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A unifying mechanism of ketogenic diet action: The multiple roles of nicotinamide adenine dinucleotide

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Abstract

The ability of a ketogenic diet to treat seizures and render a neuronal network more resistant to strong electrical activity has been observed for a century in clinics and for decades in research laboratories. Alongside ongoing efforts to understand how this therapy works to stop seizures, metabolic health is increasingly appreciated as critical buffer to resisting and recovering from acute and chronic disease. Accordingly, links between metabolism and health, and the broader emerging impact of the ketogenic diet in improving diverse metabolic, immunological and neurological conditions, have served to intensify the search for its key and/or common mechanisms. Here we review diverse evidence for increased levels of NAD^+, and thus an altered ratio of NAD^+/NADH, during metabolic therapy with a ketogenic diet. We propose this as a potential unifying mechanism, and highlight some of the evidence linking altered NAD^+/NADH with reduced seizures and with a range of short and long-term changes associated with the beneficial effects of a ketogenic diet. An increase in NAD^+/NADH is consistent with multiple lines of evidence and hypotheses, and therefore we suggest that increased NAD^+ may be a common mechanism underlying beneficial effects of ketogenic diet therapy.

Keywords

Ketogenic diet; Seizures; Epigenetic changes; Adenosine; Mitochondria; Metabolism

1. Introduction

Effective energy production and consumption is essential, and as one example carbohydrates are converted to glucose via digestive enzymes, absorbed in the intestine, and released in the blood stream to provide the main reactant for energy production (Hewitt, 1924). When dietary carbohydrates are insufficient, an alternative mechanism to maintain ATP generation is increased production and metabolism of the ketone bodies acetoacetate (AcAc) and β-

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hydroxybutyrate (β-OHB) (Laffel, 1999). It has long been known that decreased carbohydrates accompanied by increased fat will shift the dependence of energy production from glucose to ketone bodies (Wigglesworth, 1924) and promote the metabolic changes observed during what is now termed a ketogenic diet (KD).

Since the early 1900s, the KD has been used to decrease seizures (Pulford, 1927), although its use declined for many decades due to the development of anticonvulsant drugs. Recently, the diet regained popularity due to wider public awareness and additional clinical data affirming its ability to treat refractory epilepsy (Kossoff et al., 2009; Lefevre and Aronson, 2000; Neal et al., 2008). While it is most popular in pediatric epilepsy, more recent evidence indicates significant anti-seizure effects in adult patients regardless of age or seizure type (Cervenka et al., 2017; Sirven et al., 1999), and establishes beneficial effects in a range of neurological conditions (Camberos-Luna and Massieu, 2020).

To date, a fundamental mechanism that explains its diverse beneficial effects remains elusive. It may be that there is none: the KD mobilizes multiple mechanisms, and a different cohort may benefit different disorders (Masino and Rho, 2019). Alternatively, there may be a fundamental mechanism that underlies these multiple mechanisms, and could be a powerful pan-disease therapeutic target. Identifying this mechanism and its accessibility as a therapeutic target has far-reaching implications.

Based on differential utilization of nicotinamide adenine dinucleotide (NAD) during glucose-based versus ketone-based metabolism (Lodish et al., 2000), altered cellular levels of NAD^+ during ketone-based ATP generation would be expected. NAD is an essential metabolic coenzyme, a signaling molecule, a marker for mitochondrial and cellular health, and a substrate for deacetylating (sirtuins) and ribosyltransferase (PARP) enzymes implicated in longevity and DNA damage repair (Belenky et al., 2007; Lin and Guarente, 2003; Rustin et al., 1996). In this review, we highlight the role of the oxidized form of NAD (NAD^+) as a potential fundamental starting point for multiple mechanisms proposed to underly KD action in supporting metabolic health as well as treating epileptic seizures and potentially other disorders.

2. Glucose versus ketone body-based metabolism and NAD redox state

Consumption of a diet with sufficient carbohydrates will increase blood glucose (Hewitt, 1924), which readily crosses the blood-brain-barrier and enters cells through facilitated diffusion or active transport and serves as the main energy source in the central nervous system (Lodish et al., 2000; Maher et al., 1994). Generating cellular ATP from glucose starts with glycolysis, where a single 6-carbon glucose molecule is converted to two 3-carbon pyruvate molecules (Lodish et al., 2000). For each pyruvate produced, an ATP molecule is generated and a single molecule of NAD^+ is used as a cofactor and released in its reduced form, NADH. NADH is then oxidized in the electron transport chain to generate three ATP molecules (Fig. 1, upper left). Pyruvate is converted to acetyl-CoA via oxidative decarboxylation, which reduces another NAD^+ molecule to NADH. Each acetyl-CoA molecule enters the citric acid cycle to generate twelve additional ATPs (Fig. 1, lower left).

In summary, when glucose is oxidized, the following changes in NADH and ATP occur: 1. during glycolysis, 8 ATP are produced (from 2 NADH + 2 ATP); 2. during oxidative decarboxylation, 6 ATP are produced (from 2 NADH) and 3. during the citric acid cycle, with two molecules of Acetyl CoA, 24 ATP are made from 12 NADH + 4 FADH2 + 4 GTP. In total 38 ATP molecules are produced.

Note that in the electron transport chain, NADH and FADH will produce approximately three and two ATP, respectively, while GTP will produce one ATP (Ahmad and Kahwaji, 2018; Lodish et al., 2000).

In contrast, fasting or consuming a KD causes the body to undergo lipolysis (fat breakdown) in order to generate fatty acids (FAs) as energy source in muscles and other tissues (Paoli, 2014). FAs are not able to cross the blood-brain-barrier: they are taken up by the liver to synthesize the ketone bodies AcAc and β-OHB which are then released into the bloodstream. Ketone bodies do cross the blood-brain barrier and enter the cells to undergo ketolysis to generate energy for the brain. Each ketone body molecule produces two molecules of acetyl-CoA (Fukao et al., 2004) (Fig. 1, right) which subsequently enter the citric acid cycle to produce ATP (Izuta et al., 2018; Krebs, 1970). When β-HB is converted to AcAc, it reduces one NAD^+ molecule to NADH (Fig. 1, right), while AcAc molecules are converted to two acetyl-CoA molecules without the need to reduce NAD^+ molecules. Therefore, the yield of ATP for a molecule of β-OHB is 27 (24 from Acetyl CoA and 3 from NADH produced during the conversion step to AcAc), and the net yield of ATP for AcAc is 24 (from Acetyl CoA) (Fig. 1, right).

At first glance, glucose is more efficient in ATP production. To produce 1000 ATP molecules only 28 glucose molecules are needed as compared to 37 β-OHB molecules and 42 AcAc molecules. But there are significant differences in NAD^+ reduction: glucose reduces 112 molecules of NAD^+, whereas β-OHB reduces 37 and AcAc reduces none. Accordingly, to produce comparable amounts of ATP, glucose-based metabolism reduces at least thrice the NAD^+ molecules compared to ketone based-metabolism. Considering that a single cortical neuron can consume 4.7 billion ATPs per second at rest (Zhu et al., 2012), it is reasonable to assume that the above-mentioned differences in NAD^+ utilization will have a significant effect on NAD redox state and the ratio of NAD^+/NADH. It is worth noting that studies have already shown that ketones are a more efficient fuel compared to glucose. A study looking at the hydraulic work and oxygen consumption of hearts metabolizing glucose versus ketone bodies uncovered that ketones increased fuel efficiency by 25 % (Sato et al., 1995). Neurons grown in the presence of the ketone body β-OHB showed increased oxygen consumption and ATP production (Marosi et al., 2016). Multiple other studies also showed that metabolizing ketone bodies in lieu of glucose led to significant increases in ATP production (Kim et al., 2010; Murray et al., 2016; Nylen et al., 2009), further confirming the higher efficiency of ketones as energy substrates.

3. Key biological roles of NAD

Differential NAD^+ utilization is significant because NAD plays a central role in two cellular processes:

1. Redox reactions, where it acts as an oxidizing agent – accepting electrons and converting to its reduced form, NADH. These redox reactions are important in cellular respiration, generation of ATP, and neutralization of microorganisms through the formation of free radicals (Imai and Johnson, 2018; Rustin et al., 1996).

2. Enzymatic reactions where it is required as a substrate. These NAD- dependent enzymes affect multiple cellular functions, ranging from gene expression and posttranslational modification of proteins to deacetylation and ADP-ribosylation reactions in the cells (Belenky et al., 2007). Two major enzymes that use NAD^+ in the central nervous system are sirtuins and PARPs (Cantó et al., 2013).

In addition to its intracellular roles, NAD^+ has been recognized as an extracellular signaling molecule released by multiple mammalian cell types, including astrocytes (Haag et al., 2007; Verderio et al., 2001). Multiple studies have also recognized NAD^+ as a neurotransmitter released by neurons in both the central and peripheral nervous system (Breen et al., 2006; Durnin et al., 2012; Mutafova-Yambolieva et al., 2007; Smyth et al., 2004). Extracellular functions of NAD^+ include – but are not limited to – modulating calcium signaling, purinergic signaling, immune cells function, and neurotransmitter release and postsynaptic signaling (Aarhus et al., 1995; Durnin et al., 2012; Haag et al., 2007; Kuzmin et al., 2016; Verderio et al., 2001).

Regarding a direct relationship between NAD^+ and seizures, recent research shows that NAD^+ can suppress the early stages of epileptogenesis in pilocarpine-treated rats, and also prevent hippocampal apoptosis (Liu et al., 2017). This is an important finding in a progressive seizure model that replicates the clinical relationship between a precipitating event and subsequent seizure development in patients. But if altered consumption of NAD^+ by ketone-based metabolism is a starting point for the therapeutic benefits of a KD, there must be further credible links to key downstream processes that align with well-established clinical observations.

4. Ketogenic therapy in the management of epilepsy

Despite strong and consistent reports of clinical success for nearly a century, the first randomized controlled study utilizing KD in pediatric epilepsy was reported in 2008 (Neal et al., 2008). It demonstrated that refractory patients treated with KD improved significantly compared to those treated with a standard of care (who did not improve). Evidence of success continues to accumulate as more centers globally prescribe the diet and support families in its implementation as well as strive to collect long-term data (Bertoli et al., 2014; Gerges et al., 2019; Groleau et al., 2014; Heussinger et al., 2018; Tian et al., 2019). Correlations have been found between β-OHB levels in the blood and the CSF and the degree of seizure control in epileptic children treated with a ketogenic diet (Gilbert et al., 2000;Ruskin et al., 2017).

In the laboratory a variety of *in vivo* and *in vitro* models have been pursued, sometimes with mixed results. Overall it has been determined that the success of the diet in reducing neuronal excitability may depend on species, seizure model, and glucose concentration *in*

vitro (Kawamura et al., 2016, 2014). In general, chronic seizure models in rats and mice that offer more clinically-relevant models show clear benefits of KD. Overall, the ketogenic diet does not have a marked effect on baseline activity but has a large effect in reducing high intensity (i.e. ATP-consuming) electrical activity (Blaise et al., 2015; Koranda et al., 2011; Ma et al., 2007; Viggiano et al., 2016).

5. Ketogenic therapy modulates NAD+ levels

As noted, the metabolic pathways of glucose versus ketone bodies exert differential effects on NAD^+ reduction, and research has shown that a ketogenic treatment can modulate neuronal NAD redox state. *In vitro*, dissociated neurons from C57BL/6 mice incubated with β-OHB for 24 h show increased NAD^+/NADH ratio and ATP production (Marosi et al., 2016). *In vivo,* KD increases hippocampal NAD^+/NADH ratio in healthy wild-type rats, and this increase appears to be rapid (within two days) and persistent (lasting at least three weeks) (Elamin et al., 2018, 2017). Similarly, nutritional ketosis achieved via ingestion of medium chain triglyceride oils increases NAD^+/NADH ratio in healthy human subjects (measured via ^{31}P magnetic resonance spectroscopy) (Xin et al., 2018).

The relationship between KD and NAD^+ is maintained under pathological conditions. After three months of subcutaneous injections of ketone bodies, fresh cortical and hippocampal tissues extracted from mice with an Alzheimer-linked gene variant showed a significant increase in NAD^+/NADH ratio and ATP production (Yin et al., 2019). Moreover, treating hippocampal pyramidal neurons with ketone bodies resulted in an increase in NAD^+/NADH ratio and oxygen consumption rate (Hasan-Olive et al., 2019), and application of the ketone bodies β-OHB and AcAc protected rat neocortical neurons from glutamate toxicity by increasing NAD^+/NADH ratio (Maalouf et al., 2007), consistent with the ability of ketone bodies to modulate NAD redox state.

6. Common mechanisms mobilized by ketogenic therapy and altered NAD+

6.1. Enhanced mitochondrial function

NAD is an essential factor for mitochondrial ATP generation (Stein and Imai, 2012), and studies indicate NAD^+ is a rate-limiting factor for several mitochondrial processes (Alano et al., 2004; Bai et al., 2011). Indeed, increasing NAD^+ levels was found to enhance mitochondrial function and protect against oxidative stress damage (Kussmaul and Hirst, 2006; Pittelli et al., 2011). Cell lines and primary cultures exhibited an increase in mitochondrial ATP production and oxygen consumption after exogenous application of NAD (Kussmaul and Hirst, 2006; Lin and Guarente, 2003; Pittelli et al., 2011). Furthermore, circadian oscillations in mitochondrial NAD^+ synthesis were shown to be associated with enhanced mitochondrial respiration and ATP production (Peek et al., 2013).

Regarding the role of NAD^+ in ketogenic therapy, *in vitro* application of ketone bodies was shown to enhance mitochondrial respiration and protect against excitotoxicity via increasing NAD^+/NADH ratio in isolated cortical mitochondria (Maalouf et al., 2007). Neurons showed similar results and the application of β-OHB increased the NAD^+/NADH ratio while

enhancing mitochondrial oxygen consumption and ATP production (Marosi et al., 2016). As noted, ketone-based metabolism is associated with enhanced mitochondrial functions (Bough et al., 2006; Sook Noh et al., 2004), which can play a significant role in controlling epileptic seizures (Rahman, 2012; Waldbaum and Patel, 2010).

Considering the ability of NAD^+ to significantly enhance mitochondrial function in a manner similar to ketone-based metabolism (Kussmaul and Hirst, 2006; Maalouf et al., 2007; Marosi et al., 2016; Peek et al., 2013), it is reasonable to surmise that some of the beneficial effects of ketogenic therapy are mediated by NAD^+. The rapidity through which NAD^+ changes appear might partially explain the rapid efficacy of the KD observed in many patients after only a few days of KD treatment (Freeman and Vining, 1999). A rapid decrease in NAD^+ availability and the subsequent effects on neuronal excitability could also be expected upon discontinuation of treatment, and indeed, 15 % of refractory epileptic patients experienced a rapid recurrence of seizures after discontinuation of the KD (Martinez et al., 2007). However, the inhibition of seizures persisted in other patients even after diet discontinuation (Martinez et al., 2007). This differential response among patients to treatment cessation indicates the existence of multiple downstream mechanisms and epigenetic changes implicated in seizure control or prevention (Lusardi et al., 2015; Masino and Rho, 2012). Upregulation of key ketogenic enzymes, mainly mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase, after longer periods of ketogenic treatment (Cullingford et al., 2002) might play a role in this maintenance of the beneficial effects after discontinuation of the diet.

6.2. Ketogenic diet, NAD^+ and activation of sirtuin enzymes

Sirtuins are a group of deacetylase enzymes (SIRT1 - SIRT7) that use NAD^+ as a main substrate (Michishita, 2005). Increasing the deacetylating activity of sirtuin enzymes can be achieved either by increasing gene expression levels, which subsequently increases the quantity of the enzymes, or by increasing cellular levels of NAD^+ (Dali-Youcef et al., 2007; Landry et al., 2000; Revollo et al., 2004).

The most prominent and abundant member of this group is the nuclear SIRT1 whose main function is the deacetylation of several important targets that regulate apoptosis, inflammation, several growth factors, and transcription factors (Yang et al., 2006). SIRT2 is a cytosolic enzyme involved in the regulation of gluconeogenesis (Jiang et al., 2011) and central nervous system myelination (Beirowski et al., 2011), while SIRT4 is found to regulate fatty acid oxidation and mitochondrial gene expression (Nasrin et al., 2010). SIRT6 and SIRT7 are nuclear enzymes that are involved in decreasing age-associated DNA damage (McCord et al., 2009; Mostoslavsky et al., 2006; Vazquez et al., 2016). Hepatic SIRT6 is necessary for the ketogenic response to fasting and KD (Chen et al., 2019).

SIRT3 is the most abundant mitochondrial sirtuin (Michishita, 2005) that plays a pivotal role in the regulation of mitochondrial respiration and fatty acid oxidation. In fact, mice lacking SIRT3 were phenotypically normal at base line, however, they exhibited severe metabolic irregularities during fasting, highlighting the role of SIRT3 in maintaining energy homeostasis (Hirschey et al., 2010). β-OHB application to cultured hippocampal neurons elevated SIRT3 expression that had been lowered by oxidative stress, part of a pathway

enhancing mitochondrial bioenergetics (Hasan-Olive et al., 2019). β-OHB treatment *in vivo* also elevates SIRT3 levels lowered by human apolipoprotein E4 expression in hippocampus and cortex of mice (Yin et al., 2019).

Taken together, sirtuins impact a wide range of functions. Increasing NAD^+ levels will have a direct effect on sirtuins activity, and may mediate the beneficial effects associated with all seven sirtuin subtypes (Dali-Youcef et al., 2007; Landry et al., 2000; Revollo et al., 2004). SIRT1 was shown to mediate the seizure-suppressing effects of the micro RNA 199a-5p (Wang et al., 2016), highlighting the role of SIRT1 as a potential target for treating epilepsy. Administration of resveratrol, a well-known SIRT1 activator (Alcaín and Villalba, 2009), resulted in neuroprotection in a multitude of seizure models and showed synergistic effects with common anti-epileptic drugs (Pallàs et al., 2014). It has been hypothesized that one aspect of the neuroprotective effects of the KD is enhanced neuronal macroautophagy partially mediated by increased SIRT1 activity (McCarty et al., 2015).

In a temporal lobe epilepsy model, a decrease in NAD^+ and a decrease in SIRT3 protein expression were uncovered in the acute and latent phases of epileptogenesis and thought to contribute to disease pathogenesis (Gano et al., 2018). Hippocampal pyramidal neurons in KD-fed mice exhibited upregulation of SIRT3, and treatment of the dissociated cells with ketone bodies increased NAD^+/NADH ratio and oxygen consumption rates and improved mitochondrial biogenesis (Hasan-Olive et al., 2019).

It is important to note that sirtuin activity is not restricted to seizure inhibition but might also modulate neuronal death: in a model of acute acquired epilepsy, PARP-1 mediated neuronal death exposed the compromised enzymatic activity of SIRT1. NAD repletion was able to enhance SIRT1 activity and resulted in decreased neuronal death (Wang et al., 2013). Taken together these data affirm the positive role of sirtuin enzymes in the management of epilepsy, and support the idea that NAD^+-driven activation of sirtuins is an important mechanism that may mediate the anti-seizure effects of ketogenic therapy.

6.3. Poly-ADP-ribosepolymerases (PARPs), DNA damage, and reactive oxygen species

The second NAD-dependent enzyme group is poly-ADP- ribosepolymerases (PARPs), with PARP-1 being the most abundantly expressed enzyme of the PARP family (Sodhi et al., 2010). This enzyme adds polymers of ADP-ribose into proteins, a process known as ADP-ribosylation and first recognized for its important role in cell survival and DNA damage repair (de Murcia and de Murcia, 1994; Grube and Burkle, 1992). A steady-state level of DNA damage exists as a result of DNA oxidation by reactive oxygen species (ROS), a by-product of normal cellular metabolism, and was reported to occur about 10,000 times per cell per day in humans and 50,000 times per cell per day in rats (Bernstein et al., 2013). PARP-1 enzyme was previously described as a molecular sensor for this type of oxidative, naturally occurring DNA damage (de Murcia and de Murcia, 1994), and it is considered an important marker for damage as levels of oxidative DNA damage were found to have an effect on both its activity (Dantzer et al., 2006) and protein levels (Shen et al., 2016). More recently, PARP-1 was found to play a role in gene transcription and programmed cell death (Kim et al., 2005).

PARP ribosylation reactions are major consumers of NAD^+ in the brain. Despite the fact that PARP enzymes play a vital role in the DNA repair process, over-activation of PARP enzymes (and the subsequent depletion of NAD^+) have been previously linked to several pathological conditions including MPTP-induced Parkinsonism, Alzheimer's disease, ischemic brain injury, and metalloproteinase-mediated neuronal death (Endres et al., 1997; Kauppinen and Swanson, 2005; Love et al., 1999; Mandir et al., 1999).

With respect to epilepsy, PARP1 activation was shown to mediate neuronal death after status epilepticus, a type of severe continuous seizures that last more than 30 min (Kim et al., 2014). Inhibiting PARP enzymes was previously shown to increase the cellular availability of NAD^+ (Hurtado-Bagès et al., 2020; Mendelsohn and Larrick, 2017). Animals treated with a KD for either two days or three weeks showed a significant decrease in hippocampal DNA damage and PARP-1 protein levels (Elamin et al., 2018). This inhibition of PARP-1 expression would be expected to augment the increase in NAD^+ that results from utilizing ketone bodies as an energy source (Fig. 1) and further increase NAD^+ bioavailability. Indeed, KD-induced PARP inhibition was associated with increased NAD^+ (Elamin et al., 2018).

In a positive feedback manner, KD-induced increases in NAD^+should also mobilize other NAD^+-dependent mechanisms such as sirtuin enzymes to modify ROS generation and limit oxidative stress damage, which in turn should decrease PARP protein levels, making more NAD^+ available. Sirtuins such as SIRT2 can decrease oxidative stress by deacetylating FOXO3a, a well-known pro-apoptotic protein (Wang et al., 2007) and SIRT3 was found to mediate the decrease in reactive oxygen species associated with caloric restriction (Qiu et al., 2010).

Ketogenic therapy decreased the formation of reactive oxygen radicals and reversed gene expression patterns of several genes that control ROS and oxidative stress in animal and cellular models (Maalouf et al., 2007; Stafford et al., 2010; Sullivan et al., 2004). Modification of cellular oxidative stress and ROS generation is important, since increased PARP1 activation and mitochondrial oxidative stress levels were shown to play a crucial role in epilepsy-associated neuronal death and contribute to epileptogenesis (Waldbaum and Patel, 2010; Wang et al., 2013). Moreover, inhibition of PARP protected epileptic neurons in the hippocampus from cell death and preserved NAD^+ levels and mitochondrial respiration after status epilepticus (Lai et al., 2017; Yang et al., 2013). Hence, the collective paradigm of increasing NAD^+, inhibiting PARP-1, and activating sirtuins might be a coherent strategy to protect neurons from epilepsy-induced cell death and a central part of the KD mechanism of action.

6.4. Ketogenic diet, NAD^+ and adenosine

KD has been shown to increase activation of seizure-reducing adenosine A_1 receptors and increase adenosine levels (Lusardi et al., 2015; Masino et al., 2011; Masino and Rho, 2012).

While there is no direct experimental evidence that we are aware of linking NAD^+, adenosine and KD, NAD^+ can be degraded into adenosine (Okuda et al., 2010; Zhang et al., 2018) – possibly contributing to the increased adenosine levels, activation of adenosine A_1

receptors, and the seizure suppressing effects (Kawamura et al., 2010; Masino et al., 2009, 2012).

Research has shown that S-adenosylhomocysteine hydrolase, an enzyme involved in adenosine biosynthesis, contains an NAD-binding domain that modulates its activity (Kloor et al., 2003). The NAD^+ precursor nicotinamide, in combination with adenosine, was shown to have strong protective effects against audiogenic seizures (Maitre et al., 1974), another potential link between NAD and adenosine that supports their role in working together to suppress seizures.

6.5. Ketogenic diet, NAD^+ and modulation of ion channels

A plethora of evidence suggests that the KD acts directly or indirectly on ion channels. In particular, multiple lines of evidence suggest that the KD can increase activation of ATP-sensitive K^+ channels (K_{ATP} channels) to decrease neuronal excitability (Giménez-Cassina et al., 2012; Kawamura et al., 2016, 2010; Tanner et al., 2011). In parallel, NAD is a known modulator of ionic transport, and several channels contain an NAD-binding domain (Kilfoil et al., 2013). Interestingly, NAD molecules can modulate K_{ATP} channels (Dukes et al., 1994) and directly interact with and bind to K_{ATP} channel subtypes (Dabrowski et al., 2003). Because of their ability to couple energy homeostasis to neuronal firing, K_{ATP} channels have been described as metabolic sensors (Olson and Terzic, 2010; Sun and Feng, 2013), and genetic defects of these channels can cause epilepsy among other neurological phenotypes (Olson and Terzic, 2010). Hence, it is not unreasonable to assume that NAD modulation of K_{ATP} is one of the mechanisms through which ketogenic therapy exerts its anti-epileptic effects.

Regarding other potassium channel subtypes, *in vitro* application of NAD^+ increased open time and open probability of the voltage-gated potassium channels Kvα1.5 and Kvβ1.3, which play a role in setting the resting membrane potential and regulating neuronal firing (Tipparaju et al., 2005; Yellen, 2002). The effects on Kvα1.5 and Kvβ1.3 currents appear to be specific to NAD^+, as application of NADH or NADPH did not achieve similar results (Tipparaju et al., 2005). Furthermore, physiological concentrations of NAD^+, but not NADH, successfully modulated sodium-gated potassium channels (K_{Na}), Slack channels, and SLO potassium channels through direct binding (Kilfoil et al., 2013).

Finally, NAD modulation of ion channels is not exclusive to potassium. Other channels that were shown to be directly modulated by NAD include the calcium-permeable TRPM2 cation channel, the ryanodine receptor calcium release channel, and $Na_v1.5$ sodium channels (Hara et al., 2002; Kilfoil et al., 2013; Liu et al., 2009; Zima et al., 2004). All of this supports the idea that the observed modulation of ion channel function with ketones or KD could be mediated via intracellular NAD.

7. Summary and perspective

Whereas glucose or ketone bodies can each produce molecules of ATP, ketone-based metabolism is associated with diverse cellular benefits and requires the conversion of far fewer (õne-third as many) molecules of NAD^+. Published research links ketone-based

metabolism, altered levels of NAD^+, and multiple postulated anti-seizure mechanisms mobilized by ketogenic therapies (Fig. 2). We suggest that a fundamental biochemical difference in NAD^+ may be a common starting point for multiple downstream mechanisms. As such, it may explain the diverse beneficial effects of the KD in treating epilepsy and its emerging role in treating numerous additional disorders.

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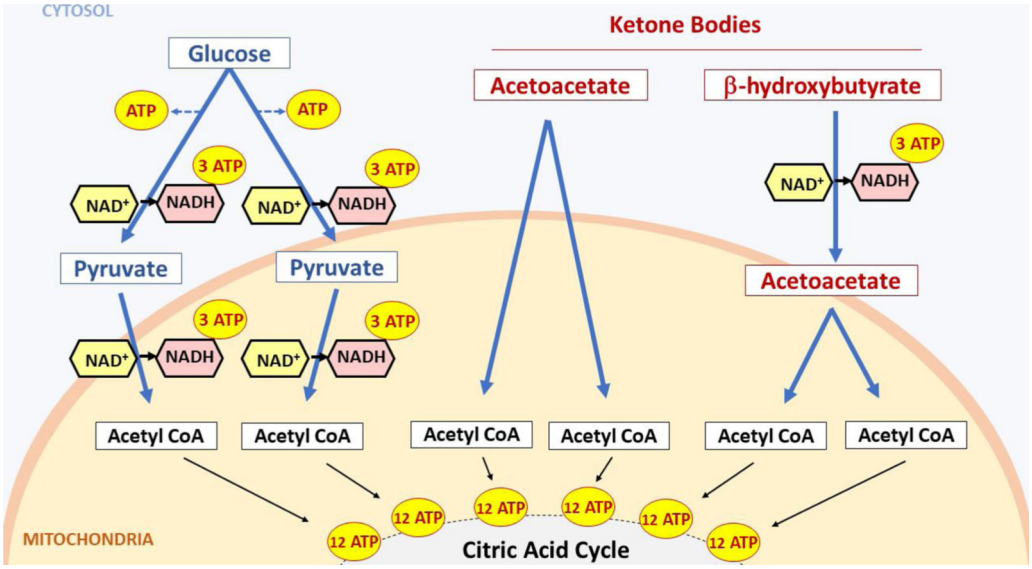

Fig. 1.

Alternate metabolic pathways whereby glucose (left) versus ketone bodies (right) are converted to ATP. Note decreased NAD^+ consumption via ketone bodies versus glucose. See text for details.

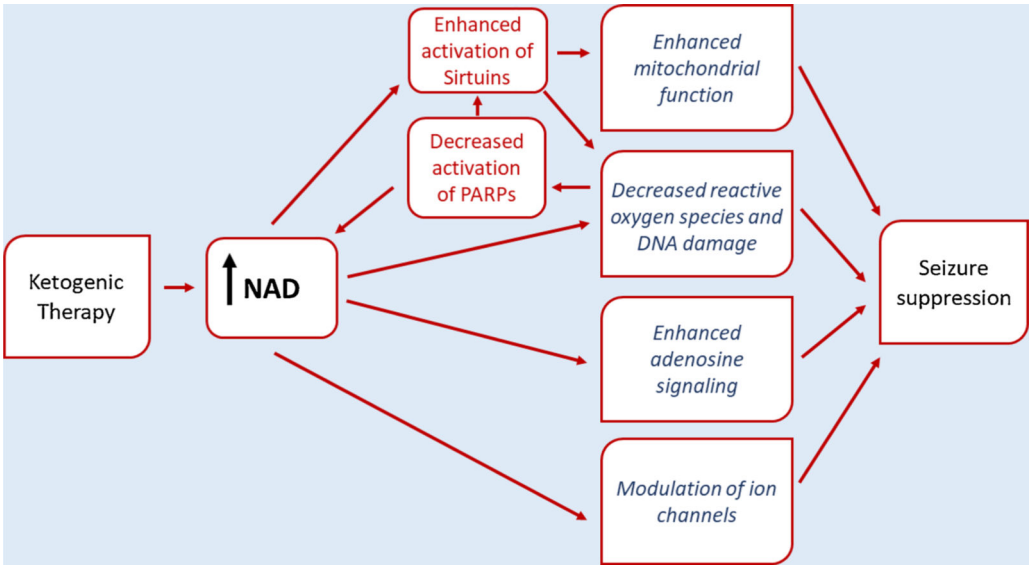

Fig. 2.

Summary of some of the proposed NAD-based links between ketogenic therapies and suppression of seizures. See text for details.