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MINI-REVIEW

Effect of the ketogenic diet in excitable tissues

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Abstract

In the past decade, ketogenic diet (KD) has gained some popularity as a potential treatment for a wide range of diseases, including neurological and metabolic disorders, thanks to a beneficial role mainly related to its anti-inflammatory properties. The high-fat and carbohydrate-restricted regimen causes changes in the metabolism, leading, through the β-oxidation of fatty acids, to the hepatic production of ketone bodies (KBs), which are used by many extrahepatic tissues as energy fuels. Once synthetized, KBs are delivered through the systemic circulation to all the tissues of the organism, where they play pleiotropic roles acting directly and indirectly on various targets, and among them ion channels and neurotransmitters. Moreover, they can operate as signaling metabolites and epigenetic modulators. Therefore, it is inappropriate to consider that the KD regimen can improve the patients' clinical condition simply by means of specific and localized effects; rather, it is more correct to think that KBs affect the organism as a whole. In this review, we tried to summarize the recent knowledge of the effects of KBs on various tissues, with a particular attention on the excitable ones, namely the nervous system, heart, and muscles.

β-hydroxybutyrate; diet therapy; excitable tissues; ketogenic diet; low carbohydrate high fat diet

INTRODUCTION

The ketogenic diet (KD) is an unbalanced high-fat, restricted-protein, and low-carbohydrate diet that simulates a starvation condition and forces the metabolism to use lipids as primary energy source. In KD, the relative glucose deprivation and the elevation in circulating free-fatty acids (FFAs) result in the production of ketone bodies [KBs, β-hydroxybutyrate (βHB), acetoacetate (ACA), and, the least physiologically abundant, acetone] generated by the liver through FFAs β-oxidation, and therefore, KBs replace glucose as peripheral primary energy source. When the organism is in this metabolic condition, the few residual circulating carbohydrates are used by erythrocytes as they lack mitochondria and thus cannot use KBs. The interplay of KBs hepatic production and extrahepatic utilization sets their systemic levels (Fig. 1), and total circulating KB concentration in healthy adult humans normally exhibits circadian oscillations (Fig. 2). KB metabolism occurs principally in the mitochondria; in particular, the activity of the oxoacid CoA-transferase 1 is highest in heart and kidney, followed by skeletal muscle and brain. However, since skeletal muscle accounts for ~40% of body mass in adult humans, it is responsible for the highest fraction of total KB metabolism at rest (1).

CELLULAR TRANSPORT OF KBs

KBs are transported by monocarboxylate transporters (MCTs) that are membrane proteins with a core domain formed by 12 transmembrane segments and ubiquitously expressed in mammalians. MCTs catalyze the transport of short-chain monocarboxylates, usually carrying two or three keto groups (pyruvate, ACA) or hydroxyl groups (lactate, βHB), and because this transport is proton-coupled, they also have a role in the acid-base balance. MCT isoforms differ for substrates and inhibitors affinity, expression regulation, intracellular localization, and tissue distribution (2). MCT1 is expressed in the endothelial cells, ependymocytes, and astrocytes, MCT4 in astrocytes, and MCT2 almost exclusively in neurons (3). MCT1 and MCT4 have also been found in the heart and the skeletal muscle (2): MCT1 is highly represented in slow oxidative type I fibers (1), such as soleus, whereas muscles with a higher proportion of fast-twitch glycolytic fibers, such as the semimembranosus and semitendinosus, are enriched in MCT4 (3).

The majority of smooth muscle (SM), including the fibers of the gastrointestinal, female genital tracts, and blood vessels, express MCT1 (3), however, MCT3 was also identified in primary aortic SM cells and MCT4 in the vascular SM of the ocular tissues (3).

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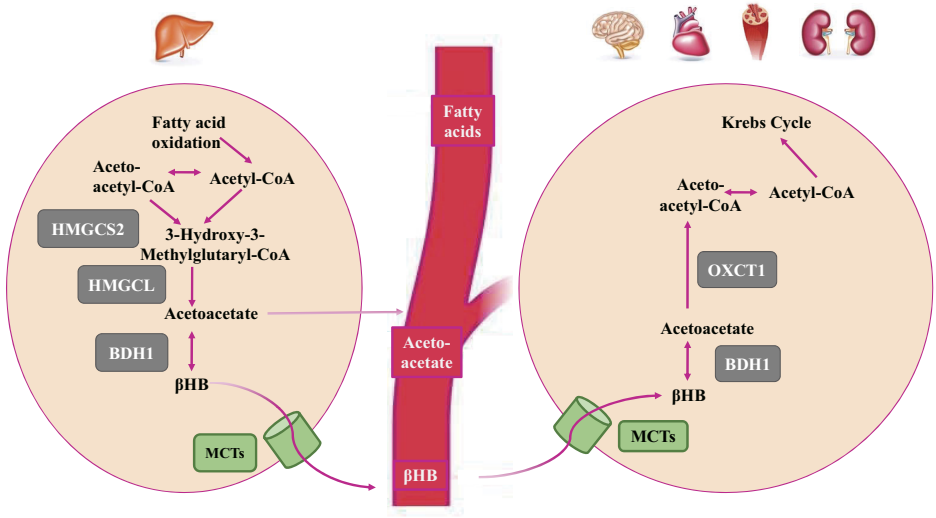

Figure 1. Overview of the metabolic pathways of ketone body (KB) metabolism in liver and in extrahepatic tissues. When fats and carbohydrates degradation are balanced, equal levels of acetyl-CoA and oxaloacetate (OAA) are produced and enter the Krebs cycle. When carbohydrates uptake is limited, OAA is used to produce glucose, thus is unavailable to react in the Krebs cycle with acetyl-CoA, that, in turn, is diverted to ACA and βHB production. KBs represent a transport form of acetyl-CoA, usable by any tissues having the machinery of ketolysis (conversion of KBs back to acetyl-CoA) and the ability to oxidize acetyl-CoA in the Krebs cycle (38). KB metabolism occurs principally in the mitochondria and is catalyzed by 3-hydroxybutyrate dehydrogenase type 1 (BDH1), acetyl-CoA acetyltransferase 1 (ACAT1), and 3-oxoacid CoA-transferase 1 (OXCT1). [Reprinted from Evans et al. (1) with permission from Wiley and Sons.]

Compared with FFA oxidation, KBs are more energetically efficient and therefore ensure a higher level of energy content to be used for ATP synthesis per molecule of oxygen invested (i.e., P/O ratio ~2,33 for FFAs and ~2,50 for KBs, where P/O represents the ATP produced per oxygen atom reduced) (2). Apart from serving as energy fuels for extrahepatic tissues, KBs also play pleiotropic roles acting as signaling metabolites or as epigenetic modulators (1).

THERAPEUTIC USE OF KD

The rationale behind the use of KD as a therapeutic option is that the human body already physiologically exploits KBs as an immediate and alternative fuel for brain, heart, and muscles during brief periods of fasting. The classic KD provides 90% fats, 6% proteins, and 4% carbohydrate (or eventually 80%, 15%, and 5%, respectively). However, due to its highly restrictive nature, patient's ease of producing KBs, and to possible side effects, other more moderate approaches suggest more conservative ratios of fats/proteins/carbohydrates as in the Modified Atkins Diet (MAD; 70/25/5), in the medium chain triglyceride (MCT)-based KD (70/10/20) or in the Low Glycemic Index (LGI)-based KD (45/28/27) (4).

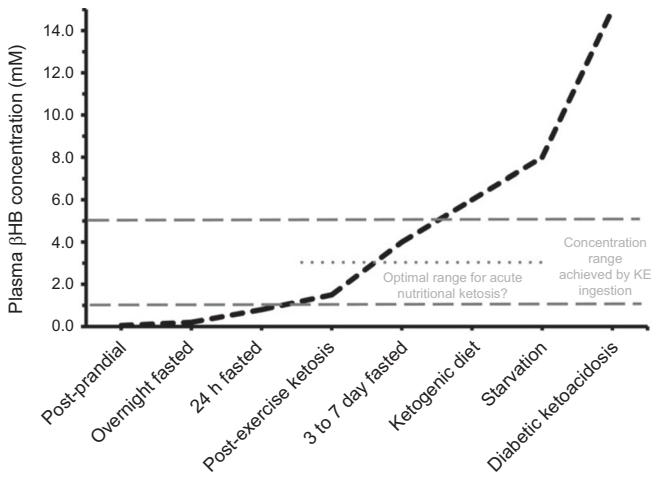

Figure 2. Plasma ketone body (KB) concentrations. Total circulating KB concentration in healthy adult humans normally exhibits circadian oscillations between approximately 100–250 μM, they increase to 1 mM after 24 h of fasting or prolonged exercise and elevate to 7–9 mM with prolonged fasting (17–24 days). Postexercise, the serum KBs can achieve concentrations of 1–2 mM and accumulate as high as 40 mM in pathological state. During KDs, their level exceeds 500 μM and can increase up to 5 mM. Ketonemia, an abnormal increase of circulating KBs, could degenerate in ketoacidosis if uncontrolled KBs production causes a dangerous dip in blood pH. [Reprinted from Evans et al. (1) with permission from Wiley and Sons.]

KD BLUNTS CELLULAR HYPEREXCITABILITY

KD decreases states of hyperexcitability by acting on various targets, such as ion channels, neurotransmitters, and the astrocyte-neuron lactate shuttle (5) (Fig. 3). Due to historical reasons, most of the studies on KD and hyperexcitability have focused on the central nervous system (CNS), however, since the molecular targets are also present in other excitable tissues, similar conclusions can be reasonably extended to these additional tissues too.

When the organism is in a KD regimen, the body's pH buffering capacity is transiently decreased due to the lower bicarbonate levels and this leads to significant acidosis. The extracellular acidic environment promotes the activity of acid sensitive ion channels (ASICs), with the subsequent activation of sodium and calcium inward currents causing calcium overload. However this action is counteracted by a direct inhibition exerted by KBs on the opening of ASICs, as it has been demonstrated in rat hippocampal neurons at pH 6.0, displaying a neuroprotective role and reducing neuronal excitability (6).

ATP-sensitive K^+ channels (K_{ATP}) are metabolic sensors modulated by the intracellular ATP/ADP ratio. In a ketogenic regimen, K_{ATP} opening probability increases because of a decrease in glycolytic ATP production due to a higher βHB concentration. Those researchers, who focus their attention on the KD-induced mitochondrial biogenesis and activity, thus supporting the idea of an increase in ATP cellular content, instead suggest that KBs favor the ATP cellular efflux via pannexins junctions and its subsequent degradation to adenosine, which, in turn, activates adenosine A1 receptors that increase K_{ATP} channels activity (5). In both the cases,

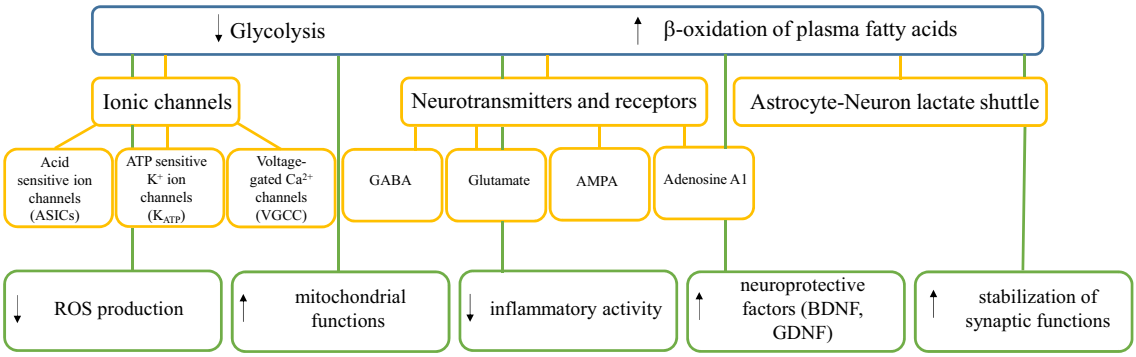

Figure 3. Schematic illustrations to tie the mechanisms by which ketogenic diet (KD) alleviates different disease symptoms. Metabolic effects of KD are shown in blue, the pathways involved in yellow, and the neuroprotective effects in green.

the increase in K_{ATP} function raises the resistance to seizures in vivo.

KBs modulate the voltage-gated Ca^{2+} channels: in particular, they inhibit the Ca^{2+} influx in CA1 pyramidal cells and reduce excitatory postsynaptic currents (EPSC). This action is particularly relevant when the brain is in a hyperexcitable state (7). In glial cells, instead, KBs cause elevations in cytosolic Ca^{2+} through L-type Ca^{2+} channels (8). N-type Ca^{2+} channels in sympathetic nerve terminals are coupled to the G-protein through the fatty acid receptor FFA3 that can be positively or negatively modulated by βHB yielding controversial results (8, 9).

M-current is a low voltage-activated potassium current mediated by the KCNQ channels. Its activation strongly antagonizes membrane depolarization and neuronal firing, whereas its suppression increases both. Medium chain fatty acids (MCFAs) present in a ketogenic regiment maintain this current as active, thus contributing to the control of the cellular excitability (3).

Chronic ketosis indirectly affects the Krebs cycle by lowering the level of the excitatory glutamate and increasing the synthesis of the inhibitory γ-aminobutyric acid (GABA). Glutamate is recovered by astrocytes from the synaptic space, it is converted to glutamine and as such it is exported to neurons, where it is transformed into GABA in a reaction that requires oxaloacetate (OAA). KD enhances OAA availability leading to an enhanced GABA synthesis (10). Other inhibitory neurotransmitters, such as adenosine, can be involved. In a mouse model of pharmacoresistant epilepsy, KD activates adenosine A1 receptors thus suppressing chronic seizures (5). KD also inhibits the synaptic excitation mediated by ionotropic glutamate receptors, including amino-3-hydroxy 5-methyl-4-isoxazole-propionate (AMPA) receptors. Indeed, decanoic acid (DA), a saturated FFA provided in MCT-KD, penetrates the blood-brain barrier and exerts a direct antagonism on AMPA receptors, which determines a reduction of the inward currents and thus of cellular excitability. At a concentration comparable with the one found in children exposed to the MCT diet, the DA reduces EPSCs in CA1 pyramidal neurons with a voltage-dependent effect, suggesting stronger inhibitory activity during prolonged seizures (11, 12).

As mitochondrial dysfunctions seem to be potentially pro-epileptic, the KD clinical benefits might also be associated with its effects on mitochondria. Indeed, in murine hippocampal neurons, the application of βHB or of DA promotes the activity of sirtuins, leading to the activation of mitochondrial respiratory chain enzymes. In vivo studies are needed to confirm this as a potential mechanism of action of KD (13).

Beyond the effects on ketones metabolism and on their circulating concentrations, the high-fat content of KD elevates FFAs levels in the body, including those of polyunsaturated FFAs (PUFAs). Although the cellular mechanisms are still unclear, KBs and PUFAs play a major role in the activation of pathways contributing to control excitability (14).

KD IN THE CNS

KBs become a source of energy for the CNS when their concentration achieves 4 mmol/L (15). KD forces the brain, a highly energy-dependent organ consuming at least 20% of the body's total caloric needs (16), to use KBs.

KD Application in Several Form of Epilepsy

From 1990s, KD has become an established therapy for refractory epilepsy due to its capability to significantly decrease seizures frequency therefore improving the patients quality of life (17). The anticonvulsant effect observed during MCT-KD may be explained by MCFAs indirect actions in preserving the M current, thus preventing aggravation of seizures (18).

In pediatric patients affected by Dravet syndrome and receiving KD treatment, a reduction of seizures frequency higher than 50% was observed in association with improved language ability, cognitive and motor functions, and behavior; only limited side effects were reported (19). This evidence thus suggests that KD might be a good therapeutic option. Interestingly, a recent study in developmental and epileptic encephalopathy pediatric patients demonstrated that the efficacy of KD in reducing seizures could differ based on which gene was causative of the disease (20). However, despite a further meta-analysis confirmed KD efficacy in drug-resistant epilepsy in children, some doubts arise in adults because of a lack of clear evidence (21).

Even though the majority of the studies support KD administration in epileptic patients, we should not disregard that KD may also affect cardiac electrical activity. Indeed, QTc prolongation was observed in children with drug-resistant epilepsy treated with KD, but the correlation involved also systemic acidosis, low serum bicarbonate, and selenium

deficiency (22, 23). Conversely, children with drug-resistant epilepsy receiving KD, controlled supplementation and stable serum KB concentration (4–5 mM) and revealed no alterations of the ECG indexes associated with cardiac repolarization. This evidence thus suggests that serum parameters as well as ECG recording (24, 25) should be constantly and carefully monitored in pediatric patients on KD . Furthermore, patients (and particularly adults) under long-term KD may develop a susceptibility to metabolic and cardiovascular disorders due to the increasing risk to develop insulin resistance (26) and alteration in thyrodic function (27). Finally, gastrointestinal adverse effects are common in epileptic children, especially during the first phases of KD administration; also, metabolic abnormalities and renal adverse effects have been reported (4).

KD Effects in Migraine, Autism, and Alzheimer's Disease

Similarly to epilepsy, KBs reduces cerebral excitability and influence migraine pathophysiology on several levels: for example, it probably reduces macrophages-induced inflammation during migraine attacks (28) and the oxidative stress caused by reactive oxygen species (ROS). Indeed, KBs enhance mitochondrial functions, reduce H_2O_2 production, and promote antioxidants and detoxification enzymes synthesis (29).

A recent 6-mo-long study on the autism spectrum disorder enrolled 45 children, equally divided into three groups. One group received a balanced nutrition whereas another received MAD; in the MAD groups 10 patients showed cognition and sociability improvements. Unfortunately, the dropout rate was high since five patients left the study due to poor compliance to the diet. The third group was treated with gluten-free and casein-free diet. These children showed a partial improvement of symptoms similarly to the MAD-group. It was thus concluded that both diet regimens may be good alternative therapeutic options (30). In a mouse model of autism, KD administration showed an antimicrobial effect and a compositional remodeling of the gut microbiome which has recently been recognized as an important element in brain health (31).

KBs act as neuroprotectors through several mechanisms in Alzheimer's disease (AD) as they reduce the glutamate concentration, stabilize the synaptic functions due to enhanced mitochondrial biogenesis, and alleviate the effects of impaired glucose metabolism due to mitochondrial dysfunction, inflammation, and oxidative stress. Furthermore, KBs reduce the deposition of amyloid plaques by reversing Aβ toxicity, as shown in vitro in rat hippocampal neurons, and ameliorate cognitive performance and memory (32). Moreover, βHB inhibits the NLR family pyrin domain containing 3 (NLRP3) inflammasome and reduces AD pathology in an AD mouse model (33). Metabolic ketosis decreases ROS production and improves mitochondrial respiration (34, 35). The neuroprotective anti-inflammatory activity of KD is exerted also by controlling the activation of the NF-kB and the expression of the iNOS (35) and by increasing the levels of neurotrophic factors (BDNF, GDNF) and of proteins that prevent aggregation of potential toxic polypeptides (15). In the clinical practice, the feasibility of the administration of this dietary regimen may be challenging in AD patients because their memory difficulties cannot guarantee compliance and the presence of disease symptoms, like disturbances in the senses of taste and smell and/or behavioral disorders, may prevent food consumption. Furthermore, energetic deficits may arise; indeed, KD may induce an insufficient protein supply, leading to catabolism of structural proteins and eventually worsening of sarcopenia, a common condition among elderly people (15).

KD IN THE PERIPHERAL NERVOUS SYSTEM

Similar to seizures, chronic pain is also thought to induce a general increase in neuronal excitability and KD display species-specific hypoalgesic effects as it prolongs thermal pain latency in juvenile and adult rats, but not in mice. This effect was unlikely due to a direct acute effect of KBs on molecular targets underlying thermal pain sensitivity, as at least 10 wk of ketogenic regimen were necessary for rats before showing hypoalgesia (36). Nevertheless, the presence of a direct KD/pain relationship is still controversial: some positive effects were observed on allodynia in a mouse model of neuropathic pain, but no benefits were reported in a chemotherapy-induced neuropathy model (36).

KD IN THE HEART

The heart has the highest energy expenditure and oxidative demand; at rest its metabolic rate exceeds 400 kcal/kg/day (33-fold higher than skeletal muscle, 14,5 kcal/kg/day, and 1.8-fold higher than the brain, 240 kcal/kg/day) (37) and its ATP turnover ranges between 6 kg/day and 35 kg/day, with 70% of energy supply originating from FFAs oxidation. Under physiological conditions, the heart is omnivorous and flexible: it oxidizes KBs in proportion to their delivery, at the expense of FFAs and glucose oxidation (38, 39).

Preliminary studies indicate that the combination of KBs with glucose elicits a higher cardiac efficiency relative to glucose alone (40). In addition, KBs seem to have cardioprotective effects in ischemia/reperfusion injury probably because they induce a reduction of myocardial oxidative stress. In murine models, a pre- or postischemia increase in KBs limits myocardial infarct size and apoptosis, improves postischemic recovery of contractile function, and decreases the duration of ventricular tachycardia during the early reperfusion (41).

KD Effects in Pathophysiological States Leading to Cardiac Dysfunction

According to a recent study (42), KD improves cardiac function and alleviates cardiac remodeling in diabetic mice with a significant diabetic cardiomyopathy. In particular, KD regulates mitochondrial morphology, number and size, prevents mitochondrial fission and improves their function, suppresses oxidative stress, attenuates cardiomyocytes apoptosis, increases left ventricular ejection fraction, and normalizes left ventricular internal dimension.

In cardiac diseases, such as heart failure (HF), the heart changes its preferences in fuel utilization. Plasma KBs concentration increases, probably as an adaptive process to supply energy for cardiac metabolism during stress, modifying the general myocardial metabolic state, and contributing to myocardial ROS decrease (39) and to mitochondrial quality

control (43). In the mouse model of HF and in the failing human heart, metabolomic profiles indicate a rise in KBs oxidation. Whether HF benefits from KD is still controversial: although ketosis can be considered a potential instrument to improve cardiac function, the perfusion of ACA decreases contractile function in rat heart (38, 44).

KD Effects in Pathophysiological States Altering Cardiac Electrical Activity

Even if acidosis promotes the activity of ASICs channels, hence in line with the risk of a QT prolongation, the blocking action by βHB on these channels and the activation of K_{ATP} should promote cardiomyocytes repolarization. Despite this reasoning, KD has a negative impact on long QT syndrome, probably due to heart metabolic changes: a woman with congenital asymptomatic LQT2 followed 3 wk of KD during which four severe cardiac episodes occurred, even if serum KBs were normal. When she resumed a regular diet, no fibrillation events occurred and the ECG showed a decreased QTc (45). Indeed, KD must be avoided in the presence of baseline ECG abnormalities as it might potentially raise the risk of malignant arrhythmias even during pregnancy, breastfeeding, and in elderly people (12, 15, 17, 46).

KD IN SKELETAL AND SMOOTH MUSCLE

Skeletal muscle has a high affinity to KBs but, because of their low circulating concentrations under normal conditions, the contribution to energy provision in muscle is less than 5% and FFAs are the main source (1). Therapeutic ketosis shows an overall anticatabolic potential in healthy skeletal muscle, especially during inflammation-related muscle atrophy (47). In a mouse model for late-onset mitochondrial myopathy with a mitochondrial respiratory chain dysfunction, long-term KD reduces the number of COX-negative fibers, a hallmark of muscular dysfunction, induces mitochondrial biogenesis, and rescues mitochondrial ultrastructure (48). Acute, subchronic, and chronic exposure to KD (and to ketone supplementation) also improves motor performance of rodents at various levels based on strain, specific ketone formulation, age, and exposure frequency/time after administration (49).

In physiological condition, the ability to use KBs is increased in exercise-trained skeletal muscle because of an increased ketolitic activity. However, ketolitic enzymes activity varies among the different types of skeletal muscle fibers. Moreover, KBs regulate metabolic processes and act as signaling metabolites to regulate gene expression (1). The metabolic and training status and the intensity of exercise affect KBs metabolism during exercise (1) but the KD regimen does not clearly ameliorates endurance exercise performance both in trained athletes and in obese individuals, although it facilitates weight loss. Moreover, KD is ineffective in increasing anaerobic performance in athletes (40).

SM cells use glucose as primary energy substrate, but they can also oxidize other substrates such as KBs. When vascular SM is incubated in a medium containing a mixture of substrates, the oxidation of one single substrate does not account for more than 8% of the total oxygen consumption. Thus, the oxidation of a particular substrate may parallel its concentration. Some recent results suggest that βHB may delay vascular aging by exerting antisenescence effects in vascular cells. Indeed, βHB suppress the expression of markers of senescence-associated phenotype (IL-6 and IL-1α) and increase the expression of Oct4, a regulator of pluripotency, in embryonic stem cells in human aortic SM cells exposed to a senescence-generating stimulus and in middle-aged mice (50).

SHOULD WE ALL FOLLOW A KETOGENIC REGIMEN?

KD is a well-established nonpharmacological treatment in neurological and metabolic disorders, however, its use is increasingly recommended also in several other diseases. KD therapy may induce an improvement of the clinical condition of patients by acting on common targets; for example, it reduces neuronal excitability in epilepsy, migraine, and chronic pain; decreases inflammation in migraine and AD; acts on mitochondrial dysfunctions in epilepsy, migraine, AD, diabetic cardiomyopathy, HF, and muscular dysfunctions; and ameliorates cellular oxidative stress in AD, ischemia/reperfusion, diabetic cardiomyopathy, and HF. However, its effectiveness is heterogeneous among the population, and this is likely also associated with its organoleptic attractiveness that may compromise long-term adherence of patients to dietary recommendations. The possibility to predict whether a patient might be a responder or a nonresponder to KD is an interesting goal to pursue to ensure a real improvement in the single patient's quality of life in a vision of a true personalized medicine. Based on current evidence, it seems reasonable to recommend a patient-to-patient tailoring made by an experienced physician that will prescribe a mandatory complete medical evaluation: physical examinations, liver, thyroid, kidneys, and heart functions together with serum chemistry panel to be performed as KD may induce metabolic, gastrointestinal, and renal side effects.

Though they are a natural fuel source that the body most likely exploited during eras when primal diets were customary, KBs represent a heterogenous and still relatively new and controversial phenomenon in research. Indeed, available studies involve small patient's cohorts and cover only short-term studies, and thus further research is needed before drawing definitive conclusions.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

C.M. and P.P. prepared figures; C.M., A.B., P.P., J.C.D., and I.R. drafted manuscript; A.B., M.B., and I.R. edited and revised manuscript; C.M., A.B., P.P., M.B., J.C.D., and I.R. approved final version of manuscript.

REFERENCES

1. **Evans M**, **Cogan KE**, **Egan B.** Metabolism of ketone bodies during exercise and training: physiological basis for exogenous supplementation. *J Physiol* 595: 2857–2871, 2017. doi:10.1113/JP273185.
2. **Halestrap AP**, **Wilson MC.** The monocarboxylate transporter family–role and regulation. *IUBMB Life* 64: 109–119, 2012. doi:10.1002/iub.572.
3. **Iwanaga T**, **Kishimoto A.** Cellular distributions of monocarboxylate transporters: a review. *Biomed Res* 36: 279–301, 2015. doi:10.2220/biomedres.36.279.
4. **Goswami JN**, **Sharma S.** Current perspectives on the role Of the ketogenic diet in epilepsy management. *Neuropsychiatr Dis Treat* 15: 3273–3285, 2019. doi:10.2147/NDT.S201862.
5. **Sada N**, **Inoue T.** Electrical control in neurons by the ketogenic diet. *Front Cell Neurosci* 12: 208, 2018. doi:10.3389/fncel.2018.00208.
6. **Zhu F**, **Shan W**, **Xu Q**, **Guo A**, **Wu J**, **Wang Q.** Ketone bodies inhibit the opening of acid-sensing ion channels (ASICs) in rat hippocampal excitatory neurons. *Front Neurol* 10: 155, 2019. doi:10.3389/fneur.2019.00155.
7. **Kadowaki A**, **Sada N**, **Juge N**, **Wakasa A**, **Moriyama Y**, **Inoue T.** Neuronal inhibition and seizure suppression by acetoacetate and its analog, 2-phenylbutyrate. *Epilepsia* 58: 845–857, 2017. doi:10.1111/epi.13718.
8. **Achanta LB**, **Rae CD.** β-Hydroxybutyrate in the brain: one molecule, multiple mechanisms. *Neurochem Res* 42: 35–49, 2017. doi:10.1007/s11064-016-2099-2.
9. **Won YJ**, **Lu VB**, **Puhl HL**, **Ikeda SR.** β-Hydroxybutyrate modulates N-type calcium channels in rat sympathetic neurons by acting as an agonist for the G-protein-coupled receptor FFA3. *J Neurosci* 33: 19314–19325, 2013. doi:10.1523/JNEUROSCI.3102-13.2013.
10. **Rogawski MA**, **Löscher W**, **Rho JM.** Mechanisms of action of antiseizure drugs and the ketogenic diet. *Cold Spring Harb Perspect Med* 6: a022780, 2016. doi:10.1101/cshperspect.a022780.
11. **Chang P**, **Augustin K**, **Boddum K**, **Williams S**, **Sun M**, **Terschak JA**, **Hardege JD**, **Chen PE**, **Walker MC**, **Williams RS.** Seizure control by decanoic acid through direct AMPA receptor inhibition. *Brain* 139: 431–443, 2016. doi:10.1093/brain/awv325.
12. **Warren EC**, **Walker MC**, **Williams RSB.** All you need is fats-for seizure control: using amoeba to advance epilepsy research. *Front Cell Neurosci* 12: 199, 2018. doi:10.3389/fnhum.2018.00199.
13. **Dabke P**, **Das AM.** Mechanism of action of ketogenic diet treatment: impact of decanoic acid and beta-hydroxybutyrate on sirtuins and energy metabolism in hippocampal murine neurons. *Nutrients* 12: 2379, 2020. doi:10.3390/nu12082379.
14. **Pifferi F**, **Tremblay S**, **Plourde M**, **Tremblay-Mercier J**, **Bentourkia M**, **Cunnane SC.** Ketones and brain function: possible link to polyunsaturated fatty acids and availability of a new brain PET tracer, 11C-acetoacetate. *Epilepsia* 49: 76–79, 2008. doi:10.1111/j.1528-1167.2008.01842.x.
15. **Włodarek D.** Role of ketogenic diets in neurodegenerative diseases (Alzheimer's Disease and Parkinson's Disease). *Nutrients* 11: 169, 2019. doi:10.3390/nu11010169.
16. **Raichle ME**, **Gusnard DA.** Appraising the brain's energy budget. *Proc Natl Acad Sci USA* 99: 10237–10239, 2002. doi:10.1073/pnas.172399499.
17. **Ułamek-Kozioł M**, **Czuczwar SJ**, **Januszewski S**, **Pluta R.** Ketogenic diet and epilepsy. *Nutrients* 11: 2510, 2019. doi:10.3390/nu11102510.
18. **Hoshi N.** M-current suppression, seizures and lipid metabolism: a potential link between neuronal Kv7 channel regulation and dietary therapies for epilepsy. *Front Physiol* 11: 513, 2020. doi:10.3389/fpls.2020.00513.
19. **Tian X**, **Chen J**, **Zhang J**, **Yang X**, **Zhang JT**, **Wu Y**, **Fang Y**, **Wu , FX.** The efficacy of ketogenic diet in 60 Chinese patients with Dravet Syndrome. *Front Neurol* 10: 625, 2019. doi:10.3389/fneur.2019.00625.
20. **Ko A**, **Jung DE**, **Kim SH**, **Kang HC**, **Lee JS**, **Lee ST**, **Choi JR**, **Kim HD.** The efficacy of ketogenic diet for specific genetic mutation in developmental and epileptic encephalopathy. *Front Neurol* 9: 530, 2018. doi:10.3389/fneur.2018.00530.
21. **Martin-McGill KJ**, **Bresnahan R**, **Levy RG**, **Cooper PN.** Ketogenic diets for drug-resistant epilepsy. *Cochrane Database Syst Rev* 6: CD001903, 2020.
22. **Aygün D**, **Aygün F**, **Nişli K**, **Baş F**, **Çıtak A.** Electrocardiographic changes in children with diabetic ketoacidosis and ketosis. *Turk Pediatri Ars* 52: 194–201, 2017. doi:10.5152/TurkPediatriArs.2017.4917.
23. **Bank IM**, **Shemie SD**, **Rosenblatt B**, **Bernard C**, **Mackie AS.** Sudden cardiac death in association with the ketogenic diet. *Pediatr Neurol* 39: 429–431, 2008. doi:10.1016/j.pediatrneurol.2008.08.013.
24. **Doksöz Ö**, **Çeleğen K**, **Güzel O**, **Yılmaz Û**, **Uysal U**, **İşgüder R**, **Çeleğen M**, **Meşe T.** The short-term effects of ketogenic diet on cardiac ventricular functions in epileptic children. *Pediatr Neurol* 53: 233–237.e1, 2015. doi:10.1016/j.pediatrneurol.2015.06.009.
25. **Özdemir R**, **Güzel O**, **Küçük M**, **Karadeniz C**, **Yılmaz Û**, **Calik T**, **Meşe T.** The impact of 3:1 ketogenic diet on cardiac repolarization changes in children with refractory seizures: a prospective follow-up study. *Neuropediatrics* 47: 157–161, 2016. doi:10.1055/s-0036-1582139.
26. **Kong J**, **Nam SO**, **Ko A**, **Byun SY**, **Lim TJ**, **Kim YM**, **Yeon GM**, **Cho JW**, **Lee YJ.** Longitudinal changes in insulin resistance in children with epilepsy on ketogenic diet: prevalence and risk factors. *Epilepsy Behav* 112: 107393, 2020. doi:10.1016/j.yebeh.2020.107393.
27. **Kose E**, **Guzel O**, **Demir K**, **Arslan N.** Changes of thyroid hormonal status in patients receiving ketogenic diet due to intractable epilepsy. *J Pediatr Endocrinol Metab* 30: 411–416, 2017. doi:10.1515/jpem-2016-0281.
28. **Gross EC**, **Klement RJ**, **Schoenen J**, **D'Agostino DP**, **Fischer D.** Potential protective mechanisms of ketone bodies in migraine prevention. *Nutrients* 11: 811, 2019. doi:10.3390/nu11040811.
29. **Milder J**, **Patel M.** Modulation of oxidative stress and mitochondrial function by the ketogenic diet. *Epilepsy Res* 100: 295–303, 2012. doi:10.1016/j.eplepsyres.2011.09.021.
30. **El-Rashidy O**, **El-Baz F**, **El-Gendy Y**, **Khalaf R**, **Reda D**, **Saad K.** Ketogenic diet versus gluten free casein free diet in autistic children: a case-control study. *Metab Brain Dis* 32: 1935–1941, 2017. doi:10.1007/s11011-017-0088-z.
31. **Paoli A**, **Mancin L**, **Bianco A**, **Thomas E**, **Mota JF**, **Piccini F.** Ketogenic diet and microbiota: friends or enemies? *Genes (Basel)* 10: 534, 2019. doi:10.3390/genes10070534.
32. **Hertz L**, **Chen Y**, **Waagepetersen HS.** Effects of ketone bodies in Alzheimer's disease in relation to neural hypometabolism, β-amyloid toxicity, and astrocyte function. *J Neurochem* 134: 7–20, 2015. doi:10.1111/jnc.13107.
33. **Shippy DC**, **Wilhelm C**, **Viharkumar PA**, **Raife TJ**, **Ulland TK.** β-Hydroxybutyrate inhibits inflammasome activation to attenuate Alzheimer's disease pathology. *J Neuroinflammation* 17: 280, 2020. doi:10.1186/s12974-020-01948-5.
34. **Lilamand M**, **Porte B**, **Cognat E**, **Hugon J**, **Mouton-Liger F**, **Paquet C.** Are ketogenic diets promising for Alzheimer's disease? A translational review. *Alzheimers Res Ther* 12: 42, 2020. doi:10.1186/s13195-020-00615-4.
35. **Rusek M**, **Pluta R**, **Ułamek-Kozioł M**, **Czuczwar SJ.** Ketogenic diet in Alzheimer's disease. *Int J Mol Sci* 20: 3892, 2019. doi:10.3390/ijms20163892.
36. **Masino SA**, **Ruskin DN.** Ketogenic diets and pain. *J Child Neurol* 28: 993–1001, 2013. doi:10.1177/0883073813487595.
37. **Javed F**, **He Q**, **Davidson LE**, **Thornton JC**, **Albu J**, **Boxt L**, **Krasnow N**, **Elia M**, **Kang P**, **Heshka S**, **Gallagher D.** Brain and high metabolic rate organ mass: contributions to resting energy expenditure beyond fat-free mass. *Am J Clin Nutr* 91: 907–912, 2010. doi:10.3945/ajcn.2009.28512.
38. **Abdul Kadir A**, **Clarke K**, **Evans RD.** Cardiac ketone body metabolism. *Biochim Biophys Acta Mol Basis Dis* 1866: 165739, 2020. doi:10.1016/j.bbadis.2020.165739.
39. **Ritterhoff J**, **Tian R.** Metabolism in cardiomyopathy: every substrate matters. *Cardiovasc Res* 113: 411–421, 2017. doi:10.1093/cvr/cvx017.
40. **Harvey KL**, **Holcomb LE**, **Kolwicz SC.** Ketogenic diets and exercise performance. *Nutrients* 11: 2296, 2019. doi:10.3390/nu11102296.
41. **Zuurbier CJ**, **Bertrand L**, **Beauloye CR**, **Andreadou I**, **Ruiz-Meana M**, **Jespersen NR**, **Kula-Alwar D**, **Prag HA**, **Eric Botker H**, **Dambrova M**, **Montessuit C**, **Kaambre T**, **Liepinsh E**, **Brookes PS**, **Krieg T.** Cardiac metabolism as a driver and therapeutic target of myocardial infarction. *J Cell Mol Med* 24: 5937–5954, 2020. doi:10.1111/jcmm.15180.
42. **Guo Y**, **Zhang C**, **Shang FF**, **Luo M**, **You Y**, **Zhai Q**, **Xia Y**, **Suxin L.** Ketogenic diet ameliorates cardiac dysfunction via balancing

mitochondrial dynamics and inhibiting apoptosis in type 2 diabetic mice. *Aging Dis* 11: 229–240, 2020. doi:10.14336/AD.2019.0510.

43. **Thai PN**, **Seidlmayer LK**, **Miller C**, **Ferrero M**, **Dorn GW**, **Schaefer S**, **Bers DM**, **Dedkova EN.** Mitochondrial quality control in aging and heart failure: influence of ketone bodies and mitofusin-stabilizing peptides. *Front Physiol* 10: 382, 2019. doi:10.3389/fphys.2019.00382.
44. **Horton JL**, **Davidson MT**, **Kurishima C**, **Vega RB**, **Powers JC**, **Matsuura TR**, **Petucci C**, **Lewandowski ED**, **Crawford PA**, **Muoio DM**, **Recchia FA Kelly DP.** The failing heart utilizes 3-hydroxybutyrate as a metabolic stress defense. *JCI Insight* 4: e124079, 2019. doi:10.1172/jci.insight.124079.
45. **Sudhakaran S**, **Yazdani L**, **Wheelan KR**, **Rao PK.** The ketogenic diet and the QT interval. *Proc (Bayl Univ Med Cent)* 33: 77–79, 2020. doi:10.1080/08998280.2019.1664220.
46. **Watanabe M**, **Tuccinardi D**, **Ernesti I**, **Basciani S**, **Mariani S**, **Genco A**, **Manfrini S**, **Lubrano C**, **Gnessi L.** Scientific evidence underlying contraindications to the ketogenic diet: an update. *Obes Rev* 21: e13053, 2020. doi:10.1111/obr.13053.
47. **Koutnik AP**, **D'Agostino DP**, **Egan B.** Anticatabolic effects of ketone bodies in skeletal muscle. *Trends Endocrinol Metab* 30: 227–229, 2019. doi:10.1016/j.tem.2019.01.006.
48. **Ahola-Erkkilä S**, **Carroll CJ**, **Peltola-Mjösund K**, **Tulkki V**, **Mattila I**, **Seppänen-Laakso T**, **Oresic M**, **Tyynismaa H**, **Suomalainen A.** Ketogenic diet slows down mitochondrial myopathy progression in mice. *Hum Mol Genet* 19: 1974–1984, 2010. doi:10.1093/hmg/ddq076.
49. **Ari C**, **Murdun C**, **Goldhagen C**, **Koutnik AP**, **Bharwani SR**, **Diamond DM**, **Kindy M**, **D'Agostino DP**, **Kovacs Z.** Exogenous ketone supplements improved motor performance in preclinical rodent models. *Nutrients* 12: 2459, 2020. doi:10.3390/nu12082459.
50. **Han YM**, **Bedarida T**, **Ding Y**, **Somba BK**, **Lu Q**, **Wang Q**, **Song P**, **Zou MH.** β-Hydroxybutyrate prevents vascular senescence through hnRNP A1-mediated upregulation of Oct4. *Mol Cell* 71: 1064–1078. e5, 2018. doi:10.1016/j.molcel.2018.07.036.