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KETOGENIC DIET IN NEURODEGENERATIVE AND NEUROMUSCOLAR DISEASES

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Abstract

The ketogenic diet is a low-carbohydrates and high-fat diet that has been used for years to treat cases of drug-resistant epilepsy. In recent years, several targets have been identified on which the KD actually acts with different mechanisms. The benefits of the ketogenic diet have been demonstrated in various metabolic disorders, such as diabetes, obesity, metabolic syndrome. Recently the neuroprotective effects of this diet have been increasingly evident, than the interest towards possible uses of the ketogenic diet in neurodegenerative and neuromuscular disorders, such as Alzheimer's disease, Parkinson's disease and Amyotrophic lateral sclerosis, is increasing.

Keywords: *Ketogenic diet, Alzheimer's disease, Parkinson's disease, Lateral amyotrophic sclerosis.*

Introduction

Ketogenic diet (KD) was born as treatment for patients with drug-resistant epilepsy. The aim of this review is to examine the literature with reference to current knowledge on the effects of KD on neuroprotection and on neuromuscular and neurodegenerative diseases.

Discussion

Since time immemorial fasting or deprivation / excess of some particular foods has been used as a "cure" in the state of illness. At the time of the Ancient Greeks epileptic seizures were considered a divine punishment and affected people were isolated from the rest of the community and forced into a life of hardship with periods of prolonged fasting that in some cases decreased the frequency of attacks. In the writing work "On the sacred disease" attributed to Hippocrates dating back to 400 BC, he denies the divine origin of epilepsy but indicates fasting as the only therapy [1]. In 1921 Woddyatt observed that in normal subjects, fasting or on a diet with a low carbohydrate intake, blood levels of acetone and β-hydroxybutyric acid increased [1]. In the same year Wilder, at the Mayo Clinic, subjected some epileptic patients to a diet high in fat and low in carbohydrates. Later he coined the term "Ketogenic Diet" [KD] [1]. Even today, this diet is considered a valid option for auxiliary treatment in childhood epilepsy.

This type of diet induces in the body a metabolic state very similar to that obtained in physiological conditions after a few days of fasting or after a drastic reduction of carbohydrates (under 20-50 g/day). The organism is forced to use other energy sources and ketone bodies are produced in the hepatic mitochondria. Ketogenesis is a metabolic process by which fatty acids are transformed into acetoacetate and β-hydroxybutyrate. Red blood cells (which do not contain mitochondria and depend on glycolysis), the brain and other glucose-dependent tissues still need glucose to function: the concentration of blood glucose, even in prolonged fasting, cannot drop below 2.2 mM/L [2]. The liver and kidney have the necessary enzymatic equipment to synthesize glucose; the liver is the major source of blood glucose after short periods of absorption: it is estimated that during the night fasting in humans the production of glucose varies from 122 to 420 g/day [3]. The precursors of gluconeogenesis are lactate, pyruvate, glycerol and glucogenic amino acids.

The physiological response of the organism to fasting varies with the continuation of this [4-9]. When blood glucose concentration is high, during the meal or in the postprandial period, the liver retains the glucose that is phosphorylated by the glucokinase and therefore cannot get out of the cells. The phosphorylation reaction_rate, being the glucokinase K_m high, increases with increasing concentration of blood glucose, when insulin secretion also increases. It is also stimulated glycogen synthase activity that leads to the formation of glycogen reserves (hepatic and muscular). On the other hand, glucose inhibits phosphorylase, the enzyme responsible for the degradation of glycogen. At the level of the adipose tissue the entry of glucose, stimulated by insulin, allows to obtain the glycerol 3-phosphate necessary for the storage of triglycerides; protein synthesis is stimulated in the muscle.

After several hours from the meal the blood glucose concentration begins to drop and increases the secretion of glucagon that stimulate, above all in the liver, the degradation of glycogen to produce the glucose that is poured into the blood to maintain adequate blood glucose [2]. When blood glucose drops, since the high availability of acetyl-CoA and citrate inhibits glycolysis, both the liver and the muscle preferentially use fatty acids released from adipose tissue allowing the blood glucose concentration to stay around 4.4 mM [2].

If fasting is prolonged, most of the energy can be provided by the mobilization of triglycerides from adipose tissue, but, since only the glycerol that derives from these can be converted in the liver to glucose (acetyl-CoA derived from fatty acids cannot be transformed into pyruvate) other sources of carbon skeletons are needed to synthesize glucose. Thus the glucogenic amino acids deriving from the degradation of proteins are used. The pool of fast-turnover proteins such as those in the intestinal

epithelium and those secreted by the pancreas is initially used [2-4].

The body, however, with the continuation of fasting, in order to reduce the consumption of body proteins (which unlike glucose and fatty acids have no body reserves), gradually reduces the use of amino acids and the extent of gluconeogenesis, resulting in greater use of ketone bodies by the brain [2,4]. The ketone bodies, unlike the fatty acids deriving from triglycerides, cross the blood-brain barrier (BEE) and do not require ATP for their oxidation. They are therefore a rapidly usable energy source.

In normal conditions and with a balanced diet the ketone bodies are produced in small quantities because the acetyl-CoA from which it derives is mainly used in the citric acid cycle to produce ATP. When glucose and insulin levels decrease, the availability of oxaloacetate (used for gluconeogenesis) also decreases: this is a critical factor for the entry of acetyl-CoA into the tricarboxylic acid cycle. Acetyl-CoA is accumulated and the liver removes it producing ketone bodies, but since the enzyme responsible for their mitochondrial oxidation is lacking in the liver, they are largely released into the blood to be used by peripheral tissues. Most ketone bodies are produced from the β-oxidation of fatty acids. Ketogenesis takes place in the mitochondria: a reversible enzyme, acetoacetyl-CoA thiolase converts two acetyl-CoA molecules into acetoacetyl-CoA, so the hepatic isoform of 3-hydroxymethylglutaryl-CoA synthase (HMGCS2) condenses an acetoacetyl-CoA molecule and one of acetyl-CoA in hydroxymethylglutaryl-CoA on which acts 3-hydroxy-3-methylglutaryl lyase (HMGCoA lyase) that cleaves it into acetoacetate (AcAc) and acetyl-CoA. AcAc can be reduced to D-β-hydroxybutyrate by the enzyme 3-hydroxybutyrate dehydrogenase type 1 (BDH1) that requires phosphatidylcholine as an allosteric activator. AcAc may spontaneously decarboxylate in acetone. Through membrane transporters the ketone bodies pass into the cytoplasm and then into the blood.

The blood levels of ketone bodies under physiological conditions rise rapidly from 0.100-0.250 mM/L in the postprandial period to 1-2 mM/L after 24 hours of fasting, but a plateau is reached with values between 6 and 10 mM/L after 2 weeks of fasting. Blood levels of bicarbonate are reduced but acidosis is compensated [7], contrary to what happens with diabetic ketoacidosis that is a potentially lethal pathological condition in which blood ketone bodies can reach or exceed 20 mM/L [10-11].

The oxidation of ketone bodies occurs mainly in the mitochondria in peripheral tissues. Here D-β-hydroxybutyrate is converted back to acetoacetate by the enzyme BDH1. AcAc is activated to acetoacetyl-CoA by the enzyme succinyl-CoA:3-ketoacid-CoA transferase (SCOT) by transferring CoA from a succinyl-CoA molecule. This is the key enzyme for mitochondrial oxidation of ketone bodies that is missing in the liver. Acetoacetate thiolase therefore produces 2 acetyl-CoA molecules that enter the tricarboxylic acid cycle to be oxidized.

The brain uses about 120 g/day of glucose on the first day of fasting. After about 3 days of fasting, about a quarter of the energy needed by the brain is obtained from ketone bodies (about 50 g/day): in these conditions the brain uses about 100 g / day of glucose. After about 40 days of fasting the consumption of glucose by the brain drops to around 40 g/day while that of ketone bodies rises to about 100 g/day [2,7]. However, excessive fasting inevitably leads to death due to the loss of function of liver, kidney or heart.

The ketogenic diet is characterized by high fat content, adequate protein intake and low carbohydrate content. In a diet of 2000 kcal per day the carbohydrates amount to 20-50 g/day: the low sugar content induces a metabolic state of "nutritional ketosis" considered safe for the body since the ketone bodies are produced in low concentrations, without changes in blood pH [11].

There are four main ketogenic diets [12, 13]. The classic Long Chain Triglyceride diet (LCT), in which fats can be obtained from standard foods, which vary in different countries and in different cultures and can be adapted to specific religious needs, food allergies, vegetarian habits, etc. [14]. Here we have a precise ratio between grams of fat and grams of

protein + carbohydrates: in the more rigorous one this ratio is 4 : 1 (fats make up 90% of daily calories), in the less rigid one the ratio can go down to 3:1 or 2:1. Another type is the Medium Chain Triglyceride (MCT) diet: the oils containing MCT give a greater yield in ketones than the LCT and allow to increase the amount of proteins and carbohydrates expanding the choice of foods; MCT diet is associated with a higher incidence of gastrointestinal effects such as nausea, diarrhoea, swelling and vomiting. The Modified Atkins Diet (MAD) is similar to classic KD but with fewer limitations in protein intake, and the Low Glycaemic Index Diet (LGID) are less rigorous dietary regimes. In the latter the carbohydrate intake reaches 40-60 g and allows to obtain a lower level of ketosis [12]. A ketogenic diet with high protein content has a high satiating power and reduces hunger [15].

In an effort to improve patient compliance by reducing the impact of these difficult dietary regimes, several researchers are evaluating the effects of administration of ketone body ester (KE), such as R, S-1,3-butanediol acetoacetate, 1,3-butenediol monoester of β-HB and glyceryl-tris-3-hydroxybutyrate (3GHB). Esters avoid blood electrolytes and blood pH alterations associated with the administration of ketone bodies as acids or salts. The objective is to obtain blood levels of ketone bodies comparable to those obtained with KD without the increase in blood lipids [16, 17].

The KD has had some success since the 1930_s of the last century, then with the introduction of antiepileptic drugs such as phenytoin into therapy, the interest of the scientific community has diminished. Since the 1990s, interest in this diet has resumed and a wide range of situations has been hypothesized in which it is possible to benefit from it. Today there are scientific evidence of neuroprotective properties and many data are available supporting KD use in different neurological and non-neurological disorders [18]. It is increasingly evident that ketone bodies play a pleiotropic role in the organism, not only having an energetic function [19] and multiple mechanisms have been proposed to explain the neuroprotective properties of KD, even if not completely clarified. The two cornerstones of KD are blood glucose reduction and ketonemia raising.

One of the proposed mechanisms is based on mitohormesis (mitochondrial hormesis)[20,21]: an excessive production of reactive oxygen species (ROS), as occurs in some mitochondrial dysfunctions, is involved in many chronic diseases (cardiovascular diseases, diabetes, etc.), on the other hand a moderate stimulus to mitochondrial ROS production is associated with a greater capacity to defend the cell against oxidative stress since in the cell an adaptive defence response is induced by different mechanisms. Mitochondria represent the main source of ROS (mtROS): normally small amounts of superoxide radical (O_2^-) are produced during oxidative phosphorylation, which is rapidly converted by manganese superoxide dismutase (SOD2) into H_2O_2 which, at least in part, diffuses outside the mitochondria [20]. Here in the presence of catalysts such as Fe^{++} (Fenton reaction) other highly reactive radicals are formed, such as $OH^{\cdot}$ ROS generate oxidative damage to proteins, lipids and DNA [20, 21]. At the neuronal level, O_2 levels can significantly increase due to calcium overload and mitochondrial damage resulting from glutamate excitotoxicity, which is responsible for many of the neurological deficits that follow stroke, head trauma, ischemia, etc.

Since KD, with a low carbohydrate content, implies a greater dependence on fat oxidation and mitochondrial respiration, in the mitochondria there is an increase in the flow of electrons through the transport chain (mtETC) and an increase in reverse electron transport (RET) which translates into greater production of mtROS. The mtROS induce the expression of the peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) that stimulates mitochondrial biogenesis and antioxidant adaptation. It increases the activity of enzymes such as superoxide dismutase (SOD) and there is an increase in uncoupling proteins (UCPs). Uncoupling dissipates the proton-motive force (Δp) that develops through the inner mitochondrial membrane and thus reduces the formation of mtROS. The initial increase in oxidative stress is then followed by its decrease and is also related to an increase in the activity of the nuclear factor

erythroid 2 (NFE2) -related factor 2 (NRF2) [20,22,23], which induces an increase in SOD, catalase, etc. The NRF2 transcription factor in unstressed conditions is kept in the cytoplasm by KEAP1 and Cullin 3 which degrade it by ubiquitination. Under oxidative stress NRF2 is not degraded but migrates into the nucleus where it forms a heterodimer with a smallMaf protein and binds to the antioxidant response element (ARE) in the upstream region of antioxidant genes and allows its transcription [23].

Paradoxically, administration of weak pro-oxidants for treatment and prevention of diseases seems to be more promising than administration of antioxidants (such as vitamin C or selenium) [22].

Another adaptation mechanism against oxidative stress is related to the signaling activity related to β-hydroxybutyrate (BHB). It inhibits the histone class I and II deacetylases [20][21] (a similar mechanism of action has been reported for valproic acid, a broad-spectrum antiepileptic,) with the result of a greater expression of forkhead box O (FOXO) 3a and metallothionein II and increase in FOXO3a, SOD2 and catalase levels [20].

BHB also has direct activity on a G protein-coupled receptor, GPR109A [19], which is also the Niacin (vit B3) target, a drug used for dyslipidemia. GPR109A is localized on neutrophils, macrophages and adipocytes. In the brain it is found mainly in the anterior cingulate cortex. BHB through this receptor exerts predominantly anti-inflammatory effects [19] which may explain part of the neuroprotective effects of KD. BHB via GPR109A blocks the inflammatory response mediated by NLRP3 inflammasome. However, BHB has proinflammatory effects in calf-hepatocytes [19]. Acetoacetate also has proinflammatory effects.

Numerous studies focus attention on KD-induced changes on the intestinal microbiota that can affect the gut-brain axis.

KD in Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disease in which there is a progressive deterioration of cognitive functions and memory, with alterations in behavior and personality. It is characterized by the extracellular deposition of beta amyloid (Aβ) and intracellular accumulation of tau protein. Aβ is the main component of senile plaques and tau is the component of neurofibrillary tangles. Less than 5% of cases are inherited and can occur before the age of 65. The vast majority of cases have sporadic onset in old age. In recent years, strong correlations are emerging between AD and a bioenergetic shift in which there is glucose hypometabolism, mitochondrial dysfunction and oxidative stress [24-26]. Several researches are highlighting a strong relationship between insulin resistance and AD.

Several studies have shown that the brain is an insulin-sensitive organ: insulin modifies neuronal activity by promoting synaptic plasticity, has neurotrophic and neuroprotective action and plays a role in cognitive processes. It passes through the BEE via a saturable transport system [25,27,30].

Individuals with AD show a decrease in glucose consumption in temporal, parietal and prefrontal cortex and in the posterior cingulate [27] that may be related to synaptic loss and neuronal death, but on the other hand, it begins to be observed many years before the onset of a measurable cognitive decline[27,30]. It could be the consequence of a decreased adrenergic stimulation of the damaged areas consequent to an early destruction of the noradrenergic cells of the locus coeruleus which stimulate, at least in the astrocytes, the glucidic metabolism [30]. Hypometabolism is related to glucose, then KB can improve brain energy deficit [27]. Furthermore in glucose-hypometabolism conditions, the brain itself implements a shift towards KDs use, also using myelin (white substance) as a source of fatty acids [28]: the degeneration of white matter is another sign of neurodegeneration associated whit AD. A cellular phospholipase A2 (cPLA2) - sphingomyelinase pathway has been proposed [28]. The amyloidogenic oligomers Aβ1-42 are themselves neurotoxic, and have also inhibitory action on the uptake of glucose by astrocytes [30]. As reported by Xiang et al [34] ketone bodies block the entry of Aβ1-42 into neurons. Reduction in the accumulation of Aβ1-42 in the cell, recovers the mitochondrial

complex I activity, reduces oxidative stress and increases synaptic plasticity.

Currently available treatments for AD (donepezil, rivastigmine, galantamine and memantine) are symptomatic and do not decelerate or prevent the progression of the disease. Regarding the effects of

KD, various studies have been carried out on cell cultures, on animal models and on humans.

Kashiwaya et al. [32] subjected 3xTgAD mice to a diet with ketone body esters. Behavioral test results at 3 and 7 months showed less anxiety in KD-treated mice compared to controls while immunohistochemical analyses showed a decrease in Aβ deposition in the subiculum, in the CA1 and CA3 regions of the hippocampus, and in the amygdala. Hyperphosphorylated Tau depositions also, in the same regions, were reduced. Van der Auwera et al. [31] in female transgenic mice carrying the "London" APP mutation (APP/V/717I) fed with a KD rich in saturated fats and low in carbohydrates, found, after 43 days, a reduction by approximatively 25% of Aβ deposits in brain. This despite the diet rich in saturated fats, considered predisposing to AD. Studzinki et al. [33] subjected aged dogs to a diet containing 2g / kg / day of medium chain triglycerides (MCTs) for 2 months. Older dogs represent a natural model of amyloidosis. Dogs treated with MCTs compared to controls showed a dramatically improved mitochondrial function, with an increase in active respiration rates. Of the studied lobes, the parietal, the frontal and the occipital, the best results were obtained in the parietal lobe. There was also a tendency towards decreasing Aβ levels. Kashiwaya et al [29] tested the effects of BHB on hyppocampal neurones in culture subjected to the neurotoxic action of Aβ1-42. The addition of BHB 4mM doubled cell survival compared to controls, increased cell size and neurite outgrowth, suggesting also a trophic function of ketone bodies. Reger et al. [36] tested the effects of BHB on 20 subjects with AD or mild cognitive impairment, who received a drink containing emulsified MTCs or a placebo. In this case only the APOE ε4 negative patients (without the apolipoprotein ε4 allele, which is the major known risk-factor gene for late-onset Alzheimer's disease) had cognitive improvements. Ota et al [35] studied the effects of a KD based on MCTs on a group of 20 Japanese subjects with mild-to-moderate AD. These, first, on separate days, underwent neurocognitive testing 120 min after consuming 50 g of a ketogenic formula containing 20 g of MCTs or placebo. Subsequently they were maintained with KD for up to 12 weeks. While in acute administration, despite the increase in ketonemia, there are no relevant effects in cognitive tests, positive effects have been shown with chronic administration of KD.

KD in Parkinson's disease

Parkinson's disease (PD) is a progressive degenerative nervous system disorder that affects movement. Symptoms start gradually: tremors are common, but the disorder also causes stiffness or slowing of movement. There are also mental and behavioral changes, sleep problems, depression, memory difficulties, and fatigue. PD symptoms worsen over time. The most significant neuropathological feature is a loss of dopaminergic neurons in the substantia nigra pars compacta, which leads to a reduction in dopamine levels in the striatum. PD is associated with the presence of Lewy bodies in the brain.

Advances in PD genetics have revealed a prominent role of mitochondrial dysfunction in the pathogenesis: the products of different genes associated with the pathology SNCA, Parkin, PINH1, dJ-1, LRRK2 and HTR2A under certain conditions show a degree of localization to mitochondria. However there is a pathophysiological heterogeneity within this disorder: mitochondrial dysfunction is not detected in all individuals with PD [37].

Several studies have been carried out on the effects of KD on PD. Tieu et al. [38] showed that BHB infusion in mice confers partial protection against dopaminergic neurodegeneration and motor deficit induced by MPTP. MTPT (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a prodrug to the neurotoxin MPP^+, which causes permanent symptoms of Parkinson's disease blocking complex I (NADH-ubiquinone oxidoreductase) of the mitochondrial electron transport chain. The effects of BHB are related to improved mitochondrial

respiration and ATP production mediated by a complex II-dependent mechanism. Kashiwawaya et al. [45] showed that the addition of 4 mM of Na D-β-hydroxybutyrate to mesencephalic cells in culture treated with MPP^+ increases their survival. VanItallien et al. [39] performed a feasibility study by subjecting seven patients with PD to a 4: 1 hyperketogenic diet (HKD) for 28 days. Saturated fats were used to prevent the increase in LDL. Five of the volunteers completed the 28-day study and in 4 of them actually there was no increase in LDL. Unified Parkinson's Disease Rating Score improved in all five during hyperketonemia, but no placebo controls were performed. Phillips et al. [40] compared a low-fat diet with ketogenic diet for 8 weeks into a hospital clinic of PD patients. Both diet group significantly improved in motor and nonmotor symptoms, but the ketogenic group showed greater improvements ibn nonmotor symptoms. Adverse effect were generally mild.

KD in Amyotrophic lateral sclerosis (ALS)

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder of motor neurons. Early symptoms of ALS usually include muscle weakness or stiffness. Most people with ALS die from respiratory failure, usually within 3 to 5 years. The mechanisms involved in the pathogenesis of ALS include glutamate neurotoxicity, oxidative stress, glial cell disorders, axonal transport, and mitochondrial dysfunction. Riluzole is the only drug to prolong survival in amyotrophic lateral sclerosis, so there is the need for new effective therapies. In ALS an adequate body mass index (BMI) is fundamental [41]: in fact, caloric reduction induces a more rapid onset of clinical symptoms and reduces lifespan.

Transgenic mice with mutations of the copper-zinc superoxide dismutase (SOD1) gene are experimental ALS models. The first study on the use of KD in ALS was conducted by Zhao Z. et al. [42] using SOD-1G93A transgenic mice. Blood ketone levels in mice fed with KD were > 3.5 higher than controls and these mice lost 50% of baseline motor neurons 25 days later than disease control. The authors also demonstrated that BHB prevents rotenone-mediated inhibition of the mitochondrial Complex I [42]. Zhao W. et al. [43] tested caprylic triglyceride, a medium chain triglyceride, as a source of ketone bodies, to increase performance and alleviate symptoms in transgenic mice SOD1-G93A. The food ration consisted of 10% (w/w) of caprylic triglyceride (caloric composition: fat 34%, carbohydrates 46%, proteins 20%) while the controls were subjected to an isocaloric diet. The progression of the weakness was attenuated and there was a reduction in the loss of neuronal motion in spinal cord, with an improvement in performance but without important benefits on survival.

Conclusions

The studies reported above show that there are promising data supporting a possible use of KD in various neuromuscular and neurodegenerative pathologies. However, contrary to what happened for epilepsy, there are still few clinical data available. Randomized controlled trials should be performed to clearly assess positive effects and side effects.

References

1. Wheless, J.W. (2008). History of the ketogenic diet. Epilepsia, 49 (sup. 8),3-5.
2. Berg, J.M., Tymoczko, J.L., Gatto jr, G.J., Stryer,L. (2015). Biochemistry, 8th ed, 816-820.
3. Owen, O.E., Felig, P., Morgan, A.P., Wahren, J., Cahill Jr., G.F. (1969). Liver and kidney metabolism during prolonged starvation. The journal of clinical investigation, 48, 574-583.
4. Felig, P., Owen, O.E., Wahren, J., Cahill Jr, G.F. (1969). Amino acid metabolism during prolonged starvation. The journal of clinical investiogation, 48, 584-592.
5. London, E.D., Margolin, R.A., …, Rapoport, S.I. (1986). Effect of fasting on ketone body concentrations in healty men of different ages. Journal of gerontology, 41 (5),599-604.
6. Cahill Jr., G.F., Owen, O.E., Morgan, A.P. (1968) The consumption of fuels during prolonged starvation. Advances in enzyme regulation, 6, 143-150.

7. Cahill Jr., G.F. (1983). President's address. Starvation. Transactions of The American Clinical and Climatological Association, 94, 1–21.
8. Reichard Jr., G.A., Owen, O.E., Haff, A.C., Paul, P., Bortz, W.M. (1974). Ketone-body production and oxidation in fasting obese humans. The journal of clinical investigation, 53, 508-515.
9. Garber, A.J., Menzel, P.H., Boden, G, Owen, O.E. (1974). Hepatic ketogenesis and gluconeogenesis in Humans. Journal of clinical investigation, 54, 981-989.
10. Masood, W., Annamaraju, P., & Uppaluri, K. R. (2020). Ketogenic Diet. In StatPearls
11. Cahill Jr., G.F., Veech, R.L. (2003). Ketoacids? Good medicine? Transactions of the American Clinical and Climatological Association, 114,149–163.
12. Kossoff, E.H., Zupec-Kania, B.A., …, Wirrel, E.C. (2018). Optimal clinical management of children receiving dietary therapies for epilepsy: Updated recommendations of the International Ketogenic Diet Study Group. Epilepsia Open, 3 (2), 175-192.
13. Liu, Y.C., Wang, H.S. (2013). Medium chain triglyceride ketogenic diet. An effective treatrment for drug-restant epilepsy and a comparison with other ketogenics diets. Biomed Journal, 36, 9-15.
14. Kossoff, E.H., Wang, H.S. (2013). Dietary Therapies for epilepsy. Biomed Journal, 36, 2-8.
15. Westerterp-Plantenga, M.S., Nieuwenhuizen, A., Tomé, D., Soenen, S., Westerterp, K.R. (2009). Dietary protein, weigth loss and weigth maintenance. The annual review of nutrition, 29, 21-41.
16. Hashim, S.A., VanItallie, T.B. (2014). Ketone body therapy: from the ketogenic diet to the oral administration of ketone ester. Journal of lipid research, 55, 1818-1826.
17. Veech, R.L. (2004). Ketone ester effects on metabolism and trascription. Journal of lipid research, 55, 2004-2006.
18. Stafstrom, C.E., Rho, J.M. (2012). The ketogenic diet as a treatment paradigm for diverse neurological disorders. Frontiers in pharmacology, 3, 1-8.
19. Puchalska, P., Crawford, P.A. (2017). Multi-dimensional roles of ketone bodies in fuel metabolism, signalling, and therapeutics. Cell metabolism, 25, 262-284.
20. Miller, V. J., Villamena, F. A., & Volek, J. S. (2018). Nutritional ketosis and mitohormesis: potential implications for mitochondrial function and human health. Journal of nutrition and metabolism, 2018.
21. Scialò F., Fernandez-Ayala, D.J., Sanz, A. (2017). Role of mitochondrial reverse electron transport in ROS signalling: potential roles in health and disease. Frontiers in physiology, 8 (428), 1-7.
22. Valerio, A., Nisoli, E. (2015). Nitric oxide, interorganelle communication, and energy flow: a novel route to slow aging. Frontiers in Cells and developmental biology, 3 (6), 1-11.
23. Ma, Q., (2013). Role of Nrf2 min oxidative stress and toxicity Annu Rev Pharmacol Toxicol., 53 401–426.
24. Neth, B.J., Craft, S. (2017). Insulin resistance and Alzheimer's disease: bioenergetic linkages. Aging Neuroscience, 9 (345), 1-20.
25. Ferreira, L.S.S., Fernandes, C.S. Vieira, M.N.N., De Felice, F. G. (2018). Insulin resistance in Alzheimer's disease. Frontiers in neuroscience, 12 (830),1-11.
26. Mosconi, L., Pupi, A., De Leon, M.J. (2008). Brain glucose hypometabolism and oxidative stress in preclinical Alzheimer's disease. Ann N Y Acad Sci., 1147, 180–195.
27. Lange, K.W., Lange, K.M., Makulska-Gertruda, E., Nakamura, Y., …, Kanaya, S., Hauser, J. (2017). Ketogenic diets and Alzheimer's disease. Food Science and Human Wellness, 6 (1), 1-9.
28. Klosinski, L.P., Yao, J., Yin, F., …, Diaz Brinton, R. (2015). White metter lipids as a ketogenic fuel supply in aging female brain: implicazione for Alzheimer's disease. EBioMedicine, 2, 1888-1904.
29. Kashiwaya, Y., Takeshima, T., Mori, N., …, Veech, R.L. (2000). D-β-hydroxybutyrate protects neurons in models of Alzheimer's

and Parkinson's disease. Pnas, 97 (10), 5440-5444.
30. Hertz, L., Chen, Y., Waagepetersen, H.S. (2015). Effects of ketone bodies in Alzheimer's disease relation to neural Hypometabolism, β-amyloid toxicity, and astrocyte function. Journal of biochemistry, 134, 7-20.
31. Van der Auwera, I., wera, S., Van Leuven, F., Henderson, S.T. (2005). A ketogenic diet reduces amyloid beta 40 and 42 in a mouse model of Alzheimer's disease. Nutrition e metabolism, 2 (28), 1-8.
32. Kashiwaya, Y., Bergman, C., Lee, J.H., …, Veech, R.L. (2013). A ketone ester diet exhibits anxiolytic and cognition-sparing properties, and lessens amyloid and tau pathologies in a mouse model of Alzheimer's disease. Neurobiol Aging, 34 (6), 1530-1539.
33. Studzinski, C.M., MacKay, W.A., …, McIntyre Burnham, W. (2008). Induction of ketosis may improve mitochondrial function and decrease steady-state amyloid-β-precursor protein (APP) levels in the aged dog. Brain research, 1226, 209-217.
34. Yin, J.X, Maalouf, M., Han, P., …, Shi, J. (2016). Ketones block amyloid entry and improve cognition in an Alzheimer's model. Neurobiology of aging, 39, 25-37.
35. Ota, M., Matsuo, J., Ishida, I., …, Kunugi, H. (2019). Effects of a medium chain triglyceride-based ketogenic formula on cognitive function in patients with mild-to-moderate Alzheimer's disease. Neuroscience letters, 690, 232-236.
36. Reger, M.A., Henderson, S.T., …, Craft, S. (2004). Effects of β-hydroxybutyrate on cognition in memory-impaired adults. Neurobiology of aging, 25, 311-314.
37. Henchcliffe, C., Flint Beal, M. (2008). Mitochondrial biology and oxidative stress in Parkinson's disease pathogenesis. Nature clinical practice Neurology, 4 (11), 600-609.
38. Tieu, K., Perier, C., Caspersen, C., …, Przebdorski, S. (2003). D-β-hydroxybutyrate rescues mitochondrial respiration and mitigates features of Parkinson's disease. The journal of clinical investigation,112 (6), 892-901.
39. VanItallie, T.B., Nonas, C., Di Rocco, A., …, Heymsfield, S.B. (2004). Treatment of Parkinson's disease with diet-induced hyperketonemia. A feasibility study. Neurology, 64, 728-730.
40. Phillips, M.C.L., Murtagh, D.K.J., …, Lynch, C.D.P. (2018). Low-fat versus ketogenic diet in Parkinson's disease: a pilot randomized trial. Movement disorders, 33 (8), 1306-1314.
41. Ngo, S.T., Steyn, F.J., McCombe, P.A. (2014). Body mass index and dietary intervention: implications for prognosis of Amyotrophic Lateral Sclerosis. Journal of the neurological sciences, 340, 5-11.
42. Zhao, Z., Lange, D.J., Voustianiouk, A., …, Pasinetti, G.M. (2006). A ketogenic diet as a potential novel therapeutic intervention in Amyotrophic Lateral Sclerosis. BMC Neuroscience, 7 (29), 1-10.
43. Zhao, W., Varghese, M., Vempati, P., …, Pasinetti, G.M. (2012). Caprylic triglyceride as a novel therapeutic approach to effectively improve the performance and attenuate the symptoms due to the motor neuron loss in ALS disease. Plos one, 7 (11), 1-8.