Ketogenic diets may provide neuroprotective benefits after unilateral chronic sciatic nerve constriction injury

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Abstract

Unilateral constrictive sciatic nerve injury (uCCI) is a common neuropathic pain (NP) model that provides reproducible results for possible treatment interventions for both spontaneous and stimulus evoked pain. However, treatment of NP remains a persistent clinical problem due to the incomplete understanding of its pathogenesis. NP often occurs as a result of diabetes, shingles, spinal cord injury, stroke, multiple sclerosis, cancer, and human immunodeficiency virus infection; as well as common conditions, such as lumbar or cervical radiculopathies, and traumatic or postsurgical nerve injuries. Previously, high fat, low carbohydrate ketogenic diets (KDs) were validated as non-pharmacological treatments for some forms of drug-resistant epilepsy. Recent studies have found important clues showing that ketone bodies may be responsible for cell damage and NP. Furthermore, KDs have recently been shown to have neuroprotective effects in animal models of several neurodegenerative diseases including amyotrophic lateral sclerosis, Alzheimer’s disease, cerebral hypoxia, cerebral artery occlusion, and traumatic brain injury. Specifically, KDs increased the number of glucose transporter-1-positive blood vessels in the lesioned penumbra and increased the expression of monocarboxylate transporter-1; ketosis also lowered the aspartate: glutamate ratio, reflecting a shift in the equilibrium of the aspartate aminotransferase reaction. Moreover, KDs, in parallel with increased brain malonyl-CoA and expression of the mitochondrial uncoupling proteins 4 and 5. Here, we hypothesized that these changes induced by KDs and ketone bodies may be directly related to neuroprotective effects after uCCI, and if proven, may offer an innovative method to treatment of NP.
Introduction

Neuropathic pain (NP) is caused by lesions or dysfunctions in the peripheral and central nervous systems [1-3] and is characterized by a diverse range of symptoms. NP is therefore difficult to treat, and despite the advent of new therapies, there is no single treatment that alleviates both the symptoms, and also targets the underlying mechanisms associated with this pain. Thus, it is important for clinicians to better understand therapies that are most effective at relieving specific pain experienced by patients and that also elicit the fewest adverse side-effects. Recently, an evidence-based algorithm to treat NP conditions was proposed [2] and more strategies like this are needed to develop new therapies for the prevention and treatment of NP.

Research has shown that dietary restrictions initiated after spinal cord injury (SCI) improve functional recovery. Specifically, several studies by Tetzlaff et al showed that every-other-day-fasting (EODF) improved neurological recovery in rats following cervical and thoracic spinal cord injury (SCI) [4-6]: EODF implemented after cervical SCI was found to be neuroprotective, promoted plasticity; and improved behavioral recovery, which were associated with increased blood levels (2–3 fold) of the ketone body, β-hydroxybutyrate (βOHB) on the fasting days [4]. Furthermore, another set of experiments found that ketogenic diets (KDs) improved forelimb motor function after SCI [7].

Indeed, there is a substantial body of data indicating KDs improve behavioral recovery, reduce lesion size, and reduce grey matter damage associated with SCI (Figure 1). KDs are known to increase glucose transporter-1- positive blood vessel density and also increase expression of the monocarboxylate transporter 1 [4, 7]. Interestingly, blocking medium chain triglyceride transport with α-cyano-4-hydroxycinnamate abolished the previously mentioned KD associated neuroprotective effects [7].

During fasting, βOHB acts as an energy carrier, transporting energy from the liver to peripheral tissues during fasting (Table 1) or exercise [8]. Recently, its signaling functions were determined: βOHB acts indirectly by promoting protein hyperacetylation by increasing the cellular pool of acetyl-CoA, which is a substrate for histone acetyltransferases; and it acts directly by inhibiting the activity of class I histone deacetylases (Figure 2, table 1) [9-11]. Moreover, the brain is known to actively metabolize ketone bodies [12, 13], and several studies showed that the ketotic state is associated with significant changes the brain’s ability to regulate amino acids, particularly the transamination of glutamic acid to aspartic acid [14].

KDs consist of a high fat, low carbohydrate intake with variable amounts of protein, and have been used extensively to treat epilepsy in children [15, 16]. As KDs limit the levels of carbohydrate intake, the body eventually switches
Hypothesis

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to fatty acid oxidation as an energy source that also results in the formation of ketone bodies. Elevated levels of ketone bodies in the blood, a state known as ketosis, have been associated with a reduction in the frequency of epileptic seizures [8]. Although, βOHB appears to have broad neuroprotective effects in many neurodegenerative disease models, the specific mechanisms associated with these effects have yet to be elucidated [17-19]. In vitro, βOHB protected cultured neurons from MPP+ (1-methyl-4-phenylpyridinium, a chemical used to induce Parkinsonism in mice) and β-amyloid toxicity [20]. In addition, βOHB reduced apoptosis after hypoxia in rat hippocampal neuron cultures [21] and enhanced survival of cultured cortical neurons after exposure to hydrogen peroxide; both under no glucose (high cell death) and normal glucose (low cell death) conditions [11]. Consequently, we put rats on a KD after unilateral chronic constrictive sciatic nerve injury (uCCI) to find the potential relationship between the neuroprotective benefits of ketone bodies and decreases in symptoms associated with NP. Results indicate that a long-term, novel therapeutic strategy for NP in humans may be possible [20]. In addition, βOHB reduced apoptosis after

Hypothesis

KDs are high-fat, high-protein, low-carbohydrate diets that have proven efficacy against epilepsy [22, 23] and SCI [7]. Although the mechanisms of action underlying such effects remain poorly understood, the high caloric value of fat and its role in metabolism suggest that the anticonvulsant efficacy of KDs may be

Figure 1 | Cellular signaling mediated by β-hydroxybutyrate (βOHB). βOHB is a ligand for cell-surface G-protein-coupled receptors that modulate lipolysis, sympathetic tone and metabolic rate. Moreover, βOHB alters protein acetylation through the following mechanisms: increasing the cellular pool of acetyl-CoA, a substrate for histone acetyltransferases, and directly inhibiting the activity of class I histone deacetylases. Abbreviations: ACLY, ATP citrate lyase; CS, citrate synthase; Foxo3, forkhead box O3.
related to enhanced energy flux in the brain due to increased β-oxidation [24]; and as βOHB has broad neuroprotective effects in several underdiagnosed, particularly in patients having neurodegenerative disease models [17-19]: We propose that KDs may prevent and ameliorate heterogeneous in nature and largely resistant to uCCI in a rat model of NP. However, as there is a large body of research that previously focused on the relationship between ketone bodies and the central nervous system, we set out to determine whether ketone bodies could provide neuroprotective actions to the peripheral nervous system.

**Evaluation of hypothesis**

NP, characterized by hyperalgesia, allodynia and spontaneous pain, often occurs as a result of injuries to the peripheral nerves, dorsal root ganglion, spinal cord or brain [25]. It is generally chronic and disabling, and is among the most challenging of injuries to treat; which may be related to the specificities of its complicated pathophysiological mechanisms [26, 27]; but also to it being underdiagnosed, particularly in patients having NP. NP can be invoked in several animal models [29-31] and resultant behaviors in these specific models are attributed to sensitization in either the peripheral nervous system (afferent fibers) or central in the central nervous system (efferent fibers) [32]. Partial injury to the peripheral nerves of rats has been used to investigate mechanisms of chronic neuropathic pain and may mimic certain human nerve injury pain syndromes [33]. Fasting has been used as an anti-convulsive therapy since ancient times, while KDs have been used as a therapy for over a century; and continue to be an effective therapy, particularly for some childhood epilepsies ventive treatment for drug-resistant epilepsy that are resistant to anticonvulsant medications [7]. This diet is known to increase mitochondrial...
uncoupling protein levels and activity [35]; and it presumed that fasting and KDs share a common mechanism in alleviating seizures, although this assumption has not been rigorously tested [36].

As KDs mimic fasting that induces ketosis, thus relieving seizures [37, 38], we hypothesized that KDs may enhance neurogenesis similar to the effects fasting; consistent to findings by Kwon et al that found that a KD promoted neurogenesis after kainic acid-induced seizures in mice [39]. In addition, ketone bodies are known to be neuroprotective and cytoprotective [8], and βOHB infusion and KDs protected against neuronal death in several animal models of brain injury by: reducing neuronal apoptosis, reducing brain edema, and improving sensorimotor and cognitive outcomes [40, 41].

We propose that feeding rats with a KD after uCCI may increase serum ketone bodies, especially βOHB, and provide neuroprotective benefits. These results may provide a promising therapy for human NP in the future.

### Table 1 | Comparison of longevity pathways regulated by ketogenic diets and calorie

<table>
<thead>
<tr>
<th>βOHB</th>
<th>Ketogenic diet *</th>
<th>Calorie restriction *</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOXO3</td>
<td>↑</td>
<td>↑</td>
<td>[33]</td>
</tr>
<tr>
<td>Protein acetylation</td>
<td>↑</td>
<td>↑</td>
<td>[33] [32]</td>
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<tr>
<td>Stress resistance</td>
<td>↑</td>
<td>↑</td>
<td>[33] [29]</td>
</tr>
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*↑, increased.

### Verification of hypothesis

Our experiments were designed to verify the hypothesis as follows:
1) Employ a typical animal model of uCCI.
2) Feed the animal with a KD accordingly throughout the experiment.
3) Test ketone body content in serum or/and ligated nerve.
4) Conduct correlation analysis between the levels of ketone bodies and the degree of nerve restoration.

### Consequence of hypothesis

KD are high fat, low carbohydrate diets that cause a metabolic state of increased hepatic ketogenesis, resulting in high levels of blood ketone bodies due to the breakdown of fatty acids. Although studies have linked KDs to a reduction in NP, the underlying mechanisms have yet to be elucidated.

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### Contributors


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References


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