REVIEW

Ketogenic diets: from cancer to mitochondrial diseases and beyond

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ABSTRACT

Background The employment of dietary strategies such as ketogenic diets, which force cells to alter their energy source, has shown efficacy in the treatment of several diseases. Ketogenic diets are composed of high fat, moderate protein and low carbohydrates, which favour mitochondrial respiration rather than glycolysis for energy metabolism.

Design This review focuses on how oncological, neurological and mitochondrial disorders have been targeted by ketogenic diets, their metabolic effects, and the possible mechanisms of action on mitochondrial energy homeostasis. The beneficial and adverse effects of the ketogenic diets are also highlighted.

Results and conclusions Although the full mechanism by which ketogenic diets improve oncological and neurological conditions still remains to be elucidated, their clinical efficacy has attracted many new followers, and ketogenic diets can be a good option as a co-adjuvant therapy, depending on the situation and the extent of the disease.

Keywords Cancer, high fat diet, Ketogenic diet, mitochondria, neurological diseases.

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Introduction

Several diseases involving alterations in mitochondrial metabolism, including diabetes mellitus type II, obesity and cancer, are exceptional candidates to benefit from dietary therapeutic strategies, such as ketogenic diets. These diets were shown to reverse redox signalling pathways that increase the malignancy of tumours [1], and to possess anticonvulsant effects in humans that could be related to increased mitochondrial mitochondrial biogenesis [2,3]. In fact, ketogenic diets can also constitute a first line of treatment for mitochondrial myopathies due to improvement of mitochondrial activity resulting from increased mitochondrial biogenesis [4,5]. Although these diets can lead to some short and long-term adverse effects (e.g. gastrointestinal disorders such as constipation and hyperlipidaemia, even though the latter is rather controversial [6,7]) they are effective and potentially nontoxic metabolic therapies for the treatment of chronic neurological disorders, also exerting a protective action against brain tumour angiogenesis and ischaemic injuries [8].

This review discusses the advantages of ketogenic diets in different pathologies in which mitochondrial dysfunction is an important component, and also the potential limitations and side effects of this nonpharmacological diet-based therapy.

Ketogenic Diets: Composition and Metabolism

Ketogenic diets are composed of high-fat, moderate protein and low-carbohydrate components, resulting in limited metabolism of carbohydrates and proteins and increased fat metabolism [9,10]. As a consequence of elevated levels of fat-derived ketone bodies and decreased levels of glucose in the blood, alterations in energy metabolism can occur. First described by Hans Krebs [11], ketosis is a metabolic state in which the body obtains its energy from the metabolism of ketone bodies, as opposed to what occurs in glycolysis, where glucose is the main energy source. Ketosis may be achieved through periods of fasting or by reducing the intake of carbohydrates in the diet [12].

There are four main categories of ketogenic diets [13], initially proposed in 1921. The most common is the long chain triglyceride (LCT)-based diet [14]. This diet consists of a classical ratio of fat to nonfat (protein and carbohydrates) of 4 : 1,

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but also possibly of 3 : 1, 2 : 1 or 1 : 1 [15]. Other ketogenic diets emerged over the years. The medium chain triglyceride (MCT) [16] diet was introduced to surpass the severe restrictions of classic ketogenic diets [17]. The main fatty acids in MCTs are caprylic acid, capric acid and, to a lesser extent, caproic acid and lauric acid [18]. This diet is not based on diet ratios but uses a percentage of calories from MCTs oils to create ketones [17]. The main advantage of MCTs over LCTs is that the former are more efficiently absorbed and quickly transported to the liver by albumin. Following hepatic uptake, those are promptly metabolized by liver mitochondria and, after fatty acid β-oxidation, converted into ketone bodies [13]. On the other hand, LCTs have to be incorporated into chylomicrons and transported via the thoracic duct into the blood circulation. LCTs need carnitine as a carrier to enter the mitochondria and then undergo cycles of β-oxidation [18]. Thus, compared with MCTs, LCT metabolism is a slower process and requires more energy expenditure to occur. Because of this, less total fat should be required in MCT diets to achieve the desired level of ketosis. Additionally, an enhancement in palatability can be achieved due to a higher content in protein and carbohydrates [13]. There have been two additional diets developed to help treat epilepsy: the modified Atkins diet and the low glycaemic index treatment (LGIT). The modified Atkins diet was originally designed and investigated at Johns Hopkins Hospital [19] and proposed as a less restrictive and more palatable dietary treatment [20]. Contrary to other ketogenic diets, it does not overly restrict protein intake or daily calories [21]. Regarding LGIT, the grounding objective was to allow a more moderate intake of carbohydrates with a glycaemic index lower than 50 [22], although without increasing ketone levels. Low glycaemic index carbohydrates induce a smaller increase in blood glucose, causing less variability in its levels throughout the day [23]. All these diets must be planned by a dietician based on different grounds including clinical diagnosis, age, gender, weight, activity level, and the expected compliance, to help each patient achieve their best ketone levels and to maximize the objective [17,24].

The high percentage of fat contained in ketogenic diets forces the body to use fats instead of carbohydrates. Ketone bodies are produced in the liver as a consequence of fatty acid oxidation, following the metabolism of acetyl-CoA formed during mitochondrial β-oxidation (Fig. 1). Acetyl-CoA can either enter the Krebs cycle for ATP production and/or be converted into the ketone bodies acetoacetate, beta-hydroxybutyrate (β-OHB), and acetone, which are transported from the blood to different tis-
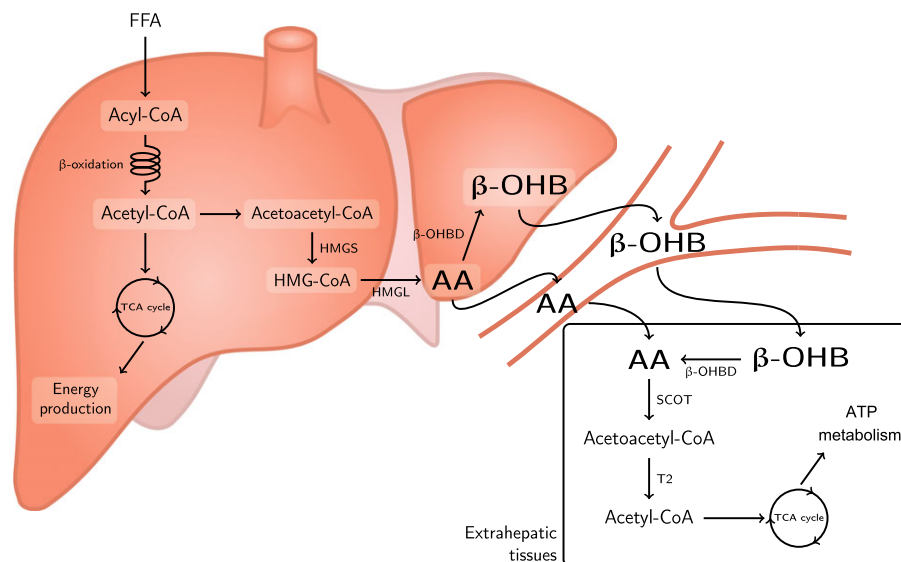

Figure 1 Ketone bodies generation and utilization. In the liver, free fatty acids (FFA) are converted into acyl-CoA which enters mitochondrial β-oxidation and is converted into acetyl-CoA. This molecule can enter the Krebs cycle to generate energy and/or be converted into the ketone bodies acetoacetate (AA), by the hydroxymethylglutaryl-lyase (HMG), β-hydroxybutyrate (β-OHB), by the β-OHB dehydrogenase (β-OHBD) and acetone, which are transported from the blood to different tissues. In brain, heart or muscle, ketone bodies produced in the liver are used as an energy source *via* acetyl-CoA. This process depends on important mitochondrial enzymes such as the β-OHBD that converts the β-OHB in AA, succinyl-CoA: 3-ketoacid CoA transferase (SCOT), involved in the formation of acetoacetyl-CoA from AA, and thiolase (T2) that converts acetoacetyl-CoA into acetyl-CoA, which then enters the Krebs cycle.

sues such as the heart and brain (Fig. 1). β-OHB is the major circulating ketone body, whereas acetoacetate is chemically very unstable and acetone is poorly metabolized [25]. Under normal conditions, the concentration of ketones in the plasma is relatively low (<0·2 mM), but during ketogenic conditions, their levels can increase up to 7–8 mM [26]. In different tissues including the brain, muscle or heart, ketone bodies are converted back to acetyl-CoA to serve as an energy source. Energy retrieval from β-OHB depends on the expression of two key mitochondrial enzymes: β-OHB dehydrogenase (β-OHBD) and succinyl-CoA: 3-ketoacid CoA transferase (SCOT) [27] (Fig. 1). Ketone bodies are energetically more efficient than pyruvate or fatty acids due to their greater hydrogen/carbon ratio and to the fact that, unlike fatty acids, they do not uncouple mitochondria [28,29]. However, increased β-oxidation may be responsible by increased expression and activity of mitochondrial uncoupling proteins in the hippocampus of juvenile mice subjected to a high-fat ketogenic diet, generating a mild uncoupling effect which may constitute a neuroprotective

mechanism aimed at reducing ROS formation by the respiratory chain [30].

Ketogenic Diets as Anticancer Approaches

The modulation of cellular metabolism by carbohydrate depletion via ketogenic diets has been suggested as an important therapeutic strategy to selectively kill cancer cells. One hallmark of nearly all cancer cells is the anomalous metabolic phenotype first described by Otto Warburg [31], which is characterized by a metabolic shift from respiration towards glycolysis, regardless of oxygen availability. In the majority of normal cells with functional mitochondria, pyruvate generated via glycolysis is shuttled to the tricarboxylic acid (TCA) cycle for mitochondrial oxidative metabolism. Cancer cells, on the other hand, use pyruvate mostly in the lactic acid fermentation pathway (Fig. 2). This metabolic phenotype provides several advantages to cancer cells. First, it allows for a more efficient generation of carbon equivalents for macromolecular synthesis
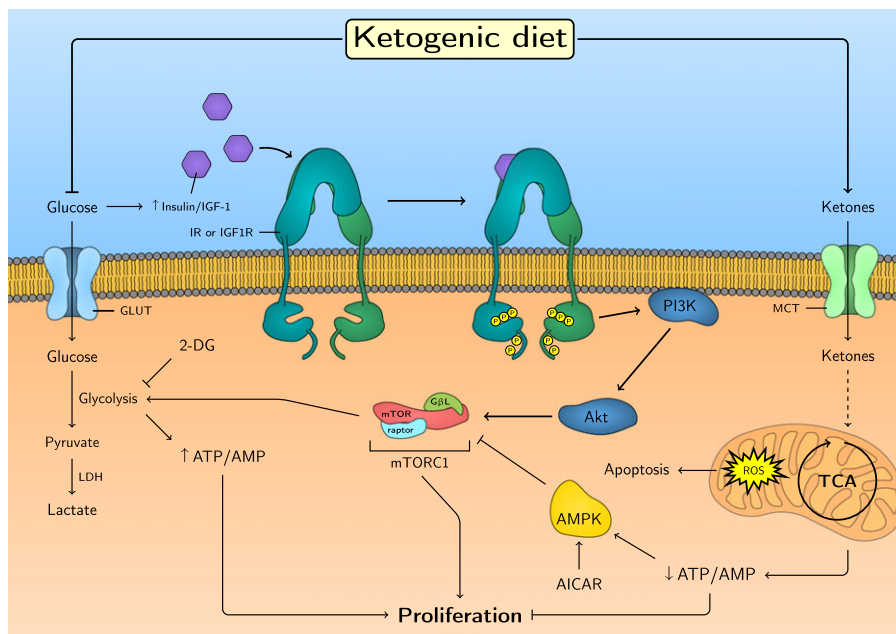

Figure 2 Ketogenic diets simultaneously target glucose metabolism and glucose-related signalling in tumour cells. A reduction in circulating glucose levels compromises energy production and macromolecular biosynthesis. The concomitant reduction in blood insulin/IGF-1 levels decreases signaling by the PI3K/Akt/mTOR pathway, thus impairing glycolytic metabolism and macromolecular biosynthesis. Moreover, in contrast with normal cells, tumour cells are unable to efficiently adapt to metabolize ketone bodies. Also shown are pharmacological disruptors of glucose metabolism and glucose-related signalling. Abbreviations: 2-DG, 2-deoxy-D-glucose; AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide; AMPK, AMP-activated protein kinase; GβL, G protein beta subunit-like; GLUT, glucose transporter; IGF-1, insulin-like growth factor 1; IR, insulin receptor; IGF-1R, IGF-1 receptor; LDH, lactate dehydrogenase; PI3K, phosphatidylinositol-3 kinase; MCT, monocarboxylate transporter; mTORC1, mammalian target of rapamycin complex 1; mTOR, mammalian target of rapamycin; raptor, regulatory- associated protein of mTOR; ROS, reactive oxygen species. Other abbreviations are described in the text.

than oxidative phosphorylation (OXPHOS), which is suitable for a proliferative phenotype [32]. Second, it bypasses mitochondrial oxidative metabolism and its concurrent production of reactive oxygen species (ROS). This confers a survival advantage since cancer cells display higher steady-state levels of oxidative stress relative to normal cells, which renders them more sensitive to ROS-mediated apoptotic stimuli [33]. Finally, an elevated glycolytic flux promotes acidification of the tumour site, which facilitates tumour invasion and progression [34].

The shift towards a glycolytic and proliferative phenotype requires an extensive metabolic transformation. This is believed to occur mostly via inappropriate overactivation of the insulin/IGF-1-dependent phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of the rapamycin (mTOR) system (Fig. 2) [35–37], not only due to mutations in the genes that code for pathway proteins [38], but also due to chronic hyperglycaemia and hyperinsulinaemia – also known hallmarks of cancer [39]. Activation of the PI3K/Akt/mTOR pathway increases glucose uptake and trapping via upregulation and membrane translocation of glucose transporters (GLUT) [37,40] and increased hexokinase II (HKII) activity [41]. Glycolytic metabolism is further reinforced by effectors downstream of mTOR (c-Myc and HIF-1α) that upregulate key glycolytic enzymes [39,41]. β-Oxidation, in turn, is inhibited via Akt-mediated downregulation of carnitine palmitoyltransferase 1A (CPT1A) [42]. The above discussion leads to the conclusion that cancer cells are highly dependent on glucose availability for growth, proliferation, energy production and transformation. Elevated glucose levels and uptake rates have indeed been consistently associated with poor prognosis in cancer patients [40,43,44]. Thus, exploiting this unique metabolic requirement, a logical therapeutic strategy is the implementation of a ketogenic diet, which reduces glucose availability to tumour cells while providing ketone bodies as an alternative fuel to normal cells (Fig. 2). This allows for the selective starvation of tumour cells, which, in contrast with normal cells, should be unable to adapt to ketone metabolism as a result of their acquired metabolic inflexibility and genomic instability [45]. Brain tumours should be particularly susceptible, since the normal brain cells from which they derive are already adapted to rely almost exclusively on glucose for energy [46]. Indeed, various tumours display reduced levels of β-OHBDH and SCOT [29,47,48], which is suggestive of an impaired ability to metabolize ketone bodies for energy. Mitochondrial abnormalities associated with cancer cells should additionally compromise efficient ketone body metabolism [45,49]. Furthermore, lower circulating levels of glucose will also lower insulin and IGF-1 levels, thereby decreasing the activation of the PI3K/Akt/mTOR pathway. A ketogenic diet has already been shown to effectively downregulate this pathway in patients with advanced cancer [50].

Although ketone bodies can be theoretically expected to be detrimental to tumour cells, work published by the Lisanti group suggests otherwise [51,52]. Under their proposed 'reverse Warburg effect' hypothesis, fibroblasts in the tumour microenvironment differentiate in a way to provide neighbouring tumour cells with energy-rich substrates (such as β-OHB) that may enter the TCA cycle and further complement ATP production. As such, under the view that tumour cells derive benefits from ketone bodies, it can be predicted that the antitumour effects of ketogenic diets are mostly mediated by a decrease in circulating glucose rather than increases in ketone bodies. Another important question is related with cancer cachexia, which is characterized by an ongoing loss of weight – particularly skeletal muscle mass – that stems from a combination of anorexia, hypermetabolism, hypercatabolism and hypoanabolism [53]. Development of cachexia is associated with decreased cancer therapy tolerance and severe impairment of respiratory function [54], both of which ultimately lead to lower survival rates. Procedures resulting in unnecessary weight loss for cancer patients are therefore highly undesirable, as they may trigger and/or exacerbate cachexia development. As such, to maximize clinical applicability, anticancer ketogenic diets should be tailored to promote weight gain or at least body weight maintenance.

Experimental therapies for the management of cancer cachexia are usually multimodal approaches that include nutritional management [53]. This strategy usually consists of supplementing pre- and early cachectic patients with the ω-3 fatty acid eicosapentaenoic acid (EPA) and branched chain amino acids (BCAAs), which possess anti-catabolic and pro-anabolic properties, respectively [54]. As it is immediately apparent, the concept of ketogenic diets can very easily accommodate both of these criteria. Studies performed in humans [55] and animals [56] showed that a noncalorie-restricted ketogenic diet slightly increases lean body mass. Animal studies conducted thus far provide conflicting lines of evidence. This is likely owing to differences in cancer experimental models, animal strains and particularly diet composition, since the concept of a ketogenic diet can accommodate an enormous variety of dietary compositions.

In a series of studies employing mouse astrocytoma allograft models in the C57BL/6J strain, Seyfried *et al.* reported decreased tumour progression rates and increased survivability in mice fed ketogenic diets under calorie-restricted regimens [29,57,58]. No beneficial effects were observed when the diets were administered *ad libitum*, possibly because they consistently failed to reduce circulating glucose levels. Interestingly, mice fed a restricted standard diet displayed the same beneficial outcomes as mice fed the restricted ketogenic diet. While these results cast doubt on whether the positive effects were mediated by carbohydrate or caloric restriction, it should

nevertheless be noted that all animals fed restricted diets displayed lower levels of circulating glucose and elevated levels of ketone bodies, suggesting that ketosis is indeed responsible for the observed positive effects. In contrast to the previous results, a collection of studies using different experimental models and ketogenic diet compositions reported equally positive antitumour effects in mice fed unrestricted ketogenic diets [1,59–61], although no reduction in circulating glucose levels was observed in some of the studies.

Clearly, given the multitude of simultaneous variables that must be considered in this topic, mixed results are unavoidable. We nevertheless wish to stress the need to standardize *in vivo* models in favour of a combination of animals and unrestricted ketogenic diets in which a ketogenic state is induced. In addition, these models would simulate a diet regimen that would be more acceptable to practitioners and patients alike, as they avoid the much-dreaded cachectic weight loss and the compliance issues associated with severe hunger from caloric restriction. Moreover, this would isolate the effects of carbohydrate limitation and eliminate caloric restriction as a confounding factor. Additional therapeutic strategies can be employed simultaneously with ketogenic diets to further exploit the reliance on glucose by cancer cells (Fig. 2). In a synergistic study, Marsh *et al.* [58] assessed the effect of combining a restricted ketogenic diet with the glucose analogue 2-deoxy-d-glucose (2-DG), a pharmacological inhibitor of glycolysis. The authors reported a significant decrease in tumour progression compared with the restricted ketogenic diet alone, which came however with adverse effects in health and vitality, in addition to increased mortality. It is likely that the combination of lower circulating glucose levels and inhibition of glycolysis is too aggressive for exclusively glycolytic cells such as erythrocytes, thereby rendering this strategy clinically unfeasible. Pharmacological activation of AMP-activated protein kinase (AMPK) by the AMP-analogue 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) (Fig. 2) is another strategy that may further hamper tumour proliferation, as AMPK inactivates mTOR by phosphorylating its upstream regulator tuberous sclerosis complex protein-2 (TSC2) [62]. While no synergistic studies have been conducted as of yet, one study reported that AICAR administration alone lead to a ≈ 50% reduction in tumour progression in a rat glioma model [63]. Ketogenic diets have also been reported to enhance the effectiveness of therapies unrelated to glucose metabolism, such as radiation therapy [60], and hyperbaric oxygen therapy [61]. Clinical studies assessing the antitumour efficacy of ketogenic diets are rare. A proof of concept case study by the group of Nebeling and coworkers was the first attempt at using a ketogenic diet as an antitumour therapy in humans [64], with favourable results. Two female paediatric patients with advanced stage glioma participated in this case report; both

successfully achieved ketosis and displayed a ≈ 22% decrease in [18F]fludeoxyglucose uptake on PET scans, along with significant clinical improvement. More recently, a case report on a 65-year old patient with glioblastoma multiforme treated with a restricted ketogenic diet while undergoing radiation and chemotherapy also reported positive results, as imaging studies were suggestive of tumour regression [65]. Another recent pilot trial in 16 patients with advanced metastatic tumours likewise reported positive results from a restricted ketogenic diet in tumour progression and blood parameters [66]. The remaining available completed trials focused mostly on the safety and feasibility of ketogenic diets in the oncological population, reporting for the most part favourable and encouraging results [50,67,68]. Ongoing trials using ketogenic diets as mono- or adjuvant therapies in cancer treatment are summarized in Table 1.

Improving Mitochondrial Diseases by Using Ketogenic Diets

Mitochondrial diseases may be a consequence of genetic defects in mtDNA and/or nuclear DNA coding mitochondrial proteins, resulting in mitochondrial dysfunction [69,70]. Although mitochondrial diseases have been considered rare diseases, epidemiological studies suggested a minimum prevalence of 1

Table 1 List of ongoing clinical trials using ketogenic diets in cancer treatment

Condition	Intervention	Identifier
Pancreatic Neoplasms	Ketogenic diet with concurrent chemoradiation	NCT01419483
Head and Neck Neoplasms	Ketogenic diet with concurrent chemoradiation	NCT01975766
Carcinoma, NonSmall-Cell Lung	Ketogenic diet with concurrent chemoradiation	NCT01419587
Glioblastoma	Energy-restricted ketogenic Diet	NCT01535911
Breast Cancer	Ketogenic diet, low glycaemic and insulinaemic diet	NCT02092753
Glioblastoma Multiforme	Ketogenic diet	NCT01865162
Cancer	Ketogenic diet	NCT01716468
Recurrent Glioblastoma	Calorie-restricted ketogenic diet and transient fasting with concurrent radiation	NCT01754350
Glioblastoma	Ketogenic diet with concurrent chemoradiation	NCT02046187

in 5000 children [71]. Mitochondrial DNA mutations can also be detected in healthy humans without the disease being manifested, due to the presence of a mixture of mutated and wild-type mtDNA, or heteroplasmy [72]. However, if the percentage of mutated mtDNA increases in germ cells, more specifically in the female ones, which are the mitochondria donors during reproduction, the probability that mitochondrial diseases will be manifested in the offspring increases [72]. Characterized by the occurrence of abnormal metabolic pathways, mitochondrial diseases lead to a decrease in energy production, as well as various clinical symptoms [73]. Being normally heterogeneous and multisystemic diseases, they preferentially affect tissues with high energy demands such as the brain, muscle, heart and endocrine system [69,73].

Several strategies have been approached to ameliorate the consequences of mtDNA defects in patients with mitochondrial diseases, including changes on diet and lifestyle, pharmacological treatments and gene therapy. These strategies can shift heteroplasmic levels of mtDNA mutations, replace the defective mitochondrial genes, scavenge toxic intermediates, optimize ATP synthetic capacity, and/or bypass defective OXPHOS components [70,74–76]. Unfortunately, an effective treatment is not yet developed, making the patients dependent on adjuvant, but not curative, interventions [77].

Since ketogenic diets stimulate mitochondrial biogenesis, improve mitochondrial function, decrease oxidative stress [1,78,79], and contribute to reducing the glycolytic rate due to increases in lipid oxidation and mitochondrial respiration [80], these diets have also been proposed as a possible treatment for mitochondrial disorders [5,81–83]. However, in this case, special care should be taken. As stated before, ketogenic diets induce a shift from carbohydrates to lipids as the main source of energy [84], and so can overcome conditions such as pyruvate oxidation disorders [85], including pyruvate dehydrogenase deficiency, a severe mitochondrial disease that results in lactic acidosis and severe impairment, precluding pyruvate metabolization into acetyl-CoA [86]. This is accomplished because ketogenic diets supply alternative sources of acetyl-CoA, as described before. Conversely, these diets are not indicated to individuals with disorders in fat metabolism such as medium-chain-acyl-CoA dehydrogenase (MCDA) deficiency [87] or pyruvate carboxylase, the mitochondrial enzyme that catalyzes the conversion of pyruvate to oxaloacetate. MCDA is a mitochondrial matrix flavoenzyme that catalyzes the initial step of medium-chain fatty acid β-oxidation. A deficiency in this protein leads to an excessive increase and accumulation of CoA and medium-chain fatty acids derivatives. This ultimately results in episodes of hypoketotic hypoglycaemia [87]. Impaired β-oxidation disturbs ATP supply, which dowregulates neoglucogenesis, and acetyl-CoA production used for ketone bodies synthesis and for urea cycle activity [88].

Disorders in fatty acid β-oxidation can thus lead to a devastating catabolic crisis in individuals that are fasting or on ketogenic diets.

Concerning pyruvate carboxylase, ketogenic diets downregulate the TCA cycle and decrease energy production [84]. Studies developed by Santra *et al.* [81] demonstrated that ketogenic treatments in cultured human cells promoted an heteroplasmic shifting, increasing the proportion of nonmutated mitochondrial DNA and increasing mitochondrial protein synthesis [81].

Moreover, in about 40% of children with mitochondrial disease, epilepsy is part of the clinical phenotype [89]. Kang *et al.* [83] demonstrated the clinical efficacy and safety of ketogenic diets in 14 children with intractable epilepsy and with established mitochondrial defects in complexes I, II and IV. Their studies found that half of these patients became seizure-free after the treatment with the ketogenic diet. However, four of those patients did not show any favourable responses and due to complications they were advised to cease the diet. Also, Jarrett *et al.* [90] demonstrated that a ketogenic diet affords protection to the mitochondrial genome against oxidative insults, increasing the levels of mitochondrial GSH, stimulating *de novo* biosynthesis of GSH, and improving mitochondrial redox status.

Alpers-Huttenlocher syndrome is a mitochondrial disease characterized by mutations in polymerase (DNA directed) gamma gene, resulting in defective oxidative phosphorylation, and consequently in intractable epilepsy, psychomotor regression and liver disease. Although an effective treatment for this syndrome has not yet been developed, one report described the efficacy of a ketogenic diet in the treatment of epileptic encephalopathy observed in a child with this syndrome [82]. Following the ketogenic diet, the patient revealed an increase in alertness, improvement of memory, control of bladder and bowel and the ability to speak in 3–4 word sentences. Although the treatment was not 100% effective for this patient, since she died at age of 66 months due to respiratory failure, an improvement in the symptoms was observed [82]. Other reports presented similar beneficial effects of KD with symptomatic improvement but premature death [91–94].

Also, another study developed in transgenic mice with accumulated mtDNA deletions (the Deletor mice), a model for a progressive late-onset mitochondrial myopathy, showed that ketogenic diets improved mitochondrial function, induced mitochondrial biogenesis, and restored metabolic and lipidomic changes induced by the progressive disease. However, no significant effects on mtDNA quality or quantity were observed [5]. Regarding lethal mitochondrial cardiomyopathy, Krebs *et al.* [95] described a missense mutation in the Mediator complex (Med), a protein complex necessary for expression of RNA polymerase II-transcribed genes that binds simultaneously to polymerase II and to gene-specific transcriptional activators,

promoting pre-initiation complex assembly. A missense mutation in Med30 causes a progressive and selective decline in the transcription of genes necessary for OXPHOS and mitochondrial integrity. Med function was shown to be associated with the induction of a metabolic program for mitochondrial OXPHOS and fatty acid oxidation, with a specific impact on cardiac function. *In vivo* studies showed that weaned mutants that were fed with ketogenic diets had a significantly increased lifespan and increased expression of cardiac OXPHOS genes.

Although several studies have suggested ketogenic diets as therapies for mitochondrial diseases, since they increase mitochondrial function and biogenesis and decrease oxidative stress and mitochondrial pathogenic mutations (Fig. 3), in our opinion more clinical studies are needed in order to understand the pathophysiology of mitochondrial diseases and to determine which individuals are likely to benefit from this therapeutic strategy.

Control of Neurological Disorders by Ketogenic Diets

Ketogenic diets are commonly used in patients suffering from neurological disorders, mostly epilepsy [96–100], but are increasingly being considered for Alzheimer's disease [101,102], Parkinson's disease (PD) [103,104], amyotrophic lateral sclerosis (ALS) [105,106], GLUT1 deficiency syndrome [107,108] and, as already referred to in section 3, PDH deficiency [86]. As previously mentioned, ketogenic diets also have
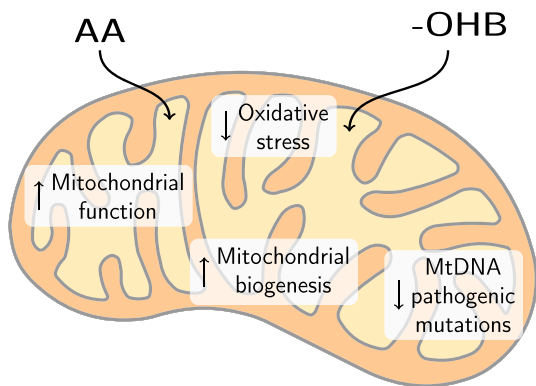
Figure 3 Mitochondrial effects of ketogenic diets. Ketogenic diets are used in the treatment of several diseases, such as epilepsy, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, cancer and brain trauma. Ketone bodies such as acetoacetate (AA) and β-hydroxybutyrate (β-OHB) lead to decreases in oxidative stress and improvements in mitochondrial biogenesis and mitochondrial function. Ketogenic diets may also induce a heteroplasmic shift, reducing pathogenic mutations on mitochondrial DNA (mtDNA).

anticonvulsant effects, leading to a significant decrease in the occurrence of seizures in epileptic patients [2,3]. Epilepsy is a neurological disorder characterized by repeated seizures of different types over time. Seizure types can be broadly divided into generalized and partial (also called focal) seizures. It is estimated that about 65 million people around the world have epilepsy, resulting from different causes [109]. The most common type is idiopathic epilepsy which is believed to have an underlying genetic basis, although the causes are still unknown. In contrast to idiopathic epilepsy, symptomatic epilepsy is known to have a variety of causes, including trauma, infection and malignancies of the brain. Epilepsy is diagnosed by manifestation of at least two types of seizures. Seizures are thought to result from an imbalance between excitatory and inhibitory neurotransmission, leading to increased excitatory neurotransmission mediated by glutamate [110]. Since the 1920s, epilepsy has been treated with ketogenic diets without much understanding of the mechanism of action. Antiepileptic medication is initially prescribed to decrease the number of seizures, but the patient can also be placed on a ketogenic diet [3]. Four mechanisms were proposed to explain the success of the ketogenic diet in helping with the alterations of neuronal excitability in epileptic patients: decreased carbohydrate intake; inhibition of glutamatergic synaptic transmission; inhibition of the mTOR pathway; and activation of ATP-sensitive potassium channels by mitochondrial metabolism [80].

Since glucose is the preferred source of energy in the brain, leading to increased neuronal excitability, and contributing to spark seizures in some patients, the lower amount of carbohydrates in the ketogenic diet may be responsible for symptomatic improvement [110]. The role of reduced carbohydrate intake in the decrease of seizure occurrences was tested using animal models exposed to the glucose analog 2-DG, which blocks glycolysis at the phosphoglucomutase step, thereby blocking carbohydrate metabolism. This analogue is taken up by high-energy demanding brain regions, which increase during seizures, encouraging further study of 2-DG as a potential antiseizure medication [80]. In this regard, the effects of 2-DG on seizure occurrence were studied in rat hippocampal slices perfused with either 4-aminopyridine, a nonselective potassium channel blocker that prevents neuronal repolarization [111], or bicuculline, a competitive antagonist of inhibitory GABA$_A$ receptors [112], before seizures were evoked *in vivo* by 6 Hz stimulation in mice. The results of this study showed that 2-DG exerts chronic antiepileptic and acute anticonvulsant actions [113].

It is believed that there is a connection between ketones and the glutamatergic synaptic transmission [3]. There are, however, contradicting studies regarding the effects of β-OHB and acetoacetate on glutamatergic synaptic transmission. Acetoacetate and β-OHB inhibit glutamate uptake into synaptic vesicles

by vesicular glutamate transporters in pre-synaptic neurons, by competing with chloride at the site of allosteric modulation of glutamate transporters, resulting in decreased glutamate release and fewer seizures [114]. Nevertheless, more research is needed at the clinical and animal levels to better understand the relationship between glutamate metabolism and ketone-mediated seizure control. Until now, *in vitro* models showed no effect of acetoacetate and β-OHB on modulating glutamate receptor function and fast excitatory synaptic transmission [80]. However, in *in vivo* models, acetoacetate was able to block glutamate release from rat hippocampal neurons [114]. Further research is needed to assess if acetoacetate could have potential clinical significance at reducing seizures by reducing glutamate release.

The mTOR pathway has a pathophysiological role in different types of seizures associated with epilepsy. This pathway responds indirectly to multiple metabolic inputs including the insulin receptor, fasting, and hypoglycaemia. Inhibition of mTOR with rapamycin or ketogenic diets has been shown to decrease seizures in mouse models. However, the diet has been shown to be highly protective against acute seizures, in contrast with rapamycin [115]. In the presence of nutrients and other growth factors, mTOR is activated by PI3K/Akt signalling, whereas in the absence of energy, mTOR is inhibited by AMPK (Fig. 2). Rats on a ketogenic diet showed reduced phosphorylation of Akt and S6, suggesting decreased mTOR activation in the hippocampus and liver, along with an increase in AMPK signalling [116]. The diet was also shown to lower insulin levels in rats and it would therefore be expected to decrease Akt and mTOR signalling [117] (Fig. 2). mTOR inhibition may also occur from other dietary effects including protein restriction, low glucose levels and poor growth. In a study on kainic acid (KA)-induced status epilepticus (SE), late mTOR hyperactivation played a role in epileptogenesis and could be a mechanism for the antiepileptogenic effect of the ketogenic diet [116].

Mitochondrial role in antiseizure effects of a ketogenic diet is logical, considering the reliance of neuronal excitability on energy metabolism [118]. The anticonvulsive effects of ketogenic diets were shown to be associated with increased mitochondrial biogenesis in the rat hippocampus [79]. The coupling of neuronal excitability and energy metabolism could be modulated by the plasma membrane ATP-sensitive potassium channel, which is an inward-facing potassium channel inhibited by intracellular ATP, and could be mediated by Bad (Bcl-2-associated Agonist of Cell Death) (Fig. 4). Even though Bad is a member of the Bcl-2 family related to apoptosis, it has also been shown to alter glucose metabolism in fibroblasts, hepatocytes, and islet β-cells [119,120]. The function of Bad is altered by the phosphorylation state of serine at position 155 and interference with Bad phosphorylation state is associated with decreased glucose mitochondrial metabolism and metabolic preference

for ketone bodies [120]. Bad interference with neuronal metabolism and excitability seems to extend to reductions in behavioural seizures, including a near absence of tonic clonic seizures. Genetic modification of the Kir6·2 pore forming subunits of the ATP-sensitive channel reversed Bad-induced changes in seizure sensitivity, indicating that the ATP sensitive channel necessary for neuronal excitation and seizure response is downstream of Bad [120]. Thus, Bad may turn out to be a drug target in epilepsy in the future, making the use of ketogenic diets no longer necessary, which would be particularly beneficial for children, who would not have to give up foods they enjoy so much.

A recent study showed consistent anti-seizure effects of ketones through inhibition of mPTP [100]. The study was carried out in Kcna1-null mice, which lack voltage-gated Kv1·1 channels as a result of deletion of the Kcna1 gene, resulting in a genetic model of human epilepsy. In this work, the beneficial effects of ketogenic diets and ketone bodies were mediated by cyclophilin D [100], a controversial component of the mitochondrial permeability transition pore (mPTP) involved in Ca^{2+} and ROS homeostasis [121,122].

Another disease that can benefit from ketogenic diets is GLUT 1 deficiency syndrome, which is characterized by impaired glucose uptake, resulting in increased extracellular glucose levels. In these patients, ketogenic diets may provide alternative cellular energy sources, while helping to control glycaemia [107,108].

In Alzheimer's disease, loss of recent memory is associated with deposition of the amyloid-β (Aβ) peptide and hippocampal neuronal death. *In vitro* studies suggest that this may be ameliorated by ketogenic diets, since β-OHB was shown to protect against the toxicity of $A\beta_{1-42}$ in cultured hippocampal neurons [123]. In PD, the degeneration of dopaminergic neurons leads to abnormalities of movement and cognition [2], and may be reproduced *in vivo* and *in vitro* by complex I inhibitors [124]. The ketone body β-OHB was shown to exert protection in mice treated with the parkinsonian toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which induces dopaminergic neurodegeneration by blocking mitochondrial complex I [28]. β-OHB is converted into acetoacetate, which is then used to turn over the TCA cycle, also resulting in increased levels of the intermediate succinate (Fig. 4). Succinate is oxidized by succinate dehydrogenase, which is part of mitochondrial complex II, and may therefore support oxygen consumption when complex I is blocked [28]. Thus, ketone bodies can be considered an alternative energy source in PD. In a study performed in SOD1-G93A transgenic ALS mice, that show death of motor neurons, ketogenic diets have been shown to improve motor neuron counts and prevent motor function loss [105].

In summary, there are a number of mechanisms to explain how ketogenic diets produce such positive effects on epileptic
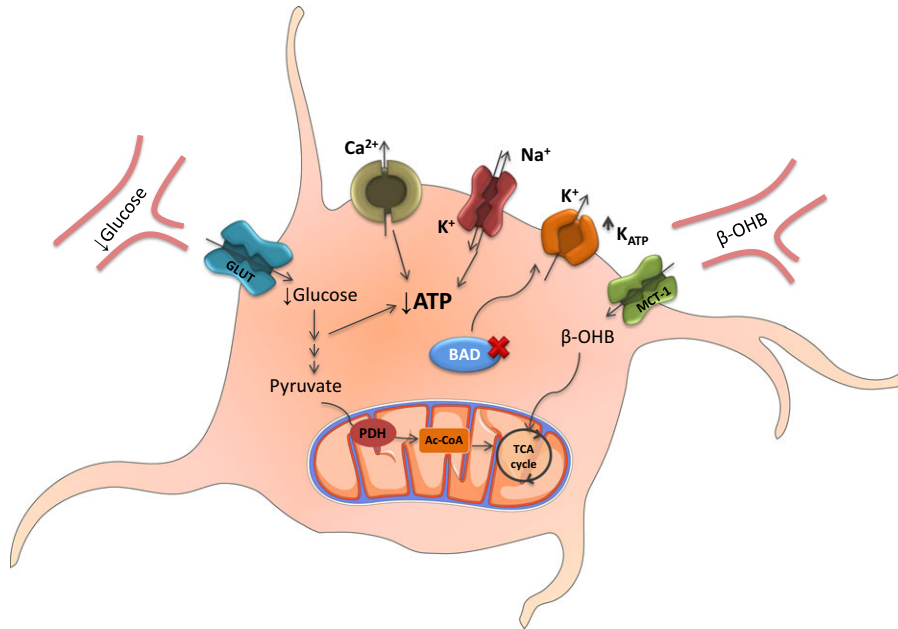
Figure 4 Mechanisms activated by ketogenic diets in the treatment of epilepsy. The administration of a ketogenic diet leads to the production of ketone bodies, such as β-hydroxybutyrate (β-OHB) that enters the cell through the monocarboxylate transporter (MCT). Due to the decrease in blood glucose and to the presence of ketone bodies, glycolytic ATP generation decreases, and ATP generation through mitochondria increases. Cellular ion pumps (Ca^{2+}, Na^+/K^+) maintain their function using ATP produced by glycolytic enzymes, leading to ATP depletion and allowing the increase in the activity of plasma membrane K-ATP channels. In the absence of intracellular ATP, K-ATP channels become active, generating a hyperpolarizing current that reduces cellular excitability. Activity of K-ATP channels is also modulated by Bcl-2-associated Agonist of Cell Death (Bad). When Bad phosphorylation status is compromised, an increase in K-ATP channel activity occurs.
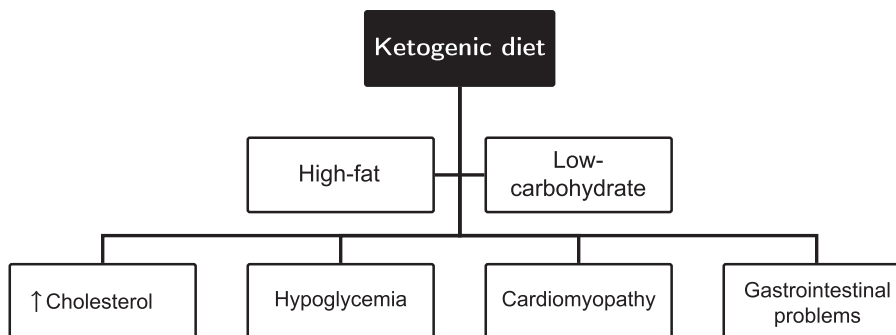

Figure 5 Adverse effects of ketogenic diets. Ketogenic diets are composed of high-fat, moderate protein and low-carbohydrate components, classically in a ratio of 4 : 1 (fat:protein+carbohydrates), which force the body to increase fat metabolism. This leads to an elevation of fat-derived ketone bodies and decreased glucose levels in the blood, resulting in metabolic alterations. These metabolic alterations can cause some undesirable adverse effects in short or long term. The former are ephemeral and easily manageable and may include gastrointestinal problems. The latter are more problematic and may encompass hypercholesterol, hypoglycaemia and cardiomyopathy. Nevertheless, it important to stress that these adverse effects hardly ever cause diet cessation.

patients, and a better understanding of each mechanism may lead to the development of agents that could bypass the use of ketogenic diets.

Not Always Good: The Risks of Ketogenic Diets

Although very helpful in a variety of pathologies, ketogenic diets also have short- and long-term adverse effects, which are easily distinguishable. Short-term side effects include gastro intestinal problems, such as gastro-oesophageal reflux and constipation, acidosis [19], hypoglycaemia [125], dehydration, and lethargy [126]. This group of effects are normally transient and easily managed [127]. On the other hand, long-term side effects include hyperlipidaemia (although with some controversy [6,7]), hypercholesterolaemia [19], nephrolithiasis [128] and cardiomyopathy [129] (Fig. 5). According to a review of 27 papers describing the adverse effects of the ketogenic diet [130], vomiting and increased serum lipid levels seem to be the most common. In a study including 52 epileptic children treated with the classic ketogenic diet, five patients experienced serious adverse effects [131]. These events, although not very frequent, can deter patients from complying with a long-term diet. Additionally, a diet high in cholesterol can lead to premature heart disease [132]. These findings show that although the diet may be useful in lessening seizure severity and occurrence, there are many adverse effects that need to be carefully monitored. Additionally, young patients must be monitored carefully to ensure they are receiving the appropriate nutrient balance. Often, patients find it difficult to maintain a diet within the restrictions of the classic ketogenic diet, with its 4 : 1 fat:carbohydrate ratio [133]. Although these adverse effects rarely lead to a cessation of the diet, they need to be recognized in due time [127].

Final Remarks

This review focused essentially on the impact of ketogenic diets on human health and how these types of diets can be applied as co-adjuvant therapies in several diseases. Due to their composition, ketogenic diets force the organism to use fat to obtain the energy. The term ketogenic comes from the ability of this type of diet to stimulate the production of ketone bodies by the liver, as a result of fatty acid beta-oxidation. Those ketone bodies are then released into the blood stream and can be used as a source of energy by other organs. Ketogenic diets have been suggested as a co-adjuvant therapy in cancer and neurological disorders. Since glucose is the main source of energy for cancer cells (the Warburg effect), a reduction in the availability of this fuel can be beneficial, controlling the proliferation and metastatic capacity. Also for some neurological disorders, ketogenic diets appear to be effective in the reduction of several symptoms, the best documented being the reduction of seizure frequency in epileptic patients. Changes in the metabolic pathways and

cellular signalling as well as increased mitochondrial biogenesis and improvement of mitochondrial function are some of the cellular effects observed after the adoption a ketogenic diet. However, the decision to adopt a ketogenic diet for mitochondrial diseases depends on the type of the disease. For example, ketogenic diets are not recommended for patients with fatty acid oxidation disturbances. Despite the well-documented advantage of ketogenic diets in the treatment of several diseases, adverse side effects should also be pointed out. In this review, the adverse effects of ketogenic diets were also discussed. In conclusion, depending on the situation and the extension of the disease, ketogenic diets can be a good option as a co-adjuvant therapy.

Conflict of interest
The authors declare no conflicts of interest.

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