

Ketosis May Promote Brain Macroautophagy via Activation of Hypoxia-Inducible Factor-1

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Abstract

Ketogenic diets are markedly neuroprotective, but the basis of this effect is still poorly understood. Recent studies demonstrate that ketone bodies increase neuronal levels of hypoxia-inducible factor-1 α (HIF-1 α), possibly owing to succinate-mediated inhibition of prolyl hydroxylase activity. Another recent study has observed reduced activity of mTORC1 in the cortex of rats fed a ketogenic diet. These effects could be expected to collaborate in the induction of neuronal autophagy. Considerable evidence points to moderate up-regulation of neuronal autophagy as a rational strategy for prevention of neurodegenerative disorders; hence, autophagy may mediate some of the neuroprotective benefits of ketogenic diets. Brain-permeable agents which activate AMP-activated kinase, such as metformin and berberine, can also boost neuronal autophagy, and may have potential for amplifying the impact of ketogenesis on this process. Since it would not be practical for most people to adhere to ketogenic diets continuously, alternative strategies are needed to harness the brain-protective potential of ketone bodies. These may include ingestion of medium-chain triglycerides, intermittent ketogenic dieting, and possibly the use of supplements that promote hepatic ketogenesis – notably carnitine and hydroxycitrate – in conjunction with dietary regimens characterized by long daily episodes of fasting or carbohydrate avoidance.

Ketogenic Diets Up-Regulate HIF-1 Activity

Puchowicz and colleagues have recently demonstrated that ketogenic diets markedly boost the protein expression of hypoxia-inducible factor-1 α (HIF-1 α) in rat brain cortex.^{1,2} This effect is likely evoked by ketone bodies, as intracerebroventricular infusion of beta-hydroxybutyrate has a similar impact on HIF-1 α . The authors speculate that this effect is mediated by a documented increase in neuronal succinate levels, which presumably stems from the fact that the first step in mitochondrial metabolism of acetoacetate, in which a CoA group is donated from succinyl-CoA, generates succinate as a by-product.³ Succinate acts as a competitive inhibitor of the prolyl hydroxylases which promote the proteasomal degradation of HIF-1 α ; hence, inhibition of these prolyl hydroxylases is expected to increase the level of HIF-1 α , enhancing its transcriptional activity.^{4,5} The authors show that i.c.v. infusion of propionate, which is metabolized to succinate, likewise boosts HIF-1 α protein expression in rat cortex.¹ Whether or not the authors are correct in identifying succinate as the mediator of the impact of ketone bodies on cortical HIF-1 α , their data clearly show that the latter is markedly enhanced in rats fed a ketogenic diet. This effect is associated with an increase in the cortical capillary density of aging

rats – likely because HIF-1 promotes transcription of the gene coding for VEGF, a prominent angiogenic factor – and an improvement in cognitive function.^{2, 6}

Another recent study demonstrates that mTORC1 activity is modestly decreased in the hippocampus of rats fed a ketogenic diet, as indicated by a reduction in the phosphorylation of ribosomal protein S6.⁷ This effect appears to reflect a small decrease in the activation of Akt; a trend toward increased AMPK activity does not achieve statistical significance, but if real could also contribute to reduced mTORC1 activity. It is not clear how ketones or ketogenic diets unfluence neuronal Akt activity.

Autophagy May Mediate Some Neuroprotective Benefits of Ketogenic Diets

It is well established that HIF-1 α mediates the activation of macroautophagy induced by hypoxia.^{8, 9} This effect comes about because HIF-1 induces transcription of BNIP3 and BNIP3L, BH3-only proteins which disrupt the inhibitory association of beclin 1 with Bcl-2 by binding to the latter. Once freed from its association with Bcl-2, beclin 1 can then fulfill its obligate role in autophagosome formation, the first step in macroautophagy.

Macroautophagy is suppressed by mTORC1 activity, via phosphorylation of ULK1.^{10, 11} This repression should be modestly relieved by the inhibition of mTORC1 activity induced by ketogenic diets. It is therefore reasonable to speculate that ketogenic diets, and ketone bodies, can activate macroautophagy in cortical neurons via the joint impact of an increase in HIF-1 α expression and a reduction in mTORC1 activity. This speculation is consistent with a recent report demonstrating a 3-4-fold increase autophagosomes in the brain neurons of mice fasted for 48 hours.^{12, 13}

There is considerable reason to believe that a moderate up-regulation of neuronal (macro)autophagy can aid in the prevention and control of a range of neurodegenerative conditions.¹³⁻²⁶ Autophagy lessens oxidative stress by disposing of damaged mitochondria,²⁷ and it also has the potential to remove pathogenic protein aggregates that are thought to play a mediating role in various neurodegenerative disorders. Hence, pharmaceutical strategies for boosting brain neuronal autophagy are being avidly researched by drug developers. Indeed, one such approach being pursued is development of small molecules which inhibit prolyl hydroxylase, which thereby boost HIF-1 α levels and activate autophagy. Such agents have shown benefit in rodent models of ischemia-reperfusion damage and Parkinson's, Alzheimer's, and Huntington's disease.²⁸⁻³³ A feasible alternative strategy for boosting cerebral autophagy is to administer brain-permeable activators of AMP-activated kinase (AMPK); this enzyme activates autophagy by inhibiting mTORC1 activation while conferring an activating phosphorylation on ULK1.^{11, 34, 35} The currently available agents metformin and berberine, both employed in the management of diabetes, appear to have potential in this regard,³⁶⁻⁴¹ resveratrol, which likewise activates AMPK in rodents, has been shown to increase cerebral autophagy in mice, thereby suppress extracellular accumulation of amyloid-beta, and also is beneficial in a

rotenone-induced Parkinsonian syndrome.^{17, 24, 42} (Unfortunately, rapid conjugation of resveratrol in humans renders it unsuitable for clinical use.^{43, 44})

These considerations suggest that ketogenic diets, particularly if used in conjunction with metformin or berberine, may have considerable practical potential for activating cerebral autophagy and thereby aiding the prevention or control of neurodegenerative disorders.

Moreover, it is unlikely that stimulation of autophagy is the only mechanism whereby ketogenic diets or ketone bodies provide worthwhile neuroprotection.^{45, 46} In particular, ketone bodies provide protection from excitotoxic oxidative stress generated by mitochondria; this effect can be demonstrated with isolated mitochondria, so it evidently is not dependent on the transcriptional activity of HIF-1 α .^{47, 48} Also, ketogenic diets promote cerebral induction of various mitochondrial uncoupling proteins, which would be expected to aid control of mitochondrial oxidative stress; whether HIF-1 plays any role in this is unclear.⁴⁹ The anti-epileptic impact of ketogenic diets has recently been traced to a suppression of adenosine kinase activity in astrocytes; this boosts extracellular adenosine levels, causing increased activation of neural adenosine A1 receptors which lessen neural excitability.⁵⁰ This mechanism also likely explains the pain relief afforded by ketogenic diets.⁵¹ Unraveling the multiple complementary mechanisms whereby ketosis provides neuroprotection will be a fascinating challenge for some years to come.

It should be noted that the down-regulatory impact of ketogenic diets on astrocyte adenosine kinase expression might exert a countervailing negative impact in certain neurodegenerative disorders, inasmuch as signaling via neuronal adenosine A2A receptors plays a pathogenic role in these disorders.⁵²⁻⁵⁷ Indeed, the neuroprotection associated epidemiologically with frequent use of caffeinated beverages has been attributed to inhibition of these receptors.⁵⁸⁻⁶¹ This suggests that a concurrent high caffeine intake might make ketogenic diets more uniformly protective for neurodegeneration – albeit caffeine would be expected to offset the benefits for ketogenic diets for control of epilepsy and pain.

Practical Implementation of Ketogenic Diets

Practical strategies for promoting ketogenesis, at least intermittently, may prove to have considerable value for preserving brain health. Administration of medium-chain triglycerides, which are immediately converted to ketone bodies in the liver (as they cannot be stored as triglyceride in adipocytes), is straightforward way to increase serum levels of ketone bodies, but gastrointestinal side effects limit the feasible daily intake of these to about 20-30 grams.⁶² Alternatively, a diet rich in coconut oil – over 60% of whose fatty acids are of medium chain length (C8-C12), and which is used for the manufacture of medium-chain triglycerides – might be a feasible way to support ketogenesis, albeit this would entail ingestion of myristic and palmitic acids as well (25-30% of the fatty acid content).

Ketogenic diets, long employed in the control of epilepsy and in the treatment of obesity, are difficult to employ on a continuous basis owing to the fact that they can be monotonous and are often difficult to tolerate once substantial weight has been lost. A potentially more practical approach would be to employ an intermittent ketogenic diet – for example, adopting a ketogenic diet for one week every month or two. Autophagy could be expected to induce improvements in the structure and function of neurons that would be sustained for some period of time even after ketosis was discontinued. For example, the increase in brain capillary density evoked by three weeks of a ketogenic diet in rats could be expected to persist for some time after the diet is discontinued. It should also be noted that temporary ketogenic dieting has been reported to sometimes achieve a lasting remission of epilepsy.^{63, 64} It would be worthwhile to conduct studies to determine whether intermittent ketogenic dieting could exert worthwhile effects in rodent models of neurodegenerative disorders, and, if so, to determine the durations of ketogenic dieting and regular dieting that would be compatible with benefit.

Qualms about the long-term consequences of low-carbohydrate diets for overall health have been raised by recent evidence that people who use low-carbohydrate diets composed primarily of animal products on a continuing basis experience increased mortality.⁶⁵ However, the same study found a *reduction* in mortality in those who use low-carb diets based primarily on plant products. Indeed, ketogenic diets composed of nuts, soy products, and low-carb vegetables are feasible.^{66, 67} Nonetheless, ketogenic diets may boost bone catabolism and entail an increased risk for nephrolithiasis; avoidance of metabolic acidosis by frequent ingestion of potassium citrate or bicarbonate solutions might be able to offset this risk.⁶⁸⁻⁷²

It seems likely that ketosis contributes importantly to the marked neuroprotective benefits associated with alternate-day fasting in rodents.^{73, 74} Strict alternate-day fasting has been studied in humans, but appears to entail too much distress and hunger to be practical for most people.^{75, 76} Modified alternate-day fasting regimens, which presumably entail a lesser degree of ketosis, have anti-inflammatory effects and are somewhat more practical, at least in obese patients.⁷⁷⁻⁷⁹

Supplemental intakes of carnitine and hydroxycitrate have the potential to boost hepatic ketone production; carnitine is a rate-limiting substrate for hepatic fatty acid oxidation, and hydroxycitrate relieves the malonyl-CoA-mediated inhibition of carnitine palmitoyltransferase-1 (CPT-1 - an enzyme rate-limiting for ketogenesis) by inhibiting citrate lyase and thereby suppressing malonyl-CoA synthesis.⁸⁰⁻⁸³ Agents which activate hepatic AMP-activated kinase also suppress levels of malonyl-CoA, via inhibition of acetyl-coA carboxylase and concurrent activation of malonyl-CoA decarboxylase.⁸⁴ However, strong activation of ketogenesis by these measures could only be expected when insulin is at fasting levels, as the insulin/glucagon balance regulates activity of CPT-1 and its sensitivity to malonyl-coA.⁸⁵⁻⁸⁷ It would be interesting to determine whether supplementation with these compounds could boost serum ketone levels when used in the context of strategies such as “mini-fast with exercise” or carbohydrate-concentrated diets, which entail long daily periods in which insulin levels are relatively low.⁸⁸⁻⁹⁰ Furthermore, carnitine/hydroxycitrate/berberine supplementation has the

potential to accelerate metabolic adaptation to fasting or a ketogenic diet, rendering the first couple of days of a ketogenic diet less stressful.⁸²

In summary, ketosis can be expected to stimulate autophagy in brain neurons by boosting HIF-1 α levels while suppressing mTORC1 activity. Concurrent administration of brain-permeable agents which activate AMPK could be expected to further enhance brain autophagy. Autophagy induction has considerable potential as a strategy for preventing and controlling neurodegeneration, and ketosis exerts additional effects that are neuroprotective. Serum ketone levels can be boosted, to a moderate degree, by daily ingestion of medium-chain triglycerides. Intermittent ketogenic dieting may prove to be a worthwhile neuroprotective strategy, and merits study in rodent models. Supplementation with carnitine/hydroxycitrate may have the potential to amplify the ketone production associated with strategies such as “mini-fast with exercise” or carbohydrate-concentrated dieting.

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