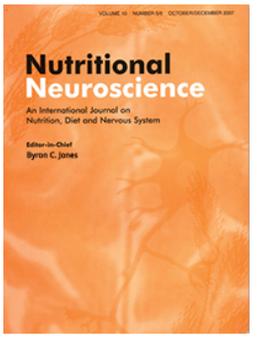

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Ketogenic diet: overview, types, and possible anti-seizure mechanisms

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ABSTRACT

The ketogenic diet (KD) has been used for a long time as a therapeutic approach for drug-resistant epilepsy. It is a high-fat, low-carbohydrate, and adequate protein diet. There are various types of KD with some differences in their compositions that mainly include classic KD, medium-chain triglyceride diet, modified Atkins diet, and low glycemic index treatment. The anti-seizure mechanisms of KDs have not yet completely understood but, some possible mechanisms can be theorized. The aim of the present study was to review the various types of KD and explain the probable biochemical mechanisms involved in its anti-seizure property.

KEYWORDS

Classic ketogenic diet; ketone bodies; low glycemic index treatment; mechanism of action; medium-chain triglyceride diet; modified Atkins diet; neuronal death; polyunsaturated fatty acids

1. Overview

Epilepsy is a common neurological disease which affects about 1% of the world's population [1]. It can be recognized by seizures caused by excessive electrical activity in the brain. Frequent seizures in this disease may lead to progressive neural damage [2]. There are a variety of anticonvulsant medications which are usually used as the primary therapeutic approach in epilepsy patients [3]. However, it has been revealed that about 20–40% of the patients suffer from drug-resistant epilepsy (DRE) that their seizures are unyielding to treatments with two or more of these drugs [2,4]. This form of the disease is challenging to manage and can cause serious clinical problems such as disability, mortality, comorbidities, as well as socioeconomic and psychological costs [2]. Currently, surgical methods and diet therapies are the most prevalent and useful approaches to manage DRE [5]. Epilepsy surgery is a procedure which aims to eliminate or reduce the seizure by excising the brain epileptic zone or limit the spread of seizure activity without causing any loss of normal brain function. Although epilepsy surgery can be highly effective, it is not always appropriate, and due to the complicated etiology and indistinct pathogenesis of DRE, surgery alone is hard to achieve a certain effect and therefore needs to be replaced with other treatments such as diet therapy [5,6].

The ketogenic diet (KD) has been used for a long time as an established non-pharmacological therapeutic approach for DRE especially in surgery unachievable children [7,8]. It is a high-fat, low-carbohydrate, and adequate protein regimen while retaining a normal amount of calories. This therapeutic diet has been used in DRE patients to simulate the metabolic profile of fasting because starvation has long been stated to diminish the frequency of seizures [9]. The anticonvulsant mechanisms of KD have not yet completely revealed. The present study is aimed to review the various types of KD and explain the probable biochemical mechanisms involved in its anti-seizure property.

2. Types of ketogenic diet

Various types of KDs that implicated in epilepsy treatment include: classic KD, medium-chain triglyceride diet (MCTD), modified Atkins diet (MAD), and low glycemic index treatment (LGIT).

2.1. Classic ketogenic diet

Classic KD was first described by Wilder in 1921 [10], as the most commonly used therapeutic diet in DRE treatment. Long-chain triglycerides (LCT) obtained from standard foods are the main portion of the fat which commonly is in a 4:1 ratio of fat to carbohydrate plus protein in this diet. This ratio could be reduced to 3.5:1 or 3:1 for children needing higher protein intake for their growth. About 80–90% of the calories are derived from fat [11,12]. Initial hospitalization of the target patients can be useful for the beginning and adaptation to the diet. The calculation of the diet is better to be achieved by an expert dietitian to educate the family and child in how to maintain the diet [13].

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Although it has been shown that the effect of KD in the controlling epileptic seizures is as good as, or better than that of any of the newer anticonvulsant drugs [14], this therapeutic regimen may lead to some complications including hypocalcemia, kidney stones, hyperuricemia, dyslipidemia, metabolic acidosis, as well as gastrointestinal conditions such as diarrhea, constipation, and vomiting [15]. In a review study by Lefevre et al. [16], he results showed that 56% and 32% of DRE children under classic KD gained >50% and >90% reductions in the seizures, respectively. Moreover, 16% of the patients became seizure-free. In a systematic review by Keene et al. [17] the determined rate for gaining total seizure control in DRE patients was 15.6%, and 33% of them gained >50% reduction in the seizures by classic KD.

2.2. Medium-chain triglyceride diet

The MCTD, introduced in the 1950s, is another type of KD which is also used as an effective treatment against DRE in children. This diet is thought to be more palatable. MCTD mainly contains octanoic (C8) and decanoic (C10) fatty acids which yield more ketones per kilocalorie of energy compared to the LCT-based diet. It can be absorbed and delivered more efficiently to the liver through portal blood. The high ketogenic potential of MCTD leads to less total fat intake and inclusion of more carbohydrate and protein. This makes MCTD more favorable and palatable for children compared to the classic KD. The effectiveness of the MCTD is great and comparable to that obtained by the classic KD, which has been verified in several studies [18–20]. The efficacies of MCTD and classic KD were compared in a study by Neal et al. [20]. There was no significant difference in mean percentages of baseline seizures between the MCTD and classic KD groups after 3, 6, and 12 months (3 months: classic KD 66.5%, MCTD 68.9%; 6 months: classic KD 48.5%, MCT 67.6%; 12 months: classic KD 40.8%, MCT 53.2%). However, because of abdominal discomfort and bloating complications, the MCTD has not been widely accepted [12,21,22].

2.3. Modified Atkins diet

The MAD was firstly introduced by a case series published in 2003 explaining the advantages of a less restrictive dietary treatment initiated as a patient without hospitalization, initial fasting, and any restrictions on calories, protein, or fluids [23]. This type of KD is a more palatable and less restrictive diet mainly for patients with behavioral problems or for children whose parents or the physicians are unwilling to administer the classic KD. About 65% of the calories in MAD are taken from fat [24,25]. In contrast to the classic KD, the MAD does not need hospitalization for the initiating the diet. This dietary treatment is usually used in adolescents and adults due to its easy self-administration especially for persons who may be carrying the burden of living. In general, MAD is better tolerated and accepted by DRE patients and their families [24,26]. The MAD may have similar or slightly less efficiency compared to classic KD [27]. The efficacies of MAD and classic KD were compared in a study by Kim et al. [28]. In their study, the patients who used classic KD had a lower mean percentage of baseline seizures compared with the MAD group after 3 months (KD, 38.6%; MAD, 47.9%; $p > 0.05$) and 6 months (KD, 33.8%; MAD, 44.6%; $p > 0.05$). Interestingly, for infants younger than 2 years of age, the outcome was much more favorable in the KD group compared with the MAD group. Tolerability was better in the MAD group with fewer side effects. Therefore, MAD can be considered as the first choice for the treatment of DRE in children, but the classic KD is a more suitable approach in infants younger than 2 years of age [28,29].

2.4. Low glycemic index treatment

The LGIT, introduced in 2005, as another effective alternative dietary approach for DRE management [30]. In this dietary treatment, the extreme carbohydrate restriction of the other KDs is liberalized. The high carbohydrate-containing foods such as rice, bread potatoes, watermelon, and bagels are restricted to the low glycemic index foods which produce relatively small changes in blood glucose. A measure of a food's tendency to cause a glucose elevation in serum is considered as the glycemic index [13,31]. The glycemic index of a specific food can be evaluated by calculating the incremental area the blood glucose response curve after administering the specified amount of that food in comparison to a same amount of the reference glucose [13]. The glycemic index of reference glucose is considered as 100 therefore, a particular food with a 50 glycemic index produces 50% of the area under the curve [31]. The diets with glycemic index less than 50 (such as meat, dairy, and some fruits and whole grain breads as well) are allowed in LGIT. This dietary treatment has nearly similar efficacy compared to the classic KD, however it is more palatable and easy to implementation [13]. The efficacy of LGIT is comparable with classic KD. Muzykewicz et al. evaluated the efficacy of LGIT in 76 DRE patients. A greater than 50% reduction in seizure frequency was recognized in 42%, 50%, 54%, 64%, and 66% of the patients after 1, 3, 6, 9, and 12 months, respectively.

Table 1. The composition of the main types of ketogenic diets [11].

Ketogenic Diets	Fat (g)	Protein (g)	Carbohydrate (g)	Fat calories (% of total)
Classic	100	17	8	90
MCTD	78	25	50	70
MAD	70	60	10	70
LGIT	60	40	40	45

LGIT, low glycemic index treatment; MAD, modified Atkins Diet; MCTD, medium-chain triglyceride diet.

The compositions of the four main types of KD are depicted in Table 1. Despite some differences, all the diets have nearly similar efficacy, and the majority of the patients have been shown to achieve at least 50% reduction in their seizure severity after the KD therapy [12,32,33].

3. Adverse effects

Although KD has been considered as one of the most effective approaches in the treatment of DRE, this diet, particularly the classic type, is not as safe as the normal diet and may have different side effects. Gastrointestinal disturbances are suggested as the most frequent adverse effects of KD that include abdominal pain, fatty diarrhea, constipation, hunger, vomiting, and gastroesophageal reflux. KD may also lead to dyslipidemia (hyperlipidemia, hypercholesterolemia, and hypertriglyceridemia) and thereby potentially enhancing the risk of cardiovascular diseases. By limiting the consumption of calorie and protein, KD can also cause growth failure in children. Moreover, other side effects such as kidney stones, hyperuricemia, lethargy, and infectious diseases have also been reported in children who initiate the diet. Severe adverse effects such as respiratory failure, thrombocytopenic purpura, and pancreatitis have been rarely reported. Although it is not sure whether these side effects are actually due to the implemented KD or perhaps an underlying disorder, it is required to be performed under careful medical supervision. Regular follow-up is necessary to address the long-term impact of KD on the overall health of children even after stopping the diet [34,35].

4. Suitability

It has been shown that KD may not be appropriate for a few groups of patients, and therefore the suitability of the diet should be carefully assessed before the administration. There are some definite contraindications that include: fatty acid oxidation defects, carnitine deficiencies, organic acidurias, pyruvate carboxylase deficiency, familial hyperlipidemia, hypoglycemia, ketogenesis/ketolysis defects, severe gastroesophageal reflux, severe liver diseases, and disorders needing a high carbohydrate diet such as acute intermittent porphyria. Additionally, diabetes mellitus, certain mitochondrial diseases, and concomitant steroid use can also be considered as possible contraindications. Therefore, before administering a KD, a careful evaluation of the patients should be conducted by an expert neurologist [36].

5. Relation with age

According to the previous studies [37,38], KD is considerably effective and well tolerated in all age groups including infants, children, adolescences, and adults. In infants younger than two years of age, the seizure control and neurodevelopmental outcome, both have a poor prognosis [39]. Nordli et al. [40] in their study evaluated the effectiveness of the KD in infantile epilepsy. The results showed that 35.5% of the patients had >50% reduction in seizure frequency, and 19.4% of them became seizure-free. The efficacy of KD in children was initially evaluated by a randomized controlled trial in 145 DRE patients [41]. In the KD group, 28 children (38%) were revealed to have greater than 50% seizure reduction compared to four children (6%) in the no diet control group. KD and its variants can also be therapeutically applied for adolescences and adults, not only those who are transferring from pediatric services, but also those who are first starting the diet [42]. Payne et al. [43] reviewed the evidence regarding the effectiveness of KD in adolescents (12–18 years old) and adults (more than18 years of age) with DRE. The data showed that 49% of adolescents and adults gained >50% seizure reduction, and 13% of them became seizure-free. Moreover, in a study by Schoeler et al. [42] the results showed that adults (16–65 years) with DRE could follow KD for long-term and the seizure reduction rate was similar to those commonly reported in pediatric cohorts. In order to evaluate the relationship between age and efficacy of KD, Coppola et al. [37] performed a prospective study to evaluate the efficacy of KD in children, adolescents and young adults (ages between 1 and 23 years) with DRE. Although any significant correlation between the efficacy and age at diet onset was not observed, the younger patients tend to have a better response to the diet.

It seems there is no age limit as to when the KD may be administered [44]. The most important factors that should be considered in designing a diet, rather than efficacy, are the patient condition and family environment. The classic type of KD appears to be more suitable and efficient in infants younger than 2 years of age.

Although this type is highly efficient, it is restrictive and time-consuming, and therefore according to the family environment, it can be replaced with other modified types of KD [44]. All types of KD are equally effective in older children, but in adolescences and adults, MAD and LGIT are less restrictive and more suitable [42,45]. In infants and children who are on enteral feeds, KD can also be applied in liquid form which is more beneficial and efficient in this condition [44,45]. Further studies are needed to determine more precisely the relationship between age and efficacy of KD.

6. Possible anti-seizure mechanisms

Although the anti-seizure mechanisms of KD have not yet completely revealed, it has been shown that enhanced ketone bodies and polyunsaturated fatty acids (PUFAs) as well, may play main roles in the anti-seizure effect of KD [7]. The possible mechanisms are depicted in Figure 1 and explained below.

6.1. Ketone bodies

During KD treatment, the metabolic efficiency of the tricarboxylic acid (TCA) cycle is reduced and body energy is generally derived from fatty acid oxidation in mitochondria that results in the generation of a large amount of acetyl-CoA. Acetyl-CoA accumulation leads to the synthesis of the two primary ketone bodies, β-hydroxybutyrate and acetoacetate, mainly in the liver that can then spill into the blood circulation. Acetone, the other major ketone, is a metabolite of acetoacetate. The ketone bodies can be used as an alternative source of energy instead of glucose in the brain. Fatty acids are not utilized due to their inability to pass through the blood–brain barrier (BBB) [12]. There are some specific monocarboxylate transporters in BBB and some mitochondrial enzymes in the brain which make the ketone bodies possible to be extracted and used by the brain [46]. It has been shown that KD by upregulating of these specific proteins can induce the using of ketone bodies by the brain [46]. After the entering to the brain, ketone bodies can be converted to acetyl-CoA and then enter the TCA cycle within brain mitochondria leading eventually to the production of adenosine triphosphate (ATP) [12]. Several hypotheses have been focused on the ketone bodies as the key mediators involved in the anticonvulsant effect of the KD. Based on the several studies [46–54], the potential mechanisms are generally center around the roles of brain energy metabolism, neurotransmitters, ion channels, and oxidative stress which are briefly discussed below.

6.1.1. Brain energy metabolism

Energy production in the brain has been proven to be enhanced remarkably by KD. Chronic ketogenic diet therapy upregulates coordinately several energy metabolism genes, enhances mitochondrial biogenesis and density, and a rise in energy reserves such as phosphocreatine. Energy production enhancement by KD increases the ability of neurons to manage metabolic challenges in the brain. This improves neuronal function and survival under stressful conditions. Therefore, brain tissue on a KD seems to become more resistant to metabolic stresses and the seizure threshold is enhanced. Taken together, an energy preservation hypothesis can be considered for the anticonvulsant effects of the KD [7,55].

6.1.2. Potassium channels

6.1.2.1. ATP-sensitive potassium channels. Potassium channels sensitive to ATP (KATP channels) are ligand-gated receptors which have key roles in insulin secretion regulation. They are also widely expressed in the central nervous system (CNS) [46,54]. ATP/Adenosine diphosphate (ADP) ratio production plays a privileged role in the regulation of these channels as well as the fueling of the ATP-driven sodium (Na^+) pumps at the plasma membrane of central neurons [46].

Under KD condition, the reduction of brain glucose utilization and glycolytic ATP production can induce KATP channels opening leading to neuron membrane hyperpolarization. This reduces the electrical excitability in the brain and increases the seizure threshold[46,56].

Therefore, KATP channels activity can be considered as a mechanism of rapid coupling between metabolism and electrical excitability that helps to prevent excessive neuronal firing and seizure threshold regulation in the brain[46,54,56,57].

6.1.2.2. Two-pore domain potassium channels. Two-pore domain potassium (K2P) channels, also known as potassium leak channels, are a major and distinct subclass of the potassium channel superfamily. They have been discovered in a wide variety of mammalian cells including neurons, myocytes, glia, and many different types of epithelial cells. These channels are structurally different from most other classes of potassium channels which form a functional tetramer. It has been shown that each subunit of K2P channels has two pore-forming regions and four trans-membrane segments, and thus, they form functional dimers. Functionally, these channels are spontaneously active leading to continuous efflux of potassium ions through the cell membrane which is necessary for setting a hyperpolarized resting potential of the cell membrane. Therefore, K2P channels

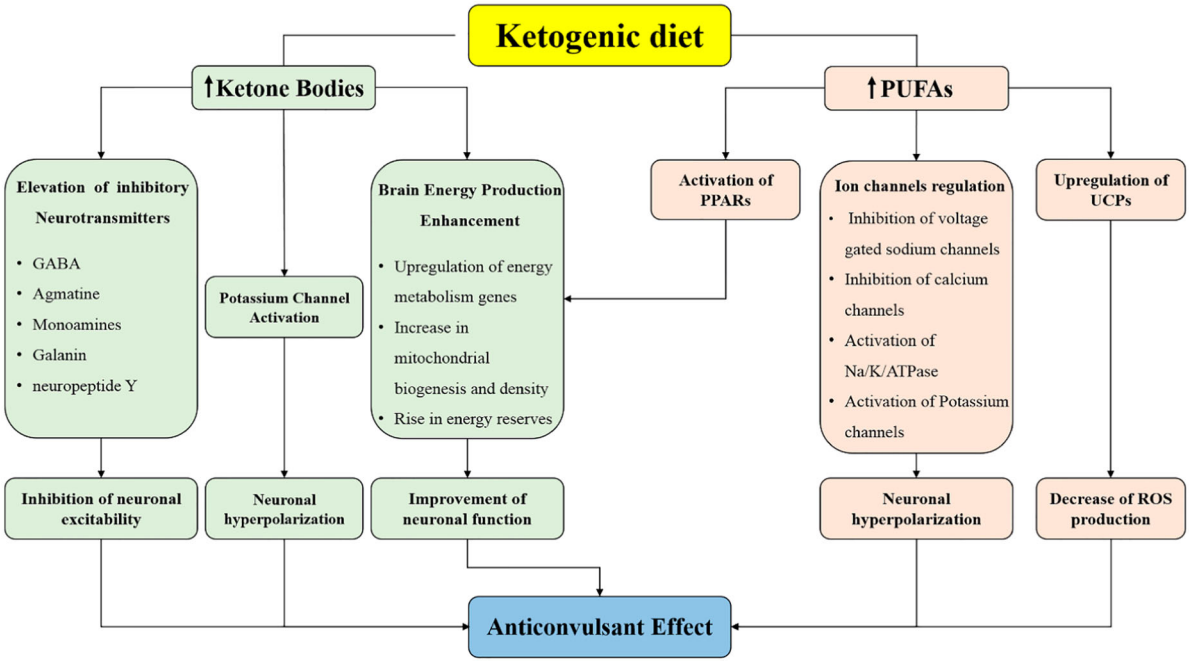

Figure 1. Possible anticonvulsant mechanisms of the ketogenic diet (KD). Ketogenic diet enhances the serum levels of ketone bodies and polyunsaturated fatty acids (PUFAs). Enhanced ketone bodies though elevation in inhibitory neurotransmitters, activation of potassium channels, and increase in energy production of the nervous system could eventually enhance the brain seizure threshold. On the other hand, increased PUFAs levels lead to activation of peroxisome proliferator-activated receptors (PPARs), inhibition of voltage-gated sodium and calcium channels, activation of potassium channels, activation of sodium/potassium/adenosine triphosphatase (Na+/K/ATPase), and upregulation and activation of uncoupling proteins (UCPs). Elevated PPARs activity causes coordinate up-regulation of energy transcripts, enhances energy reserves, and stabilizes synaptic function that eventually prevents neuronal hyperexcitability. The PUFAs-altering ion channels activity could hyperpolarize the neurons. Furthermore, upregulation of UCPs through uncoupling of the electron transfer chain could decrease reactive oxygen species (ROS) production and oxidative stress. These changes could eventually limit the seizures.

may directly influence the duration and frequency of action potential firings [56,58–60]. These channels can be also modulated by a variety of physical, chemical and natural factors including voltage, temperature, mechanical pressure, protons (pH), and volatile anesthetics. It has been suggested that K2P channels may also be activated by ketone bodies and certain fatty acids as well [59,61]. Thus, KD-induced raises in blood ketone bodies and fatty acids as well may regulate neuron membrane excitability by activating K2P channels, and this can be assumed as another probable anticonvulsant mechanism of KD.

6.1.3. Neurotransmitters

6.1.3.1. Gamma-aminobutyric acid. Gamma (γ)-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the brain. Reducing the neuronal excitability is the most prominent role of GABA and therefore, it has a key role in the initiation and spread of seizure activity in the brain [48,49,62].

It has been observed that KD can lead to glutamic acid decarboxylase upregulation which induces GABA synthesis [63]. It has also been revealed that this diet could alter GABA transaminase activity that inhibit GABA degradation [51]. These enzymatic alterations lead eventually to enhance extracellular GABA. Moreover, according to Bough et al. study [7], the elevation in energy metabolism by KD could compensate the metabolic insufficiencies and temporary failure of GABAergic inhibition that would prevent the beginning and spread of the seizures. Therefore, another important mechanism of KD-induced anticonvulsant effect is possibly mediated via the GABAergic system [49].

6.1.3.2. Glutamate. Glutamate is one of the chief excitatory neurotransmitters in the CNS. High amounts of glutamate can make the brain susceptible to seizures and therefore may be associated with epilepsy [49]. The influence of the KD on brain glutamate metabolism and its levels has not yet exactly revealed. In a study, it was shown that KD could enhance glutamate levels in the brain synaptosomes [64] while, some other studies have not found any effect [49,50], or a reduction in brain glutamate content was detected after KD administration [65]. Further studies are needed in this regard.

6.1.3.3. Agmatine. Agmatine is a metabolite derived from L-arginine through arginine decarboxylation. This small molecule is found throughout the brain, mainly in the hippocampus. Agmatine has also been partially found in synapses and can be considered as an inhibitory neurotransmitter. It may exert anticonvulsant effects probably by inhibiting different brain excitatory receptors including N-methyl-D-aspartate (NMDA), adrenaline, and histamine receptors. It has been shown in a study by Calderón et al. that KD could increase in the hippocampus agmatine levels of the studied rats [49]. This study could support the notion that KD-induced enhancement in the brain levels of agmatine which has neuroprotection effects can be considered as another anti-seizure mechanism of this therapeutic diet.

6.1.3.4. Monoamines. Monoamine neurotransmitters including norepinephrine, serotonin, and dopamine have been considered to have important roles in the control of neuronal excitability and seizure [52,53,66,67]. Norepinephrine has been shown to have potent anticonvulsant properties in several studies [52,53,67]. Many neuronal networks have also been shown to express serotonin as well as dopamine receptors that are involved in epilepsy. These neurotransmitters have different subtypes of receptors. Some subtypes have proconvulsant, and some others have anticonvulsant properties; therefore, the effects can be different depending on which subtype is activated [66,67]. It has been shown that KD can enhance norepinephrine in the extracellular fluid in the hippocampus of studied rats compared to controls [53]. It was shown in a study by Szot et al. [52] that the animals with no functional noradrenergic system could not achieve anti-seizure ability under KD compared to controls. Dahlin et al. in their study [66] also reported that cerebrospinal fluid (CSF) levels of serotonin and dopamine could be influenced by the KD in a cohort in children with DRE.

6.1.3.5. Other neurotransmitters. Noradrenergic neurons in the locus ceruleus contain galanin and neuropeptide Y [68]. These orexigenic neuropeptides are potent endogenous anticonvulsant neuromodulators which can inhibit excessive neuronal excitability[47,53,69]. It has been shown that glucose, insulin, and leptin have suppressive effects on galanin and neuropeptide Y; thus, their expression can be regulated by nutritional status. Galanin expression can also be upregulated by high fat intake. The KD initially mimics the starvation effects. The levels of insulin, glucose, and leptin in the circulation are reduced under this dietary therapy. Therefore, KD may upregulate the expression of neuropeptide Y and galanin by changing the levels of the molecules which maintain energy homeostasis that lead eventually to its anti-seizure effect [53].

Several neurotransmitter systems may be affected by KD [49–51,53,63–66]. The mechanisms behind these changes have not yet been completely understood. Further investigations are required to prove the contribution of different neurotransmitters to the anticonvulsant effect of the KD.

6.2. Polyunsaturated fatty acids

In addition to the ketone bodies, it has been suggested that fatty acids, particularly polyunsaturated fatty acids (PUFAs) are also involved in the anticonvulsant mechanisms of KD [70,71] which are detected to be raised in both serum and brain of animals and patients under KD treatment [72,73]. PUFAs are dietary fatty acids which have more than one double bond in the hydrocarbon chain [74]. There are two groups of PUFAs: n-3 (omega-3) and *n*-6 (omega-6). This classification relates to the position of the double bond comparative to the methyl terminal of the molecule [71,74]. These two classes of PUFAs have varied characteristics. Omega-3s such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have anti-inflammatory properties but, linoleic acid (LA) and arachidonic acid (AA) as omega-6 PUFAs are considered as precursors of pro-inflammatory compounds [3]. High levels of omega-6s and an increased omega-6/omega-3 ratio promote the pathogenesis of cardiovascular diseases, while a high amount of n-3 PUFAs (a low omega-6/omega-3 ratio) exert suppressive effects [74–76].

In the brain, both types of PUFAs have similar roles in mediating anticonvulsant effect of KD. KD is considered to engage peroxisome proliferator-activated receptors (PPARs), a group of nuclear transcription factors which play essential roles in the regulation of various cellular and molecular processes such as oxidative stress, inflammation, cellular differentiation, development, metabolism, and energy production [56]. Interestingly, the ligand binding pocket of one PPAR isoform, PPARγ, is large which provides a specific binding site for not only various *n*-3 and *n*-6 PUFAs, but also the saturated fatty acids such as decanoic acid can bind and activate it at physiologically appropriate concentrations [70,77]. It has been revealed that the activating potential of fatty acids is stronger with growing carbon chain length and an increasing number of double-bonds [78]. Therefore, it seems that fatty acids, particularly PUFAs provided by a KD may activate PPARγ that regulate anti-inflammatory, anti-oxidant and mitochondrial genes that lead to enhancing energy reserves, stabilize synaptic function and restrict hyperexcitability [56].

Further studies are necessary to distinguish the downstream mechanisms by which PPARs diminish seizures and discover how PPARs associate with several other hypotheses of the KD [70]. Furthermore, it has been revealed that PUFAs can block voltage-gated sodium and calcium channels, activate the K2P channels, and improve the Na+/K+/ATPase activity. These changes can reduce neuronal excitability and alleviate seizures [56]. These fatty acids can also reduce seizure indirectly by inducing the expression of uncoupling proteins (UCPs). These proteins, located in the inner membrane of mitochondrial, uncouple electron transfer chain and eventually decrease reactive oxygen species (ROS) production and oxidative stress [56,79] which are involved in the pathogenesis of epilepsy [80–83].

These results can indicate that raise in the fatty acids levels, and PUFAs in particular, may diminish neuronal hyperexcitability and seizure through various direct and indirect mechanisms. Given that *n*-3 PUFAs are more effective than *n*-6 PUFAs in decreasing inflammation and cholesterol levels; it can be possible to more effectively diminish seizures and the risk of cardiovascular diseases by reducing the omega-6/omega-3 ratio in the KD composition [78].

7. Possible effects of KD on neuronal death

The role of seizure in neuronal damage and the involved underlying mechanisms have been discussed for decades. It has been revealed that isolated slight seizures probably could not kill neurons; however, severe and prolonged seizures not only can cause neuronal damage, but also may cause neuronal death [84]. The cognitive impairments and seizure severity in DRE patients, both can be affected by the amount of neuronal damage caused by seizures [85]. This damage may be mediated mainly by excitotoxicity. In this pathological process, the prolonged seizures lead to extreme presynaptic glutamate release which activates an excessive number of postsynaptic NMDA receptors and opens their cationic sodium and calcium channels. Excessive sodium influx can cause osmotic stress leading to neuronal swelling and rupture. The increased intraneuronal calcium amount enhances the activation of calcium-dependent proteases, phospholipases, and nitric oxide synthase elevating free radicals that all lead to DNA degradation and organelles destruction culminating in necrosis of the postsynaptic neurons [85,86].

On the other hand, the increased amount of calcium and free radicals following neuronal damage may lead to the opening of the mitochondrial permeability transition pore and releasing cytochrome c into the cytoplasm [87]. Cytochrome c is a crucial member of the mitochondrial electron transport chain. The translocation of it into cytoplasm can be considered as a signal and has been revealed to initiate a neuronal apoptosis cascade (mitochondrial pathway). In the cytoplasm, this cytochrome in conjunction with apoptotic protease-activating factor 1 (Apaf-1) activates caspase-3 and caspase-9 as the main effector enzymes in neuronal apoptosis [88], followed by enzymatical cleavage of intraneuronal organelles, proteins, and DNA. The neuron corpses are then packaged in preparation for phagocytosis by microglia [84].

As mentioned above, it seems that excitotoxicity and apoptosis are the main mechanisms involved in seizure-induced neuronal damage and death. It has been suggested that the adverse consequences of these pathologic processes can be ameliorated by KD [89]. It has been revealed that KD can upregulate calbindin which has neuroprotective potential through its capability to buffer intracellular calcium [90]. Other neuroprotective effects of KD may be mediated by inhibition of the pro-apoptotic factors such as caspase-3 [87,88,90,91]. Moreover, the opening of the mitochondrial permeability transition pore can also be inhibited by KD [87].

Recently known mechanisms of cell death including autophagy, phagoptosis, necroptosis, and pyroptosis [84,92], in addition to the other probable neuronal apoptosis pathways mediated by different factors such as clusterin, P53, poly (ADP-ribose) polymerases, chaperones, tumor necrosis factor superfamily, and Bcl-2 family members [84–87] may also have roles in the seizure-inducing neuronal injury and death. The probable effects of KD on these factors and mechanisms await future studies.

8. Conclusion

Different types of KD including classic, MAD, MCTD, and LGIT have been used therapeutically in the management of DRE. There are some differences in the composition of these main types of KD, but, it has been proven that they have nearly similar efficacy. Although all the mechanisms involved in the reducing seizure induced by KD have not yet been completely understood, it has been shown that the ketone bodies and PUFAs, which can be enhanced under KD, may play main roles in its anti-seizure effect. The ketone bodies by increasing inhibitory neurotransmitters, activating of potassium channels, and enhancing the energy production of the nervous system can enhance the brain seizure threshold. On the other hand, fatty acids and PUFAs, particularly *n*–3 PUFAs, have been revealed to activate PPARs, in

particular PPARγ, leading to up-regulation of energy transcripts, enhancement of energy reserves, and stabilization of synaptic function that eventually prevents neuronal hyperexcitability. PUFAs can also alter ion-channels activities that cause neuronal hyperpolarization. Furthermore, upregulation of UCPs induced by PUFAs can diminish oxidative stress. In addition to these mentioned mechanisms, KD may alleviate the seizure-induced neuronal damage through several probable anti-apoptosis and anti-necrosis mechanisms. Overall, KD and its variants not only can eventually limit the seizures, but also may alleviate their adverse effects on neurons through different mechanisms. However, further studies are necessary to confirm them and determine other involved mechanisms which would be useful in clinical application.

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