to vehicle (Fig. 6C); however, this group at this time point did not differ significantly from drug-injected rats on the other two diets. Nevertheless, there is no evidence that ketogenic diets alleviate neuropathic pain in this model. Notably, a suggestion of generally increased tactile sensitivity in mice on the 6.6:1 diet (Fig. 5) does not occur in rats on this diet (Fig. 6C, "baseline"), highlighting species differences in ketogenic diet responsiveness.

Ketogenic Diets, Pain and Inflammation: Progress on Models and Mechanisms

Pain remains a vast data space, with a plethora of models and time courses as well as ongoing mechanisms and underlying causes. In parallel, the ketogenic diet remains relatively uncharacterized in terms of optimal formulation and underlying mechanisms even for treatment of epilepsy, despite its consistent clinical use for more than 90 years. Within this framework it is neither to be assumed that mechanisms and optimal therapeutic formula(e) will be common among the epilepsies, nor that epilepsy-based protocols will translate perfectly to painful conditions.

To date many research studies are done after three weeks on the ketogenic diet, based loosely on published anticonvulsant effects in rodents. Yet different paradigms may require different treatment time courses: for example, in the thermal pain tests with a hotplate, the first behavioral effects on an acute pain response were observed after 10 days of diet treatment. Neuropathic pain develops as a chronic pain model, and different time courses of

pain development and treatment response are to be expected. Although we did not find significant effects of the ketogenic diet in ameliorating allodynia due to sciatic nerve constriction or chemotherapy-induced neuropathy these represent just two models. Additional types of neuropathic pain have not been explored to date. Considering the magnitude of the unmet need for effective and non-addictive pain treatments, more work is needed.

Caveats regarding species differences and translatability remain a challenge in metabolic work, even among rodents. Here we highlighted reduced thermal pain in rats but not in mice after similar diet treatment protocols and experimental procedures. As an additional example, established anticonvulsant effects of ketogenic diets in humans and rats are countered by only mixed success in mice in various seizure models.[54, 55, 56] Therefore, mice might be a challenging model for exploring the relationship between a ketogenic diet and pain and inflammation. This is disappointing with the vast array of genetic approaches available in mice; knockout rats, while limited in number so far, may prove useful. An important take-home message, however, is that negative (or positive) effects of metabolic manipulations in mice might not be representative of responses in other species – perhaps echoing recent findings regarding the utility of mice in medical research.[57]

Implications, Comorbidities and Multiple Benefits

Use of the ketogenic diet has spread internationally in recent years, and therefore it has become a therapy increasingly accessible to many different cultural traditions.[58] Across all cultures, patients with chronic pain have among the lowest reported quality-of-life scores of any medical condition, and it has a striking negative impact on economies. In one

representative country, chronic pain takes up one-fifth of the entire health care expenditure, whereas in another it is three times as costly as all types of cancer together.[59] One strategy which remains unexplored and could yield important insights is to combine a ketogenic diet with a known hypoalgesic pharmacological agent (e.g. amitriptyline) and note additive or synergistic effects, as have been noted for seizure control but only for some drugs.[60]

At this time much more research is needed to determine if mechanisms mobilized by the ketogenic diet - a proven strategy for epilepsy - could benefit painful conditions, and perhaps also some of their comorbidities. For example, anxiety and depression are comorbid with both pain and epilepsy, as are sleep disorders. Restoring homeostasis in any of these realms can improve others and overall quality of life. There is a potential for similar multiple benefits, particularly given its metabolic underpinnings, in using a ketogenic diet to treat neuropathy associated with diabetes. A ketogenic diet has not been but should be explored in diabetic neuropathy, particularly since recent evidence already suggests that a ketogenic diet can reverse diabetic nephropathy in a rat.[61] Furthermore, because it stabilizes a lower blood glucose, a ketogenic diet could limit or eliminate the need for insulin or other medications. Thus a ketogenic diet could treat pain as well as multiple symptoms and consequences of diabetes. For more information about low carbohydrate diets and diabetes, please refer to the article in this supplement coauthored by Dr. Jeff Volek.

Inflammation is increasingly appreciated as part of the epileptogenic process, and becomes ever more strongly associated with neurological problems in young and old alike. For example, autism is associated increasingly with maternal inflammation,[62] and seizures are often comorbid.[63] Inflammation and seizures are similarly comorbid with Alzheimer's disease,[64, 65]

and recent work has highlighted benefits of metabolic therapies in Alzheimer's and Parkinson's disease.[66] Recent reviews have highlighted the potential for ketogenic diets in diverse disorders.[21, 67] Aside from disease-based processes, cognitive impairment has been observed alongside prediabetes even in adolescents,[68] thus underscoring the ability for altered metabolic homeostasis to affect brain function throughout the lifetime. A major research focus should be on how metabolic interventions such as a ketogenic diet can ameliorate common, comorbid, and difficult-to-treat conditions such as pain and inflammation.

Acknowledgments

We thank Tracey A.C.S. Suter and Taleen Hanania for technical assistance.

Author Contributions

S.A.M. conceived of the experiments; D.N.R. performed experiments; S.A.M. and D.N.R. wrote the paper.

Declaration of Conflicting Interests

The authors declare no competing financial interests.

Funding

NIH grants NS065446, NS065957, NS066932; NSF grant IOS-0843585; and Trinity College.

Ethical Approval

All experiments were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Trinity College animal care and use committee (IACUC A3869-01).

References

1. Elliott AM, Smith BH, Penny KI, et al. The epidemiology of chronic pain in the community. *Lancet.* 1999;354:1248-1252.

2. Becker N, Bondegaard-Thomsen A, Olsen AK, et al. Pain epidemiology and health related quality of life in chronic non-malignant pain patients referred to a Danish multidisciplinary pain center. *Pain.* 1997;73:393-400.

3. Gureje O, Von Korff M, Simon GE, Gater R. Persistent pain and well-being: a World Health Organization study in primary care. *J. Am. Med. Assoc.* 1998;280:147-151.

4. Wilder RM. The effects of ketonemia on the course of epilepsy. *Mayo Clin. Bull.* 1921;2:307.

5. Wilder RM. High fat diets in epilepsy. *Mayo Clin. Bull.* 1921;2:308.

6. Woolf CJ. Evidence for a central component of post-injury pain hypersensitivity. *Nature.* 1983;306:686-688.

7. Raja SN, Meyer RA, Campbell JN. Peripheral mechanisms of somatic pain. *Anesthesiology.* 1988;68:571-590.

8. Stafstrom CE, Ockuly JC, Murphree L, et al. Anticonvulsant and antiepileptic actions of 2-deoxy-D-glucose in epilepsy models. *Ann. Neurol.* 2009;65:435-447.

9. Fisher MC, Bodnar RJ. 2-Deoxy-D-glucose antinociception and serotonin receptor subtype antagonists: test-specific effects in rats. *Pharmacol. Biochem. Behav.* 1992;43:1241-1246.

10. de los Santos-Arteaga M, Sierra-Domínguez SA, Fontanella GH, et al. Analgesia induced by dietary restriction is mediated by the κ-opioid system. *J. Neurosci.* 2003;23:11120-11126.

11. Yarbrough GG, McGuffin-Clineschmidt JC. In vivo behavioral assessment of central nervous system purinergic receptors. *Eur. J. Pharmacol.* 1981;76:137-144.

12. Schmidt AP, Böhmer AE, Antunes C, et al. Anti-nociceptive properties of the xanthine oxidase inhibitor allopurinol in mice: role of A_1 adenosine receptors. *Br. J. Pharmacol.* 2009;156:163-172.

13. Goldman N, Chen M, Fujita T, et al. Adenosine A1 receptors mediate local anti-nociceptive effects of acupuncture. *Nat. Neurosci.* Jul 2010;13:883-888.

14. Takano T, Chen X, Luo F, et al. Traditional acupuncture triggers a local increase in adenosine in human subjects. *J. Pain.* 2012;13:1215-1223.

15. Zhao YT, Tekkök S, Krnjević K. 2-Deoxy-D-glucose-induced changes in membrane potential, input resistance, and excitatory postsynaptic potentials of CA1 hippocampal neurons. *Can. J. Physiol. Pharmacol.* 1997;75:368-374.

16. Minor TR, Rowe MK, Soames-Job RF, Ferguson EC. Escape deficits induced by inescapable shock and metabolic stress are reversed by adenosine receptor antagonists. *Behav. Brain Res.* 2001;120:203-212.

17. Kawamura M, Jr., Ruskin DN, Masino SA. Metabolic autocrine regulation of neurons involves cooperation among pannexin hemichannels, adenosine receptors and K_{ATP} channels. *J. Neurosci.* 2010;30:3886-3895.

18. Jinka TR, Carlson ZA, Moore JT, Drew KL. Altered thermoregulation via sensitization of A_1 adenosine receptors in dietary-restricted rats. *Psychopharmacology.* 2010;209:217-224.

19. Masino SA, Li T, Theofilas P, et al. A ketogenic diet suppresses seizures in mice through adenosine A_1 receptors. *J. Clin. Invest.* 2011;121:2679-2683.

20. Martins DF, Mazzardo-Martins L, Soldi F, et al. High-intensity swimming exercise reduces neuropathic pain in an animal model of complex regional pain syndrome type I: evidence for a role of the adenosinergic system. *Neuroscience.* 2013;234:69-76.

21. Ruskin DN, Masino SA. The nervous system and metabolic dysregulation: emerging evidence converges on ketogenic diet therapy. *Front. Neurosci.* 2012;6:33.

22. Ruskin DN, Kawamura M, Jr., Masino SA. Reduced pain and inflammation in juvenile and adult rats fed a ketogenic diet. *PLoS One.* 2009;4:e8349.

23. Ruskin DN, Suter TACS, Ross JL, Masino SA. Ketogenic diets and thermal pain: dissociation of hypoalgesia, elevated ketones, and lowered glucose in rats. *J. Pain.* 2013; in press, doi: 10.1016/j.jpain.2012.12.015.

24. Ruskin DN, Ross JL, Kawamura M, Jr., et al. A ketogenic diet delays weight loss and does not impair working memory or motor function in the R6/2 1J mouse model of Huntington's disease. *Physiol. Behav.* 2011;103:501-507.

25. Ziegler DR, Gamaro GD, Araújo E, et al. Nociception and locomotor activity are increased in ketogenic diet fed rats. *Physiol. Behav.* 2005;84:421-427.

26. Likhodii SS, Musa K, Mendonca A, et al. Dietary fat, ketosis, and seizure resistance in rats on the ketogenic diet. *Epilepsia.* 2000;41:1400-1410.

27. Veech RL. The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. *Prostaglandins Leukot. Essent. Fatty Acids.* 2004;70:309-319.

28. Kim DY, Davis LM, Sullivan PG, et al. Ketone bodies are protective against oxidative stress in neocortical neurons. *J. Neurochem.* 2007;101:1316-1326.

29. Kim DY, Vallejo J, Rho JM. Ketones prevent synaptic dysfunction induced by mitochondrial respiratory complex inhibitors. *J. Neurochem.* 2010;114:130-141.

30. Noh HS, Hah Y-S, Nilufar R, et al. Acetoacetate protects neuronal cells from oxidative glutamate toxicity. *J. Neurosci. Res.* 2006;83:702-709.

31. Maalouf M, Rho JM. Oxidative impairment of hippocampal long-term potentiation involves activation of protein phosphatase 2A and is prevented by ketone bodies. *J. Neurosci. Res.* 2008;86:3322-3330.

32. Maalouf M, Sullivan PG, Davis L, et al. Ketones inhibit mitochondrial production of reactive oxygen species production following glutamate excitotoxicity by increasing NADH oxidation. *Neuroscience.* 2007;145:256-264.

33. Haces ML, Hernandez-Fonseca K, Medina-Campos ON, et al. Antioxidant capacity contributes to protection of ketone bodies against oxidative damage induced during hypoglycemic conditions. *Exp. Neurol.* 2008;211:85-96.

34. Sullivan PG, Rippy NA, Dorenbos K, et al. The ketogenic diet increases mitochondrial uncoupling protein levels and activity. *Ann. Neurol.* 2004;55:576-580.

35. Kim DY, Hao J, Liu R, et al. Inflammation-mediated memory dysfunction and effects of a ketogenic diet in a murine model of multiple sclerosis. *PLoS One.* 2012;7:e35476.

36. Tendler D, Lin S, Yancy WS, Jr., et al. The effect of a low-carbohydrate, ketogenic diet on nonalcoholic fatty liver disease: a pilot study. *Dig. Dis. Sci.* 2007;52:589-593.

37. Pérez-Guisado J, Muñoz-Serrano A. The effect of the Spanish Ketogenic Mediterranean Diet on nonalcoholic fatty liver disease: a pilot study. *J. Med Food.* 2011;14:677-680.

38. Schugar RC, Crawford PA. Low-carbohydrate ketogenic diets, glucose homeostasis, and nonalcoholic fatty liver disease. *Curr. Opin. Clin. Nutr. Metab. Care.* 2012;15:374-380.

39. Michalik L, Auwerx J, Berger JP, et al. International Union of Pharmacology. LXI. Perixosome proliferator-activated receptors. *Pharmacol. Rev.* 2006;58:726-741.

40. Cuzzocrea S, Pisano B, Dugo L, et al. Rosiglitazone, a ligand of the peroxisome proliferator-activated receptor-gamma, reduces acute inflammation. *Eur. J. Pharmacol.* 2004;483:79-93.

41. Cuzzocrea S, Mazzon E, Dugo L, et al. Reduction in the evolution of murine type II collagen-induced arthritis by treatment with rosiglitazone, a ligand of the peroxisome proliferator-activated receptor gamma. *Arthritis Rheum.* 2003;48:3544-3556.

42. LoVerme J, Fu J, Astarita G, et al. The nuclear receptor peroxisome proliferator-activated receptor-alpha mediates the anti-inflammatory actions of palmitoylethanolamide. *Mol. Pharmacol.* 2005;67:15-19.

43. Cuzzocrea S, Mazzon E, Di Paolo R, et al. The role of the peroxisome proliferator-activated receptor-α (PPAR-α) in the regulation of acute inflammation. *J. Leukoc. Biol.* 2006;79:999-1010.

44. Blanquart C, Barbier O, Fruchart JC, et al. Peroxisome proliferator-activated receptors: regulation of transcriptional activities and roles in inflammation. *J. Steroid Biochem. Mol. Biol.* 2003;85:267-273.

45. Kowaluk EA, Jarvis MF. Therapeutic potential of adenosine kinase inhibitors. *Expert Opin. Investig. Drugs.* 2000;9:551-564.

46. Linden J. Molecular approach to adenosine receptors: receptor-mediated mechanisms of tissue protection. *Ann. Rev. Pharmacol. Toxicol.* 2001;41:775-787.

47. Ngamsri K-C, Wagner R, Vollmer I, et al. Adenosine receptor A1 regulates polymorphonuclear cell trafficking and microvascular permeability in lipopolysaccharide-induced lung injury. *J. Immunol.* 2010;185:4374-4384.

48. Wang J, Huxley VH. Adenosine A_{2A} receptor modulation of juvenile female rat skeletal muscle microvessel permeability. *Am. J. Physiol. Heart Circ. Physiol.* 2006;291:H3094-H3105.

49. Sorkin LS, Moore J, Boyle DL, et al. Regulation of peripheral inflammation by spinal adenosine: role of somatic afferent fibers. *Exp. Neurol.* 2003;184:162-168.

50. Campbell JN, Meyer RA. Mechanisms of neuropathic pain. *Neuron.* 2006;52:77-92.

51. Portenoy RK, Foley KM, Inturrisi CE. The nature of opioid responsiveness and its implications for neuropathic pain: new hypotheses derived from studies of opioid infusions. *Pain.* 1990;43:273-286.

52. Dickenson AH, Suzuki R. Opioids in neuropathic pain: clues from animal studies. *Eur. J. Pain.* 2005;9:113-116.

53. Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain.* 1988;33:87-107.

54. Uhlemann ER, Neims AH. Anticonvulsant properties of the ketogenic diet in mice. *J. Pharmacol. Exp. Ther.* 1972;180:231-238.

55. Samala R, Willis S, Borges K. Anticonvulsant profile of a balanced ketogenic diet in acute mouse seizure models. *Epilepsy Res.* 2008;81:119-127.

56. Hartman AL, Zheng X, Bergbower E, et al. Seizure tests distinguish intermittent fasting from the ketogenic diet. *Epilepsia.* 2010;51:1395-1402.

57. Seok J, Warren HS, Cuenca AG, et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc. Natl. Acad. Sci. USA.* 2013;110:3507-3512.

58. Rho JM, Zupec-Kania B, Masino SA. Ketogenic diet and epilepsy: the role of adenosine. In: Masino SA, Boison D, eds. *Adenosine: A Key Link between Metabolism and Brain Activity*. New York: Springer; 2013.

59. Phillips CJ. Economic burden of chronic pain. *Expert Rev. Pharmacoecon. Outcomes Res.* 2006;6:591-601.

60. Morrison PF, Pyzik PL, Hamdy R, et al. The influence of concurrent anticonvulsants on the efficacy of the ketogenic diet. *Epilepsia.* 2009;50:1999-2001.

61. Poplawski MM, Mastaitis JW, Isoda F, et al. Reversal of diabetic nephropathy by a ketogenic diet. *PLoS One.* 2011;6:e18604.

62. Atladottir HO, Thorsen P, Schendel DE, et al. Association of hospitalization for infection in childhood with diagnosis of autism spectrum disorders: a Danish cohort study. *Arch. Pediatr. adolesc. Med.* 2010;164:470-477.

63. Canitano R. Epilepsy in autism spectrum disorders. *Eur. Child Adolesc. Psychiatry.* 2007;16:61-66.

64. Heneka MT, O'Banion MK. Inflammatory processes in Alzheimer's disease. *J Neuroimmunol.* 2007;184:69-91.

65. Pandis D, Scarmeas N. Seizures in Alzheimer disease: clinical and epidemiological data. *Epilepsy Curr.* 2012;12:184-187.

66. Cai H, Cong WN, Ji S, et al. Metabolic dysfunction in Alzheimer's disease and related neurodegenerative disorders. *Curr. Alzheimer Res.* 2012;9:5-17.

67. Stafstrom CE, Rho JM. The ketogenic diet as a treatment paradigm for diverse neurological disorders. *Front. Pharmacol.* 2012;3:59.

68. Yau PL, Castro MG, Tagani A, et al. Obesity and metabolic syndrome and functional and structural brain impairments in adolescence. *Pediatrics.* 2012;130:e856-864.

Figures and Legends

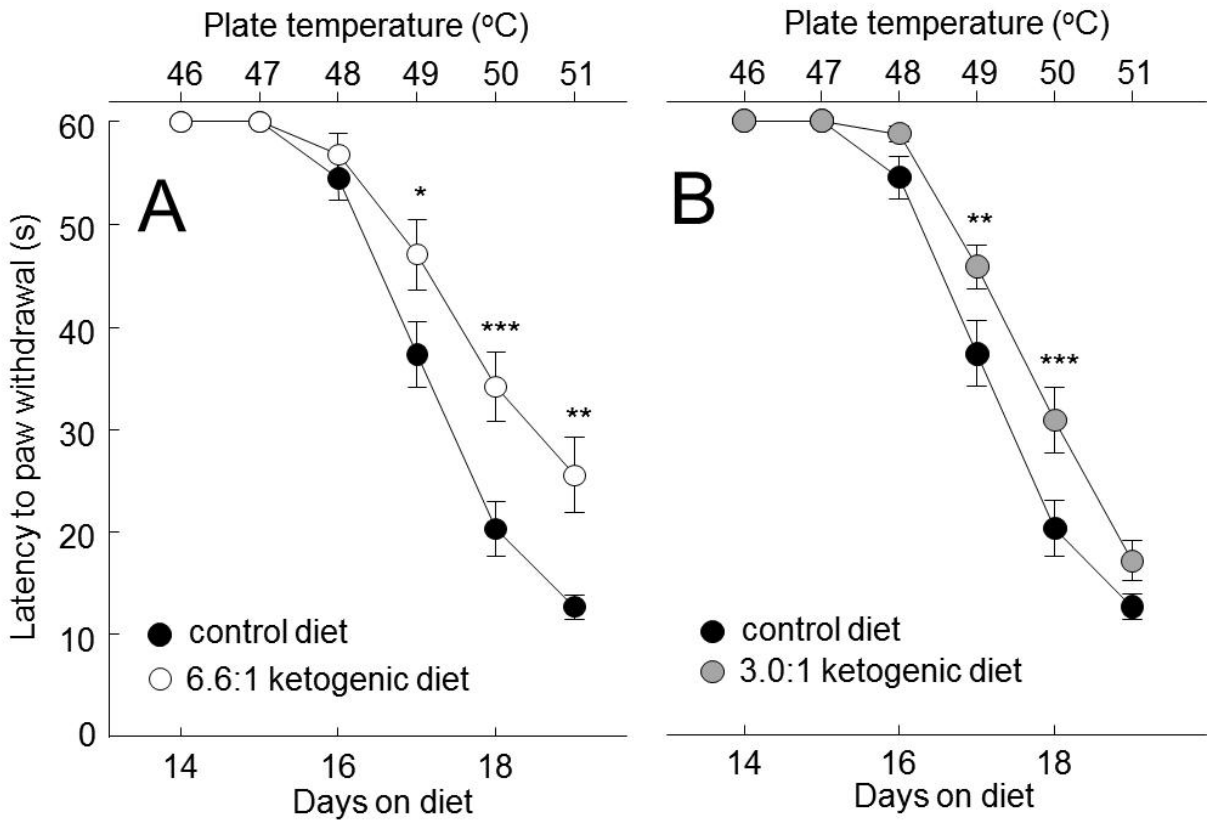

Figure 1. Temperature-response curves from hotplate tests in rats on two differing ketogenic diets. Top X-axes indicate plate temperature, those on the bottom indicate days on diet. *A)* Rats on control diet or a 6.6:1 ketogenic diet. *B)* Rats on control diet or a 3.0:1 ketogenic diet. Both diets produced significant hypoalgesia. Number of subjects was 18-20. *p<0.05 **p<0.01, ***p<0.001 versus control diet. Adapted from published work.[23]

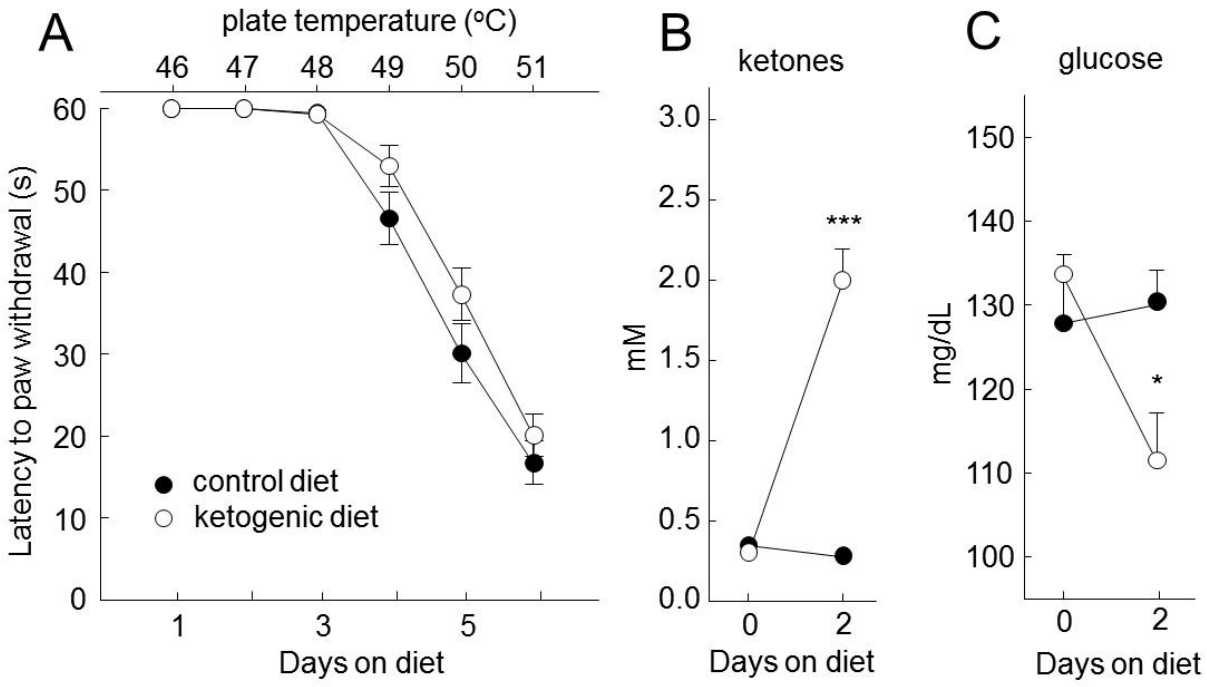

Figure 2. Elevated ketones and reduced glucose without thermal hypoalgesia. Rats were fed on a 6.6:1 ketogenic diet for one day before daily hotplate testing commenced (*A*). Separate groups of rats were used for sampling of tail whole blood (*B, C*). *p<0.05, ***p<0.001 versus control diet. Adapted from published work.[23]

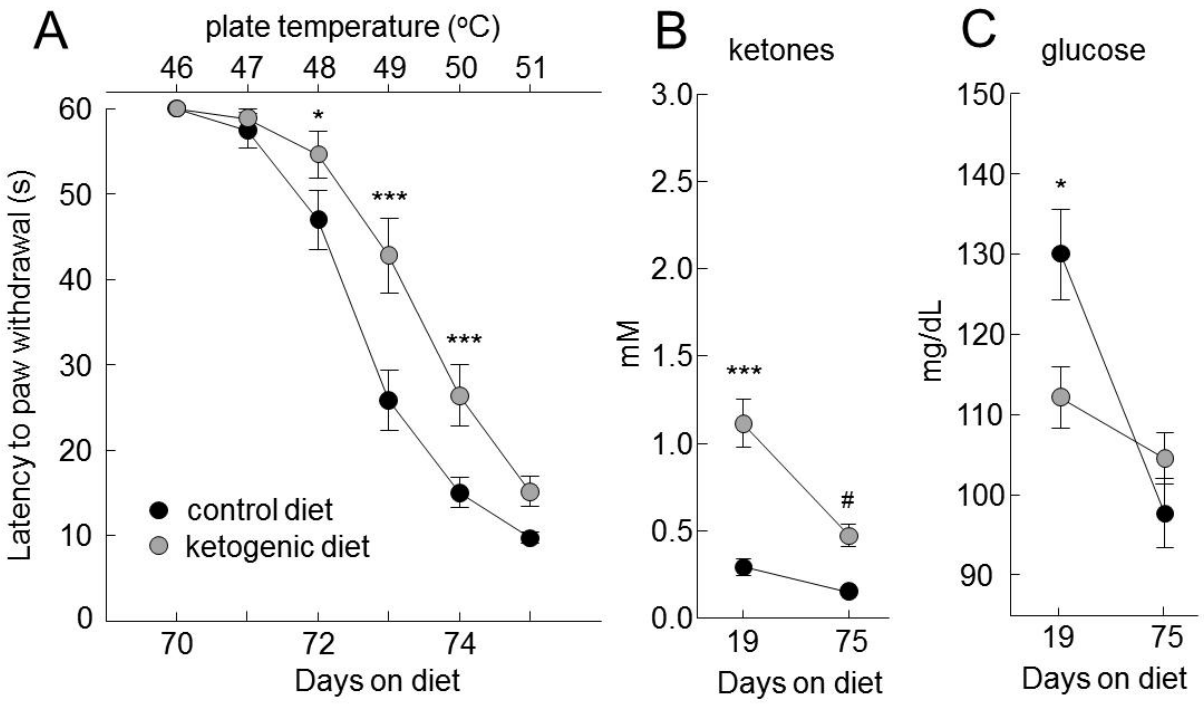

Figure 3.Thermal hypoalgesia without reduced glucose. Rats were on a 3.0:1 ketogenic diet for 70 days before daily hotplate testing (*A*). Separate groups of rats were used for sampling of tail whole blood (*B,C*). #p=0.055, *p<0.05, **p<0.01, ***p<0.001 versus control diet. Adapted from published work.[23]

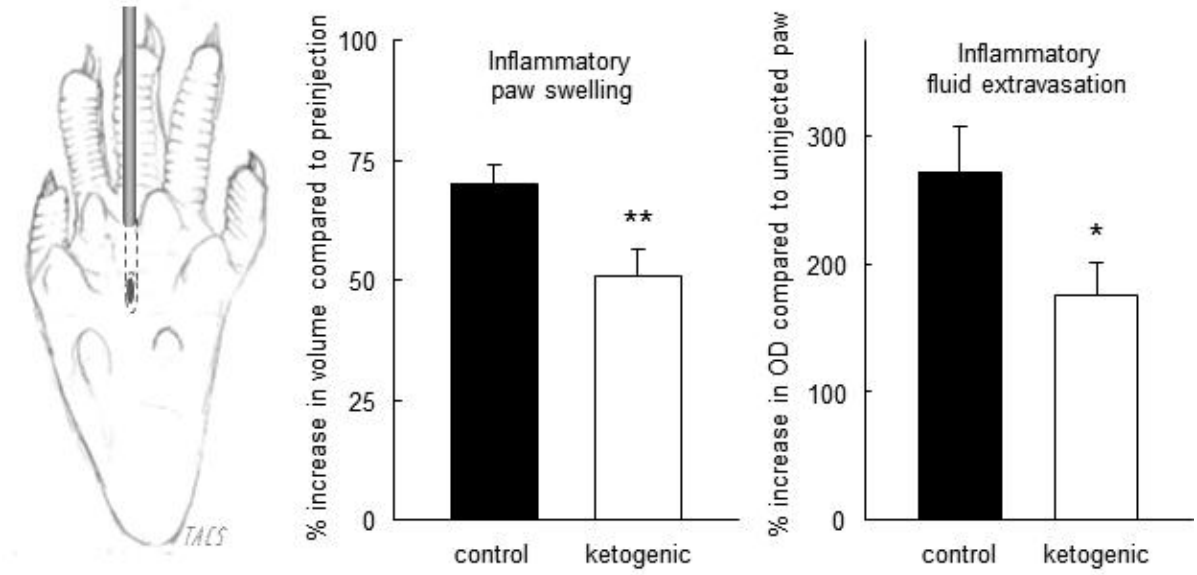

Figure 4. Effects of a 6.6:1 ketogenic diet on experimental inflammation in juvenile rats. *Left:* schematic of the subcutaneous injection of complete Freunds' adjuvant between the tori of a rat hindpaw. *Middle:*hindpaw swelling was significantly reduced in animals on a ketogenic diet. *Right:* plasma extravasation was significantly reduced in animals on a ketogenic diet. Adapted from published studies.[22]

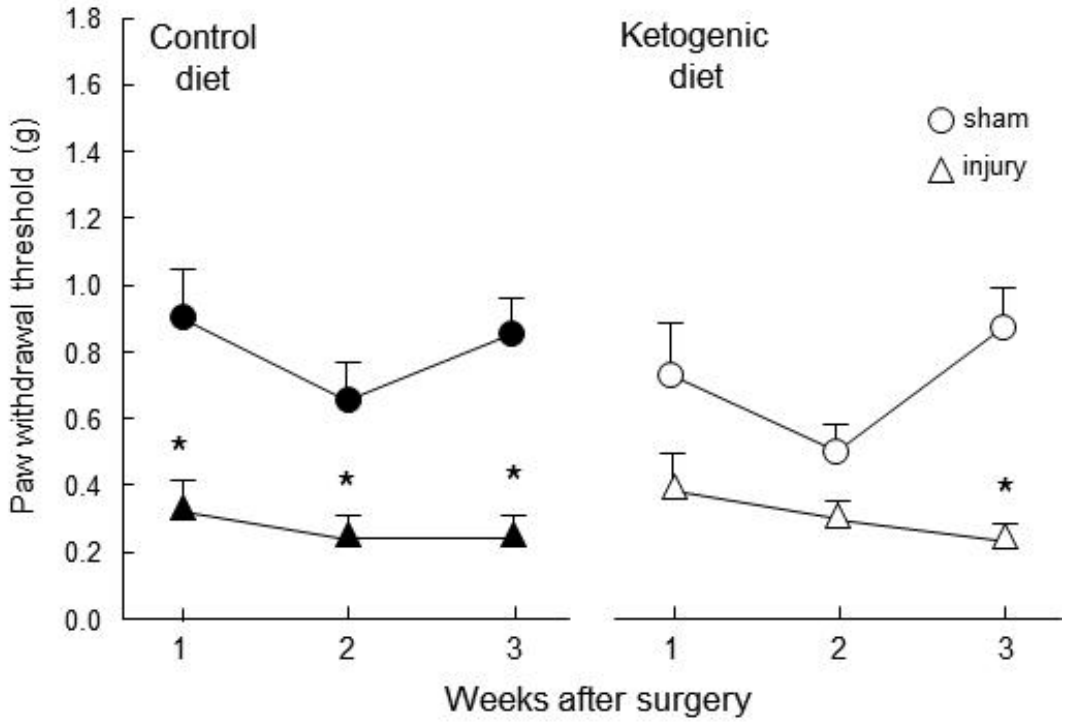

Figure 5. A ketogenic diet has little effect on neuropathic pain due to sciatic nerve constriction injury in mice. All data are from the hindpaw ipsilateral to the sham or actual surgery. The ketogenic diet was 6.6:1. Number of subjects is 10-11. *$p<0.05$ versus sham surgery.Unpublished data.

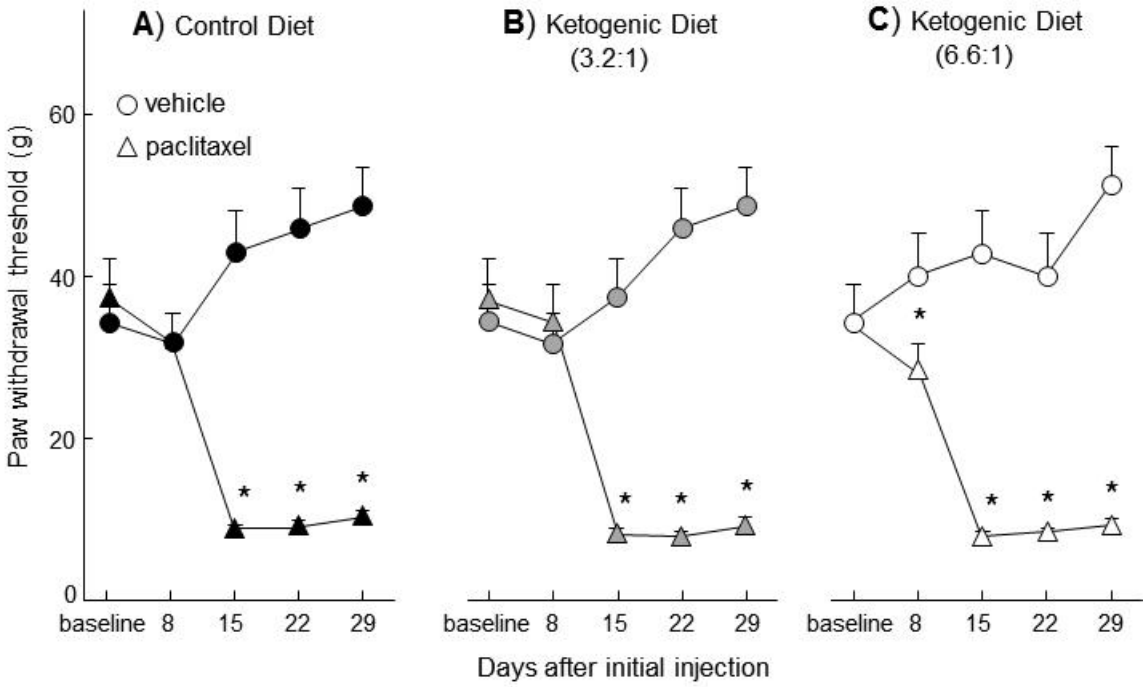

Figure 6. Ketogenic diets have little effect in rats on the neuropathic pain due to the chemotherapy drug paclitaxel. Baseline measurements were taken just prior to injections. Number of subjects is 12 in all groups. *p<0.05 versus vehicle.Unpublished data.