Published in final edited form as:

Epilepsy Res. 2012 July ; 100(3): 295–303. doi:10.1016/j.epilepsyres.2011.09.021.

Modulation of oxidative stress and mitochondrial function by the ketogenic diet

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Abstract

The ketogenic diet (KD) is a high-fat, low carbohydrate diet that is used as a therapy for intractable epilepsy. However, the mechanism(s) by which the KD achieves neuroprotection and/or seizure control are not yet known. The broad efficacy of the KD in diverse epilepsies coupled with its profound influence on metabolism suggests that mitochondrial functions may be critical in its mechanism(s) of seizure control. Mitochondria subserve important cellular functions that include the production of cellular ATP, control of apoptosis, maintenance of calcium homeostasis and the production and elimination of reactive oxygen species (ROS). This review will focus on recent literature reporting the regulation of mitochondrial functions and redox signaling by the KD. The review highlights a potential mechanism of the KD involving the production of low levels of redox signaling molecules such as H₂O₂ and electrophiles e.g. 4-hydroxynonenal (4-HNE), which in turn activate adaptive pathways such as the protective transcription factor, NF E2-related factor 2 (Nrf2). This can ultimately result in increased production of antioxidants (e.g. GSH) and detoxification enzymes which may be critical in mediating the protective effects of the KD.

Keywords

reactive oxygen species; seizure; hyperexcitability; epilepsy; mitochondria; redox; glutathione

The ketogenic diet (KD) is a high-fat/low-carbohydrate diet, most often in a 4:1 fat:nonfat ratio, used to treat intractable seizures in children and adolescents. Astoundingly, the KD appears to have broader efficacy than any currently available anti-epileptic drug, suggesting a more general mechanism of action that protects the brain. In fact, a meta-analysis of studies conducted from 1925–1998 revealed that out of 720 patients placed on the KD, 37% experienced greater than 90% reduction in their seizures, and an additional 30% achieved 50–90% seizure control (Thiele, 2003). More recently, a randomized controlled trial of the diet revealed that after 3 months, children in the diet group experienced a significantly lower mean percentage of baseline seizures compared to controls (62.0% vs 136.9%) (Neal et al., 2008). This type of broad efficacy is highly suggestive of a central mechanism capable of underlying diverse epilepsies such as bioenergetic control. Whereas theories regarding the diet's mechanism are primarily focused on the potential protective role of the ketone bodies

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that accumulate during ketosis, there has recently been a small body of work that suggests alterations in mitochondrial bioenergetics by the KD. This review will focus on the regulation of mitochondrial functions, particularly redox signaling by the KD.

Mitochondrial bioenergetics

Mitochondria are the organelles responsible for the majority of the ATP production in non-photosynthetic organisms. The number of mitochondria varies amongst organs and tissue types. Mitochondria produce energy by oxidizing carbohydrates and fats through the TCA cycle and β -oxidation, respectively. Electrons from NADH and FADH₂ produced by the TCA cycle are transferred to the electron transport chain (ETC) in the inner mitochondrial membrane. The ETC is comprised of complex I (NADH ubiquinone oxidoreductase), complex II (succinate dehydrogenase), complex III (ubiquinol cytochrome *c* oxidoreductase), and complex IV (cytochrome *c* oxidase). The reactions that occur at complexes I, III, and IV produce an electrochemical potential difference across the inner mitochondrial membrane, creating a driving force for protons that allows for ATP generation by the F₁F₀-ATP synthase (complex V). Although the primary function of the mitochondria is to produce ATP, they are also critically involved in the control of apoptosis, calcium homeostasis and the production and detoxification of reactive oxygen species (ROS). As mitochondria are intimately tied to such a wide array of cellular processes, it is not surprising that they have been implicated in a variety of diseases.

Reactive oxygen and nitrogen species

As electrons are transferred along the ETC, some can escape and reduce molecular oxygen to form ROS. A free radical is a species that contains one or more unpaired electrons, making it reactive and relatively unstable. The most well-known oxygen radical is superoxide, O₂^{•−}, the product of the one-electron reduction of ground-state O₂. O₂^{•−} cannot easily penetrate the inner mitochondrial membrane, so O₂^{•−} produced in the mitochondrial matrix usually acts locally. O₂^{•−} production increases dramatically when electron flow is inhibited at either complex I or complex III. The significance of detoxifying endogenous mitochondrial O₂^{•−} is made clear by the severe pathologies present in the mitochondrial superoxide dismutase (MnSOD)-null mouse (Li et al., 1995). Lacking MnSOD is either embryonic or neonatal lethal, depending on the genetic background (Huang et al., 2001). ROS collectively refers to both radical and non-radical oxygen species, such as hydrogen peroxide (H₂O₂). The conversion of mitochondrially generated O₂^{•−} into more reactive species, such as hydroxyl radical (•OH) or peroxynitrite (ONOO[−]), probably accounts for much of its toxicity. O₂^{•−} spontaneously dismutates to H₂O₂, a reaction catalyzed by SODs. H₂O₂ is more stable than O₂^{•−}, with a longer half-life and the ability to diffuse through membranes, allowing it to function as a signaling molecule (for review see (Day and Veal, 2010)). In the presence of free iron, H₂O₂ can react via the Fenton reaction to form hydroxyl radical, a very potent oxidant. Hydroxyl radical readily reacts with DNA, membrane lipids, and protein.

Reactive nitrogen species (RNS) also play a role in oxidative stress. Nitric oxide (NO) acts as a signal transduction molecule in vasodilation (Tor-Agbidye et al., 1998), (Ignarro et al., 1987)) and neuronal signaling (Arancio et al., 1996). Nitrosylation is proposed to be a redox-sensitive protein modification involved in signal transduction (reviewed in (Stamler et al., 2001; Raines et al., 2007)). NO also reacts with O₂^{•−} in a reaction that is diffusion limited, forming peroxynitrite (ONOO[−]), a powerful oxidant. Both O₂^{•−} and NO are produced in the course of the inflammatory response, leading to the formation of nitrotyrosine which can be used as a molecular footprint of nitrosative stress in inflammation (Kaur and Halliwell, 1994). Nitrotyrosine has been considered a hallmark of oxidative

stress, and is increased in many disease states, including Parkinson's disease (Matalon et al., 1984) and Alzheimer's disease (Hensley et al., 1998).

ROS are a consequence of normal metabolism, and under homeostatic conditions are kept in check by abundant and overlapping endogenous antioxidant defenses that include detoxifying enzymes such as SOD, catalase, and glutathione peroxidase (GPx). Oxidative stress is described as a pathological condition in which the balance of oxidant generation and detoxification is tipped towards a pro-oxidant state, antioxidant defenses are overwhelmed, reactive species accumulate, and damage to nucleic acids, proteins, and membrane lipids ensues. Three phenomena leading to oxidative stress in the brain, and implicated in neurodegenerative disease, include the inhibition of mitochondrial metabolism, neuronal excitotoxicity, and neuroinflammation.

Glutathione antioxidant system

There are many systems by which peroxides are detoxified within the cell, one of which involves glutathione (GSH). GSH (γ -Glu-Cys-Gly) is the most abundant non-protein thiol in the cell, often found in millimolar concentrations. GSH plays a major role in maintaining cellular redox status through the GPx and glutathione reductase (GR) enzymes. GPx detoxifies H_2O_2 formed in the cell, reducing it to H_2O . In order to do this, GPx uses GSH and forms glutathione disulfide (GSSG). GR then regenerates GSH at the expense of NADPH in order to prevent loss of GSH. This GSH redox cycle allows the use of the GSH/GSSG ratio as a readout of cellular redox status. When GSSG levels are high, the cellular environment is more oxidized; when GSH levels are high, the cellular environment is more reduced. The GSH/GSSG redox couple is an example of one major thiol/disulfide couple in the cell that helps to establish the overall redox state. The redox potential (E_h) of each redox couple can be calculated by the Nernst equation, and the result can be used as a measure of the tendency to accept or donate electrons. Data from various *in vivo* studies have found that E_h values for GSH/GSSG are in the range of -260 to -200 mV (Samiec et al., 1998; Jonas et al., 1999; Tian et al., 2007), with values varying slightly amongst organs, as well as between different subcellular compartments. Studies within the mitochondria have found the GSH/GSSG redox potential to be slightly more negative (approximately -280 mV) than the cytoplasm, indicating a more reduced environment (Cai and Jones, 1998; Rebrin and Sohal, 2004).

GSH is synthesized in two ATP-dependent, enzymatic steps. The first is the formation of γ -glutamylcysteine by the enzyme, glutamate cysteine ligase (GCL). This is the rate-limiting step in GSH biosynthesis. The addition of glycine occurs through the glutathione synthetase (GS) enzyme. It is widely accepted that most cells cannot take up GSH, so it has to be broken down and resynthesized in the cytoplasm of the cell. There are three main factors that regulate *de novo* GSH biosynthesis: amount of GCL present, availability of substrates (particularly cysteine), and feedback inhibition of GSH on GCL. GCL is a heterodimer comprised of a catalytic subunit (GCLC) and a modulatory subunit (GCLM). Increased GCL activity typically results from increased availability of subunits, which is usually the result of increased transcription of the subunit genes. GCLC is the heavy subunit (approximately 73 kDa), and as its name implies, possesses the catalytic activity of the enzyme. GCLM, the light subunit (approximately 28 kDa), increases the efficiency of the GCL holoenzyme by reducing the K_m for glutamate and elevating the K_i for GSH feedback inhibition (Richman and Meister, 1975; Huang et al., 1993). GSH levels have been an important indicator of mitochondrial and cellular health in studies of seizures and epilepsy, and mitochondrial dysfunction has been implicated as both a potential cause and consequence of seizures.

Mitochondrial dysfunction and epilepsy

Precedence for the role of mitochondrial dysfunction in epilepsy comes from the knowledge that epilepsy is a presenting feature of most inherited mitochondrial disorders such as myoclonic epilepsy with ragged red fibers (MERRF) and those associated with childhood encephalopathies. MERRF has been directly linked with a mutation in the tRNA^{Lys} of the mitochondrial genome (Shoffner et al., 1990), and these patients suffer from generalized seizures. Partial seizures frequently occur in mitochondrial encephalopathies, such as mitochondrial encephalopathy with lactic acidosis and strokelike episodes (MELAS) (Pavlakakis et al., 1984). These syndromes suggest that mitochondrial dysfunction may be a cause of some epilepsy syndromes. It was also found that activity of complex I of the ETC was decreased in the seizure focus of patients who had undergone surgical resection of epileptic tissue (Kunz et al., 2000). Whether the ETC defect preceded the seizures and may be the trigger for ROS generation is unknown. A role of mitochondrial oxidative stress in lowering seizure threshold comes from studies in mice partially deficient in a critical mitochondrial antioxidant (Sod2^{-/+}) that have age-dependent spontaneous seizures (Liang and Patel, 2004).

The occurrence of epilepsy in humans also increases with age, and mitochondrial oxidative stress is a leading mechanism of aging and age-related degenerative disease, suggesting a further involvement of mitochondrial dysfunction in seizure generation. Over the past decade there has been accumulating evidence for the role of oxidative stress in acquired epilepsy and most work has been conducted in animal models. One of the most commonly used models is that of injecting a single high dose of kainic acid (KA) into the rat, which initiates status epilepticus (SE), a prolonged period of convulsive seizures. It was shown that 16 hours after KA injection, the TCA cycle enzyme aconitase was significantly inactivated (Liang et al., 2000). Aconitase is a redox sensitive enzyme that can be used as a marker of intracellular O₂^{•-} production (Gardner and Fridovich, 1992). In addition to aconitase inactivation, evidence of oxidative DNA damage, as well as extensive neuronal death in the CA3 region of the hippocampus occurred after KA-induced SE. Neuronal survival following KA-induced SE was significantly improved in mice overexpressing MnSOD (SOD2), the mitochondrial SOD (Liang et al., 2000). This finding implies that O₂^{•-} production may be a causative event in the massive neuronal death following SE. Additional studies have now identified a number of alterations to mitochondrial redox status following KA-induced SE. GSH levels were depleted in the rat hippocampus following SE, along with a significant increase in GSSG, indicating a more oxidizing environment (Liang and Patel, 2006). Additionally, it has been reported that the production of isoprostanes and isofurans, products of lipid peroxidation, was increased following KA-induced SE, and that peak production of isofurans correlated with the emergence of mitochondrial oxidative stress (Patel et al., 2008). Collectively these data suggest that seizures cause mitochondrial dysfunction, and so recent reports that the KD improves mitochondrial function are important in evaluating potential mechanisms underlying protection afforded by the KD.

Mitochondria and the ketogenic diet

A brief report in 2004 revealed that mice fed for 10–12 days with a KD possessed increased levels of uncoupling proteins in the hippocampus, and therefore mitochondria from these mice produced less ROS (Sullivan et al., 2004). Rats maintained on a KD for at least 4 weeks were found to have significantly more mitochondria in their hippocampi compared to controls, suggesting that mitochondrial biogenesis is stimulated by consumption of a KD (Bough et al., 2006). How the diet might alter mitochondrial function is unknown, but it has been shown that *in vitro*, the ketone bodies β -hydroxybutyrate and acetoacetate decreased ROS production upon glutamate exposure (Maalouf et al., 2007). Additionally, the same

ketone bodies were found to prevent cell death in acute cortical slices from rat brain when exposed to H_2O_2 (Kim do et al., 2007). While the role of ketone bodies in the anticonvulsant effect of the KD remains undecided, the consensus is that they improve mitochondrial function and possess neuroprotective properties.

Improvement of mitochondrial glutathione by the KD

Several markers of redox status have since been found to show improvement in the hippocampus of rats fed a KD. When mitochondrial GSH/GSSG ratios were measured from hippocampi of rats fed a KD for 3 weeks, an improvement was observed (i.e. increased GSH/GSSG ratios) (Jarrett et al., 2008). Such a finding suggests increased GSH biosynthesis, and this is, in fact, what was found. The activity of GCL, the rate-limiting enzyme in GSH biosynthesis, was found to be increased in the hippocampi of rats fed a KD. A coordinate increase was found in the protein levels of both subunits of GCL, GCLC and GCLM. Additionally, measurement of a second redox couple (CoASH/CoASSG) was used to verify the GSH finding. Importantly, CoASH is primarily localized within mitochondria, so the observed increase in CoASH/CoASSG is presumed to be specifically mitochondrial. These data further suggest a specific improvement in mitochondrial redox status in rats fed a KD. Hippocampal mitochondria from rats fed a KD have also been found to produce less H_2O_2 than controls, suggesting a functional improvement as a result of consuming a KD.

The Nrf2 signaling pathway: a potential mechanism to elevate cellular glutathione

One possible mechanism by which GSH biosynthesis may be increased is through activation of the Nrf2 transcription factor pathway. Nrf2 plays a primary role in responding to cellular stress by initiating transcription of detoxification genes. Normally, Nrf2 is sequestered in the cytosol by an inhibitory binding partner Kelch-like ECH-associated protein 1 (Keap1). Nrf2 and Keap1 are associated via a direct protein-protein interaction between the C-terminal Kelch repeat domain of Keap1 and the N-terminal Neh2 regulatory domain of Nrf2 (Itoh et al., 1999). In the cytosol, Keap1 does not passively bind Nrf2 but acts as an adaptor for an E3 ubiquitin ligase, actively targeting Nrf2 to the proteasome for degradation (Kobayashi et al., 2004). Under conditions of cellular stress, the Nrf2-Keap1 interaction is disrupted, allowing Nrf2 to be stabilized so it can translocate into the nucleus. The mechanism by which Nrf2 is released from Keap1 has been studied in detail and two processes have been proposed: cysteine modification of Keap1 and phosphorylation of Nrf2. The first clue that cysteine residues of Keap1 might function as a redox sensor was the observation that the potency of an Nrf2 inducer was directly correlated with its reactivity with sulfhydryl groups (Dinkova-Kostova et al., 2001). Subsequently, it was found that Keap1 possesses 25 conserved cysteine residues and two of them (Cys273 and Cys288) were necessary for its basal repression and ubiquitination of Nrf2 (Zhang and Hannink, 2003). This same study identified a separate cysteine residue, Cys151, as required for the escape of Nrf2 from Keap1-mediated repression in response to an inducer. Absence of either Cys273 or Cys288 was found to be sufficient to disrupt Keap1 repression of Nrf2 (Wakabayashi et al., 2004). It is believed that when these cysteine residues are oxidized a conformational change occurs in the protein, releasing it from Nrf2. In this manner, the Nrf2-Keap1 complex can be viewed as a sensor of cellular redox status, in which the sensor for activation acts as a repressor under basal conditions.

There is competing literature demonstrating that a critical step in releasing Nrf2 from Keap1 is phosphorylation of Nrf2. A body of work has identified protein kinase C (PKC) as a potential player in Nrf2-driven gene transcription and that it is specifically due to phosphorylation of Ser40 in the Neh domain of Nrf2 (Huang et al., 2000, , 2002). Their

work *in vitro* found that PKC inducers stimulated Nrf2-driven transcription and that PKC inhibitors blocked Nrf2 nuclear translocation. A recent paper identified PKC- δ as the isoform responsible for phosphorylation of Ser40 on Nrf2 (Niture et al., 2009). An additional goal of this study was to determine whether oxidation of Cys151 on Keap1 or phosphorylation of Ser40 on Nrf2 was the essential step in dissociation of the Nrf2-Keap1 complex. Interestingly, it was found that treatment with an oxidant was necessary for PKC- δ -induced phosphorylation of Ser40 on Nrf2, as well as induction of Nrf2 target genes. Finally this study demonstrated that using a Keap1 Cys151Ala mutant blocked the release of Nrf2 from Keap1, even in the presence of oxidants. The authors proposed that both modifications are necessary but in a sequential fashion: Cys151 gets oxidized, inducing a conformational change that exposes Ser40 on Nrf2, allowing it to be phosphorylated and released from Keap1. Phosphorylation of Nrf2 is then proposed to stabilize it so that it can translocate into the nucleus.

Once in the nucleus, Nrf2 binds to a consensus sequence in the promoter region of target genes known as the antioxidant response element (ARE) or electrophile response element (EpRE). This core sequence in the 5'-region of a number of genes was identified as 5'-TGACNNNGC-3' and found to be essential for both basal and inducible expression (Rushmore et al., 1991). It has been shown that binding of Nrf2 to the ARE requires heterodimerization with other basic leucine zipper proteins, such as Jun and small Maf proteins. However, controversy remains regarding the exact role of the Nrf2-small Maf heterodimeric complex. Some literature reports that this complex acts as a transcriptional repressor under basal conditions, but that inducible expression of ARE-mediated genes is able to occur regardless of the presence of small Maf proteins (Alam et al., 1999; Nguyen et al., 2000). In contrast, it was recently shown that when Nrf2 is bound with MafG, the sequence on Nrf2 responsible for nuclear export is masked, thereby stabilizing Nrf2 in the nucleus (Li et al., 2008). This mechanism may be one way to maintain Nrf2 in the nucleus and allow further activation of ARE-mediated gene expression. While formation of Nrf2-small Maf protein complexes has been extensively studied (Itoh et al., 1997; Marini et al., 1997; Toki et al., 1997), the exact function of this complex remains disputable.

While Nrf2 is often activated in response to an injury, inducing its expression may prove beneficial. Some of the best evidence comes from work utilizing two potent Nrf2 inducers. Dietary supplementation with *tert*-butylhydroquinone (tBHQ) was shown to attenuate the effects of 3-nitropropionic acid, a mitochondrial complex II inhibitor, in a mouse model of Huntington's disease (Shih et al., 2005b). Critically, this effect was absent in *Nrf2*^{-/-} mice, suggesting that Nrf2 is required for protection by tBHQ. The same group performed intracerebroventricular injections of tBHQ in a rat model of ischemia-reperfusion and found that pretreatment with the compound resulted in a reduction of infarct volume (Shih et al., 2005a). A second Nrf2 inducer that has become increasingly popular is sulforaphane, which is naturally occurring in cruciferous vegetables. Sulforaphane can be administered intraperitoneally and has been shown to increase Nrf2 target genes (mRNA and activity) within 18 hours of injection and to preserve integrity of the blood-brain barrier in a model of traumatic brain injury (Zhao et al., 2007a). It has also been shown to decrease infarct volume following cerebral ischemia (Zhao et al., 2006), improve cognitive function following traumatic brain injury (Dash et al., 2009), and reduce oxidative damage after intracerebral hemorrhage (Zhao et al., 2007b) in animal models. These data suggest that inducing Nrf2 activation may be a worthwhile strategy for development of new therapeutics. Additionally, the Nrf2 pathway is activated following administration of KA in mice (Kraft et al., 2006). This is not surprising as the Nrf2 pathway is a primary responder to cellular stress. In this study, *Nrf2*^{-/-} mice were found to be more sensitive to KA-induced seizures, i.e. the knock-out animals experienced significantly lengthened and more severe seizures. These data suggest that sensitivity to KA may be dependent upon Nrf2 expression and

activation, making this a potentially important pathway to study with respect to seizure susceptibility, and thus protection by the KD.

Activation of the Nrf2 pathway by the KD: adaptive control

Accumulation of Nrf2 protein has been observed in nuclear fractions from hippocampus of rats fed a KD for up to 3 weeks, suggesting chronic Nrf2 activation and nuclear translocation (Milder et al., 2010). Activity of the Nrf2 target enzyme, NQO1 (NAD(P)H quinone oxidoreductase 1), was also observed to be increased in the KD group. Additionally, Nrf2 activation was suggested by a finding in a previous report that protein levels of GCLC and GCLM, both of which are Nrf2 targets, were increased by consumption of a KD (Jarrett et al., 2008). These data may relate to increased GSH as Nrf2 has been found to be a primary inducer of GSH biosynthesis. Both subunits of GCL possess ARE-like sequences in their promoters (Mulcahy et al., 1997; Moinova and Mulcahy, 1998; Erickson et al., 2002), making them targets for Nrf2-induced transcription. It has not yet been studied whether induction of Nrf2 is necessary for increased GSH biosynthesis in the KD; however, given the documented protective effects of both Nrf2 and GSH, the precise mechanism is worth further investigation.

How Nrf2 might be activated by consumption of a KD also remains to be determined, but the presence of potential acute and chronic stress in KD-fed rats has been suggested. Since the Nrf2-Keap1 complex can serve as a redox sensor, redox signaling by H₂O₂ and lipid peroxidation products may serve as activators of the pathway. Although hippocampal mitochondria have been found to produce less ROS in animals fed a KD for at least 1 week (Sullivan et al., 2004; Jarrett et al., 2008), substrate-driven H₂O₂ production was observed to be increased in hippocampal mitochondria from KD-fed rats compared to controls after only 1 day on the diet (Milder et al., 2010). Acute production of H₂O₂ may play an important role in the KD, as it has been shown to serve a redox signaling role, in addition to its more notable role as a damaging species (Figure 1). H₂O₂ has been shown to activate a number of important transcription factors and thereby initiate signaling cascades. One study reported that an array of mechanisms known to activate NF-kappa B were all dependent on production of H₂O₂, as several thiol antioxidants were found to block NF-kappa B activation (Schreck et al., 1991). Additionally, H₂O₂ has been shown to increase DNA binding of Nrf2 to the ARE (Wilson et al., 2005).

Lipid peroxidation byproducts have also been found to potentially activate Nrf2. 4-hydroxy-2-nonenal (4-HNE) is an α,β -unsaturated aldehyde formed by the reaction of ROS or RNS with arachidonic and linoleic acid and can readily react with proteins and DNA, leading to harmful consequences. It can be especially impactful due to its relative stability and ability to pass through membranes. Thus, it can move through different subcellular compartments to react with targets. 4-HNE is normally present in its free form at very low levels (less than 1 μ M in human plasma) (Selley et al., 1989; Michel et al., 1997; Gil et al., 2006), and studies have shown that it can increase 10 times or more during periods of oxidative stress (Yoshino et al., 1986; Esterbauer et al., 1987). 4-HNE and its products have been found to be increased in Alzheimer's disease (AD) (Sayre et al., 1997; Ando et al., 1998), Parkinson's disease (PD) (Yoritaka et al., 1996), and amyotrophic lateral sclerosis (ALS) (Shibata et al., 2001). Additionally, seizure-induced increases in 4-HNE have been reported (Jacobsson et al., 1999). In this context, 4-HNE was produced as a result of injury and used as a marker of damage. However, it has recently been reported that at subtoxic concentrations, 4-HNE can act as a signaling molecule to produce an adaptive response (Uchida et al., 1999; Ishii et al., 2004; Levonen et al., 2004; Chen et al., 2005).

4-HNE has been shown to be relevant in the transcription of genes involved in increasing cellular GSH levels (Liu et al., 1998; Dickinson et al., 2002), and therefore may play a role in stimulating GSH biosynthesis during consumption of a KD. Stimulation of GSH biosynthesis by 4-HNE is most likely through activation of the Nrf2 pathway, as 4-HNE has been shown to play a role in the adaptive response to toxins through inducing dissociation of Nrf2 from Keap1 (Chen et al., 2005). It is its purported signaling role that is of interest in the KD, as it was recently reported that 4-HNE levels were increased in the hippocampus of rats fed a KD for 3 days (Milder et al., 2010). These data suggest production of an acute low level of 4-HNE following initiation of a KD, which may serve to activate pathways important for the chronic protection the KD affords (Figure 1).

The most intriguing question to arise from the current literature is how increased mitochondrial levels of GSH might contribute to a change in seizure susceptibility. The importance of determining the role of the GSH system in seizure-related pathology and epilepsies is underscored by the finding that GSH depletion occurs in multiple animal models of temporal lobe epilepsy (Liang and Patel, 2006; Steven et al., 2006), as well as in human epilepsy patients (Mueller et al., 2001). A fundamental unanswered question is the significance of GSH depletion in human and experimental epilepsies and its role in the progression of the disease. Finding GSH biosynthesis increased after 3 weeks on a KD suggests that it is somehow chronically maintained (Jarrett et al., 2008), and thus makes it a target worth studying with respect to potential anticonvulsant effects. Several studies have addressed this question pharmacologically and found evidence suggestive of a possible anticonvulsant role for GSH (Abe et al., 1999, , 2000).

Conclusion

This body of work suggests a potential mechanism by which consumption of a KD results in the production of low levels of redox signaling molecules such as H₂O₂ and 4-HNE, which in turn activate adaptive pathways such as the protective transcription factor, Nrf2. The ultimate result is increased production of antioxidants (e.g. GSH) and detoxification enzymes. While the mechanism has yet to be studied in a detailed cause-effect manner, the data available suggest that it is worth investigating in an attempt to further elucidate the mechanism(s) underlying protection by the KD.

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Figure 1.

Proposed temporal schematic of Nrf2 activation by the ketogenic diet. Early mild increase in ROS production and electrophilic stress (i.e. 4-HNE) by the ketogenic diet is hypothesized to activate Nrf2 which is normally sequestered in the cytosol by Keap1. Oxidative and/or electrophilic modification of key cysteine residues of Keap1 and subsequent dissociation of Nrf2/Keap1 complex can result in the nuclear translocation of Nrf2 and activation of a number of gene products controlling the antioxidant response. Chronic activation of such an adaptive antioxidant response can lead to a decrease in ROS production.