PRACA POGLĄDOWA

Characteristics of ketogenic diet and its therapeutic properties in central nervous system disorders

Charakterystyka diety ketogennej i jej właściwości terapeutycznych w chorobach centralnego układu nerwowego

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ABSTRACT

A fat-rich and low-carbohydrate ketogenic diet has been successfully used in epilepsy treatment in children and adults for many years. Lately, advances have been made in the use of ketogenics as therapy for other disorders such the tuberous sclerosis complex, brain tumors and neurodegenerative diseases: Alzheimer's disease and Parkinson's disease. Many studies have also shown its neuroprotective abilities. This neuroprotection is connected with the molecular mechanisms of a ketogenic diet and ketone metabolism. This review shows how a ketogenic diet induces ketosis, how it works and how the molecular mechanisms of a ketogenic diet may be used in the therapy of central nervous system disorders.

KEY WORDS

ketogenic diet, ketone bodies, neurodegenerative disorders, neuroprotection

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STRESZCZENIE

Wysokotłuszczowa, niskowęglowodanowa dieta ketogenna jest terapią, która od wielu lat z sukcesem jest stosowana u dzieci i dorosłych w leczeniu epilepsji. Późniejsze badania pozwoliły na rozszerzenie poszukiwań jej terapeutycznego zastosowania o stwardnienie guzowate, guzy mózgu i schorzenia neurodegeneracyjne, jak choroba Alzheimera i choroba Parkinsona. Wiele badań wykazało również neuroprotekcyjne właściwości tej terapii. Indukowanie neuroprotekcji jest związane z molekularnymi mechanizmami działania diety ketogennej i metabolizmem ciał ketonowych. Niniejsza praca opisuje działanie diety ketogennej oraz jej mechanizmy molekularne, które mogą być wykorzystywane w terapii chorób centralnego układu nerwowego.

INTRODUCTION

Under homeostatic unsettled conditions, the human body seeks alternative ways of action. When the body has a reduced level of products derived from glucose metabolism, which is accompanied by a poor binding of acetylo-CoA to the tricarboxylic acid (TCA) cycle, the organism switches from its normal glucose metabolism to an alternative energy source [1]. Ketone bodies (KB), like acetoacetate, are an alternative energy source of substrates for the TCA cycle mainly in the brain. KB act as a metabolic fuel because their oxidation is accompanied by an acetyl-CoA release [2]. This feature is the background of the ketogenic diet.

CHARACTERISTICS OF KETOGENIC DIET

A ketogenic diet depends mostly on the limitation of carbohydrate intake along with high fat consumption and normal protein delivery. The ratio of fat (80%) to carbohydrates (5%) and proteins (15%) is approximately $4 : 1$ ($3 : 1; 2 : 1$ alternatively) and depends on the symptoms produced and expected by this diet. The main fat sources are long chain triglycerides (classic diet by Widler in 1921) and medium chain triglycerides (latter modification in 1971 by Huttenlocher). A medium chain diet leads to higher ketosis, but also shows more severe side effects (e.g. stomach ache). However, a combination of a traditional and medium-chain triglyceride diet was similarly effective in antiepileptic therapy as the classic one [3].

Under KD treatment, the organism switches its energy metabolism in a similar way to a starvation state. Thus KD therapy often starts with fasting which facilitates lipolysis by reducing the insulin-to-glucagon ratio. A higher glucagon level leads to the mobilization of glucose from the glycogen stores. After two, three days of glucose deprivation, liver glycogenolysis ends and an adequate level of blood glucose is maintained by means of gluconeogenesis using amino acids from muscles [4]. Oxaloacetate is one of the gluconeogenesis reaction compounds originating from pyruvate. Oxaloacetate and pyruvate (during glucose deprivation) are obtained from the export of alanine and glutamic acid from muscle [5].

A critical moment in the adaptation of the organism during the late phase of starvation is an increase in lipolysis in fatty tissue and the delivery of fatty acids to the liver. Thus, in the central nervous system, ketone bodies derived from fats are the most important energy substrates instead of glicolytic products of glucose. Along with the increase in KB levels in the blood (which is characteristic for KD and starvation state), their metabolization substitutes the process of glucose acquisition from muscle proteins, which inhibits muscle proteolysis [6].

Freeman describes a different duration of fasting applied over the years as an initial phase of KD therapy. Initially, the diet was applied after a 10% lost of the patient's body weight and reaching a ketone concentration of 160 mg/dL (27.5 mM) in the urine. From 1960 to 1990, 48-hour fasting preceded KD therapy. In present times, this period is shortened to 24 hours. Some teams, however, neglect fasting as an initial phase of KD [7,8].

The use of fasting before the beginning of the diet is not necessary to develop ketosis, and both types of diets (KD with and KD without fasting), produce ketosis within five days, however, after initial fasting, ketosis occurs earlier. Children who had KD applied without prior fasting, showed less susceptibility to weight loss and appearance of hypoglycemia with a lower risk of acidosis and dehydration [9].

During KD therapy, more attention should be paid to the control of β-hydroxybutyrate concentration in the serum than to ketone levels in the urine, because the KB level in urine poorly correlates with the amount of KB in the blood [10], and there is also a better (inversely proportional) correlation between the number of seizures and KB levels in the blood than in urine [11]. Even so, the amount of KB is elevated both in the blood and in urine [9]. It was reported that a 3–5 mM β-hydroxybutyrate blood level is sufficient to obtain satisfactory results of KD therapy [12]. The blood ketone content is measured as the concentration of β-hydroxybutyrate in the serum, which under physiological conditions is approximately 0.05 mM, and rises to about 0.4 mM after awakening (night fasting).

One-to-two-day fasting leads to a mild ketosis of 1–2 mM. Prolonged starvation leads to 6–8 mM blood KB levels [13].

By providing large amounts of fats or proteins, while limiting the amount of carbohydrates, the ketogenic diet is associated with a significant increase in the level of ketones in the body [14], leading to benign ketosis [8] and to a minimization of glucose metabolism because of the use of ketones (acetone, acetoacetate, β-hydroxybutyrate) as an alternative energy source [15]. The natural ability of the body to modify its energetic metabolism is used in this process.

BIOCHEMICAL ASPECTS OF KETOSIS STATE INDUCTION UNDER KD

Known changes in the cellular metabolism of the body due to the use of a ketogenic diet concern the pathways of energy production. Apart from glycolysis (due to limited availability of glucose, e.g. low carbohydrate diet), KD initiates its operation from the TCA cycle. Fatty acids, which are supplied in abundance during KD, are converted to acetyl-CoA in β-oxidation reactions (Fig. 1). CoA (coenzyme A) molecules bind fatty acids devoted to energetic transformation. The resulting acyl-CoA is a metabolically active form of fatty acids and can be next used in the cell. In the process of β-oxidation occurring in the mitochondria, two carbon residues connected with CoA are released from acyl-CoA which results in the production of acetyl-CoA. In this form, fatty

Fig. 1. Involvement of different energetic substrates in TCA cycle. Description of scheme is in the text.

Ryc. 1. Miejsce włączania substratów energetycznych do cyklu Krebsa. Opis do schematu znajduje się w tekście.

acids can be used as a source of energy in the TCA cycle [16].

Acetyl-CoA formed during the oxidation of fatty acids is incorporated into the TCA cycle if the degradation of fats and carbohydrates is balanced. This is because the incorporation of acetyl-CoA into the TCA cycle depends on the availability of oxaloacetate, necessary for binding acetyl-CoA. In hepatocytes, the oxaloacetate concentration decreases when carbohydrates are unavailable or improperly used (e.g. in diabetics, fasting people or KD), and it is then that the cell content of oxaloacetate is used for gluconeogenesis. Due to the lack of oxaloacetate, the TCA cycle decreases its productivity which leads to an accumulation of acetyl-CoA [2]. The intensification of fatty acid oxidation and oxaloacetate deprivation leads to a high production of ketones, formed in the liver [1].

Two molecules of acetyl-CoA condense with each other giving acetoacetylo-CoA, which gives rise to acetoacetic acid. Acetoacetic acid undergoes spontaneous decarboxylation to acetone or converts into β-hydroxybutyrate, which is oxidized by one molecule of NADH to give NAD^+. Acetoacetic acid and β-hydroxybutyrate are mutually transformed into one another [2]. The ratio of β-hydroxybutyrate to acetoacetic acid depends on the ratio of NADH / NAD^+ inside the mitochondria [17]. Ketones are not oxidized in the liver, but are released from the liver and consumed by the brain, skeletal muscles and the kidneys [18]. Ketones are transported through the body mostly in the form of β-hydroxybutyrate, which is more stable than acetoacetic acid. As a result of β-hydroxybutyrate oxidation with the NADH formation in tissues outside the liver, a metabolically active acetoacetic acid is formed, where as the acetyl-CoA-ketone body is used as a source of energy [19]. Acetoacetic acid reacts with succinyl-CoA (Fig. 1), thereby forming free succinic acid and acetoacetylo succinate-CoA, which is then degraded to acetyl-CoA and oxidized in the TCA cycle [20].

Ketone bodies are also formed in the process of deamination and oxidation of amino acids, where keto acids are formed such as pyruvic acid, the acetyl-CoA precursor. This gives additional ketogenic properties to the high-protein low-carbohydrate diet, during which the concentration of β-hydroxybutyrate in plasma after 65 days was 1.52 mM (before starting the diet it was at 0.2 mM) [14].
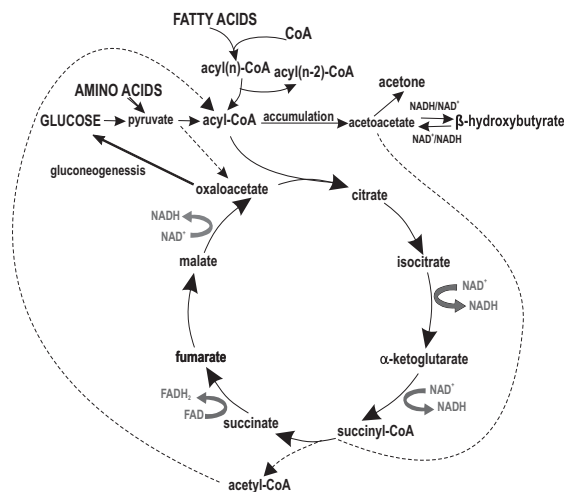
KETOGENIC DIET IN USE

The interruption of ketosis occurs easily after incidental and unplanned carbohydrate intake [21]. Insulin levels increase after the accidental administration of larger amounts of carbohydrates during KD and the achievement of ketosis becomes impossible. In this scenario, fasting can be reinitiated to restore ketosis. Food ingredients that may be included in the diet are: sour cream, bacon, eggs, mayonnaise, tuna, shrimp and vegetables, cheese and fatty meat [22]. Foods rich in simple and refined sugars must be excluded from the diet. At present, food preparations (KetoCal, Ross CarbohydrateFree) to be used in KD are readily available, and preparing a diet personally is simple thanks to the wide availability of information about KD and even computer programs available on the web (KetoCalculator) [8].

KD can be applied in children but growth should be carefully controlled. Children younger than two years of age on a ketogenic diet may show slower growth than older ones and if so the diet needs to be modified [23] by changing the ratio of individual nutrients. Certainly, diminished growth in KD is associated with an IGF-1 (insulin like growth factor) blood level reduction [24]. Children who were treated with KD, showed a trend in increasing the levels of lipids and cholesterol in the blood (20% of patients), spontaneously gradually decreasing after some time [25]. Diverse data concerning the blood cholesterol levels published, may be a consequence of the different health status of the examined patients. The overall observed trend shows a slight increase in cholesterol in the first stage of the diet with a subsequent decline to the physiological values [26,27,28]. However, avoidance of KD treatment because of the risk of high cholesterol levels seems to be a mistake because of effective modern pharmacotherapy. In long-term use of KD, atherosclerosis has not been demonstrated, and children subjected to KD exhibited a normalization of plasma lipid levels several years after and did not manifest a higher ratio of coronary disease [29].

KD application should be accompanied by supplementation with water soluble vitamins such as thiamine (B1), riboflavin (B2), niacin, vitamin B6, folic acid, biotin, pantothenic acid, in the form of sugar-free compounds. In addition, supplementation with minerals such as zinc, selenium and calcium should be applied [8]. The administration of carnitine in a dose of 100 mg/kg/day from the beginning of the diet and additional supplementation of omega-3 acids of 4 g/day (dose prescribed for children) after one month should be used [24].

Patients before KD therapy should be tested to rule out metabolic disorders [30], because the use of KD contributes to intensification of the negative effects of pathological states such as pyruvate carboxylase deficiency, porphyria, carnitine deficiency, mitochondrial dysfunction and metabolic defects associated with fatty acids oxidation [31].

NEUROPROTECTIVE PROPERTIES OF KETOGENIC DIET

A fat-rich and low-carbohydrate ketogenic diet has been successfully used in epilepsy treatment in children and adults for many years [32]. Current studies on KD also show its neuroprotective abilities.

KD neuroprotective activity is analyzed at three levels: caloric restriction, whole ketogenic diet and isolated ketone bodies [33]. With no doubt, the common feature of the first two factors (caloric restriction, KD) is the presence of increased levels of ketones in the blood, which limits the field of exploration in metabolic mechanisms. In this paper, the mechanism of the ketogenic diet influence on the brain will be discussed on the basis of ketone bodies metabolism.

Ketone bodies cause a decrease in the production of reactive oxygen species by improving the efficiency of the mitochondrial respiratory chain complex I. This was shown in experiments presenting a decrease in NADH in rat neurons and isolated mitochondria after the application of ketones, acetoacetate, and β-hydroxybutyrate (1 mM each). However, they did not show the effect of KB on the amount of glutathione [34]. It should be noted that the ketogenic diet increases the amounts of glutathione and the activity of glutathione peroxidase, being important factors of the antioxidant action of the diet [35]. β-hydroxybutyrate increases the amount of intracellular NADH, while acetoacetic acid causes a decrease in NADH concentration [36]. Maalouf et al. have demonstrated that the neuroprotection of ketones depended on the reduction of the ratio of NADH to NAD^+, which decreased the amount of *ad hoc* created, oxygen free radicals limiting their production in the mitochondria [33]. The mitochondrial respiration was also better, which

suggests a neuroprotective effect of β-hydroxybutyrate that caused a significant increase in ATP production in isolated mitochondria and in brain homogenates [37]. β-hydroxybutyrate may provide a more efficient energy source for the brain per unit of oxygen than glucose [3]. The administration of β-hydroxybutyrate results in significant prolongation of neuron survival time in hypoxia and anoxia by improving cellular respiration in the mitochondria and increased ATP production [37].

The neuroprotective characteristic of KD may also be related to its ability to modify apoptosis. KD exhibits protective action during the application of glutamate and kainate receptor agonists, reducing the amount of apoptosis markers like caspase-3. A high concentration of caspase-3 is connected with cellular degradation during epileptic seizures and following hypoxia [38]. Furthermore, KD leads to an increase in the concentration of calbindin, a calcium-binding protein that via a decreasing Ca^{2+} level, may abate apoptosis [39]. A decrease in apoptosis during KD may also be an effect of increased synthesis of anti-apoptotic protein Bcl-2 [40].

An interesting aspect of ketone metabolism is its importance during the early postnatal period. During labor, the neonatal brain consumes 60–70% of the total body energy, and half of this energy comes from the β-hydroxybutyrate turnover. The concentration of β-hydroxybutyrate in neonatal blood approximates 2–3 mM. Ketone metabolism is maintained for some time after birth as colostrum contains a high level of triglycerides and proteins but little lactose. The lactose content in the mother's milk increases after 2–3 days of lactation, leading to the switching of metabolism from ketone bodies to glucose. Such evolutionary adjustment is suggested to be connected with the neuroprotection of newborns from hypoxia that may accompany labor [1].

KETOGENIC DIET IN THERAPY

Epilepsy and anticonvulsant mechanism of ketogenic diet

Fasting as a type of anti-epileptic therapy was described already in biblical times. In the description of the case of a "possessed" boy who manifested all the symptoms of *grand mal*, "This kind can come forth by nothing, but by prayer and fasting" (Bible, King James Version, Gospel of Mark, 9:29; http://www.earlychristianwritings.com/text/mark-kjv.html). The first medical reports on the effectiveness of fasting in the treatment of epilepsy were published in 1911 by two renowned French doctors, Marie and Guelpa. However, the credit for the scientific basis of fasting in epilepsy belongs to the American pediatrician Rawle Geyelin. In 1921, at the meeting of the American Medical Association, he presented the case of the recovery of a boy with epilepsy following a dozen or so days fasting. From that time, fasting during the treatment of epilepsy became increasingly popular in the U.S., especially in child therapy [7]. Since long-term treatment using starvation appeared impossible, other ways to achieve the same effect were intensively sought. Research, conducted primarily in the clinical hospital in Rochester, led to the elaboration of the ketogenic diet. To some extent, its metabolic effects are similar to the results of starvation and inhibit seizures in an analogous way. The use of a ketogenic diet in the treatment of epilepsy has been practiced since the 1920s. The introduction of antiepileptic drugs to the clinical practice, phenobarbital as the first, significantly reduced the interest in the ketogenic diet as it was more complicated to apply. Recently however, there has been a renaissance of the ketogenic diet. This is owed largely to the media, as a huge growth of interest in the ketogenic diet was observed after the release of the Hollywood movie "First do no harm", describing the effective dietary treatment of epilepsy in a child [8].

Currently, the ketogenic diet is used in 50 countries worldwide [41]. It has been widely promoted during world congresses of epileptology and neurology. It also is beginning to be applied in the treatment of some metabolic and degenerative diseases or even brain tumors [8].

The popularity of the ketogenic diet is proved by a more than six-fold increase in the number of publications devoted to this therapeutic method in the last decade, compared to the previous one. The ultimate mechanism of the anticonvulsant action of KD has not been established yet and there is a variety of concepts concerning this subject. One of them is the assumption that chronic ketosis, which leads to the modification of the tricarboxylic acid cycle, increases the synthesis of γ-aminobutyric acid (GABA), and reduces the incidence of seizures in this way [42]. The lack of oxaloacetate is supplemented in the enzymatic pathway from α-ketoglutarate and aspartate with the forma-

tion of glutamate. The resultant glutamate is the precursor of GABA [2].

GABA is the major inhibitory neurotransmitter in the mammal nervous system [43]. Many anticonvulsant drugs are directed toward the functioning of the GABAergic system, leading to an increase in its inhibitory action. Increased GABA concentration in the cerebrospinal fluid found during KD application may be the cause of the inhibition of seizures [8].

Neither acetoacetate nor beta-hydroxybutyrate had anticonvulsant properties but acetone clearly exhibited anticonvulsant efficacy at therapeutically relevant and nontoxic concentrations [44]. Many researchers, however, reject the meaning of high levels of ketone bodies in the treatment of epilepsy, indicating that the antiepileptic action of KD is not correlated with the actual degree of ketosis [24].

Another, quite controversial, theory concerning the mechanism of anticonvulsant activity of the ketogenic diet is linked to the alternating levels of ATP following the ketosis state. The ketogenic diet increases the amount of mitochondrial ATP (and, thus, total ATP) but leads to a reduced level of cytoplasmic ATP. The inhibition of glycolysis with the utilization of ATP to maintain the action of sodium-potassium pumps leads to a decrease in its concentration near the cell membrane. The cell membrane (including neurons) contains ATP-linked potassium channels. During the application of KD, these channels open and K^+ ions flow out of the cell, leading to a reduced electric excitability of neurons [21].

KD is effective in the treatment of drug-resistant epilepsy; therefore, its action mechanism should differ from pharmaceuticals already known and used in therapy. One theory is related to the effects of purines – precisely adenosine, which is a product of ATP hydrolysis [20]. Adenosine acts on G protein-linked receptors (A1, A2A, A2B, and A3), regulating, integrating and tuning the activity of neurons, and it influences important brain functions such as sleep, arousal, cognition and memory, as well as the damage and degeneration of neurons [45]. KD increases the production of mitochondrial ATP both in neurons and glial cells [17,46]. Glial influences neuron cells by releasing ATP into the extracellular matrix, where it is decomposed. Vesicular ATP released from astrocytes is a source of extracellular ATP which is dephosphorylated to adenosine that influences neuronal activity [47]. Such an elevated adenosine concentration in the synaptic cleft as well as in the cerebral neurons *per se* stimulates the adenosine receptors located on the neurolemma. The activation of presynaptic adenosine receptors results in a decrease in glutamate release in the synapse while postsynaptic receptors lead to the opening of K^+ channels and membrane hyperpolarization. These both cause a decrease in neuronal excitability [20].

It was found that a ketogenic diet can successfully reduce the number of flexion seizures, partial seizures, myoclonus and unconsciousness seizures. Its effectiveness has been demonstrated to be on par with new antiepileptic drugs in the treatment of most drug-resistant epileptic syndromes, such as West syndrome, Lennox-Gastaut syndrome or Dravet syndrome. A twelve month KD in 150 children 1 to 16 years of age with an average of 410 attacks per month showed at least a 50% reduction in seizures in 75 patients (50%), and 41 children (27%) were seizure free or with a reduction of seizures by more than 90% [8,48]. Hong et al have shown that the ketogenic diet is appropriate therapy in the treatment of childhood flexion seizures, even in cases when other anticonvulsant drugs are ineffective [49]. Flexion seizures (called infantile spasms, West syndrome) is an epileptic condition manifested in children below one year of age (usually between 3 and 10 months), consisting of a series of sudden muscle jerks (flexor and extensor) [27]. Three months after the application of KD in 104 children with West syndrome (average age of 1.2 years), the number of seizures in all the children decreased and in 18% a complete recovery was observed. Further treatment resulted in a complete return to health in 33% of patients after 24 months. In a further 44% of respondents, a more than 50 percent reduction in the number of seizures was obtained [49]. Similar results were obtained by Kossoff et al., examining a group of 27 children aged from 5 months to 2 years, where at least a 50 percent improvement was observed in all the children after twelve months of KD application, and in thirteen patients a complete elimination of seizures was found [30].

BRAIN TUMORS

Brain tumors are the second most common cause of death of children suffering from cancer [50]. Operative resection followed by radiation and / or chemotherapy has been the standard therapy used for more than five decades. Staf-

ford et al proved that KD inhibited the development of gliomas, slowed down tumor growth and decreased the amount of reactive oxygen species that usually promote tumor growth [51]. This diet not only acts by reducing the amount of readily available glucose, but its effects are also related to the modulation of both an intracellular signaling cascade and homeostatic mechanisms. While normal brain cells can easily adapt their metabolism to use ketones as an alternative energy source, tumor cells exhibit a lower plasticity in energy metabolism. The cells of gliomas as well as most other tumor types, cannot bypass glycolysis and utilize the ketone bodies in the TCA cycle, and their metabolism is dependent on the glycolytic pathway. Therefore it is suggested that therapies aimed at the genetic and metabolic weakness of brain tumor cells may be effective in restraining neoplasm expansion [52]. It is believed that the application of KD can be useful in the regression of brain tumors and may exhibit a neuroprotective function for normal brain cells during tumor treatment i.e. under chemotherapy [51]. In neoplasm tissue there is an intensive growth of cells, accompanied by the formation of new blood vessels. However, the growth of tumor cells precedes angiogenesis, and part of the overgrown tissue is not provided with oxygen. To be able to continue growth, invasive tissue cells must draw energy from anaerobic glycolysis. The inhibition of glycolysis during the application of KD can be an important factor in inhibiting the development of cancer cells [52].

In mice implanted with astrocytomas, KD associated with calorie restrictions showed an 80% decrease in tumor mass and decreased vascularization of tumors [53]. In two pediatric patients with advanced astrocytomas (astrocyte tumors), sustained KD decreased the glucose uptake by the tumor, however, no significant decrease in the tumor mass was observed. One patient exhibited enlargement of the tumors [54]. Other reports indicate that the use of a ketogenic diet led to growth arrest of brain gliomas in an adult patient [55]. Some authors consider the applicability of KD for the treatment of malignant brain tumors, as an effective and nontoxic therapy [56].

The influence on the reduction of tumors is rather applied to the ketogenic diet as a whole, not to ketone bodies *per se*. Research with the use of pure ketones in mice models of human breast cancer did not show a decrease in tumor growth [57].

TUBEROUS SCLEROSIS

Tuberous sclerosis or the tuberous sclerosis complex (TSC) is a neuroectomezodermal dysplasia (phakomatosis), with an average frequency of 1 : 6000 individuals in the child population [58]. It is a genetic, autosomal dominant disease. Its etiology involves a mutation in one of two suppressor genes involved in cellular development: TSC1 or TSC2 [59]. These genes encode the proteins tuberin (TSC2) and hamartin (TSC1), which function in the cell as a complex involved in the regulation of cell division, maturation, differentiation, and growth. This complex mediates the inhibition of Rheb-GTP, which regulates the activity of the mTOR (mammalian target of rapamycin) pathway, a major regulator of cell growth and proliferation [60,61].In the course of the disease, benign tumors called hamartomas form in various organs including the brain, skin, lungs, liver, kidney and retina. Lesions located in the brain can cause seizures and/or mental retardation, which affect about half of the patients with TSC. In most children, epilepsy being an effect of TSC has a drug-resistant form [62] that makes it a putative target for KD.

Kossoff et al. examined twelve children aged 8 months to 18 years with TSC treated with KD. Six months after the introduction of the diet, they found an improvement in seizure protection. In eleven patients (92%), a decrease in the number of seizures by more than 50% was observed, including eight patients (67%) where the frequency of attacks fell by more than 90%. The researchers concluded that KD is a favorable therapeutic option for patients with TSC and associated epilepsy [63].

The efficiency of KD in patients with TSC is similar to other drug-resistant epilepsies [8]. The inclusion of a ketogenic diet in the therapy of TSC may not only reduce the number and severity of the seizures, but also might change the cell metabolism leading to a reduction in tumor growth. Chu-Shore and Thiele led research on the impact of KD on the growth of SEGA (subependymal giant cell astrocytoma) tumors in the brain and AML (angiomyolipoma) tumors in the kidneys in five patients with TSC [64]. They based their research on the assumption that the bioavailability of nutrients regulated the anabolic mTORC1-S6K1 pathway in animals [65], which suggested the possible therapeutic effects of KD. In one patient they revealed tumor regression after concomitant sirolimus,

a pharmacological inhibitor of mTORC1, treatment assuming that the drug can inhibit the development of tumors in patients with TSC. However, they did not show clearly that KD induced the regression of tumors occurring in TSC, or stopped their growth. Despite this, according to the author himself, because of the relatively small sample (5 patients), the result of this experiment does not seem to be fully justified and further investigations should be conducted on animals [64].

The small number of patients does not necessarily indicate the invalidity of abovementioned experiment. During the analysis of these data, the lack of uniformity of the research group in terms of disease etiology (mutations in TSC1 or TSC2) and age structure (from 8 to 52 years) should be taken into account. Additionally, the observed blood ketone levels in patients underwent considerable fluctuations during the experiment, which may indicate that the patients did not fully abide to the restrictions of the ketogenic diet and relieved themselves from the state of ketosis. Despite the lack of regression of tumors, three of the patients manifested stabilization in the growth of brain tumors and two patients showed stabilization in the growth of renal tumors [64], which may indicate the necessity to further deal with a ketogenic diet in the therapy of TSC.

An interesting potential linkage of the mTOR pathway with ketosis generated during the use of KD can be found in the work of Sengupta et al. They measured fasting liver ketone levels in mice with TSC1 deficits as well as healthy ones. In the control group, the level of ketone bodies during fasting was increased compared to animals fed normally and this is consistent with the physiology of starvation. In fasting animals with TSC1 deficits, ketosis was significantly smaller (approximately threefold). Such results may suggest that some elements of the mTOR pathway have a significant impact on the production of ketones during fasting [66] and therefore further examination of the relationship between the functioning of the mTOR pathway, and the concentration of KB in the body may be very interesting.

KD inhibits the mTOR pathway signaling in the brain and liver of healthy rats. This mTOR inhibition may be the basis of some of the physiologic effects of KD, including tumor growth impairment and anticonvulsant actions [67]. This effect may be linked to the insulin-growth factor 1 (IGF-1). IGF-1 stimulates the activity of mTOR toward cellular proliferation and initiates mechanisms of angiogenesis [60]. During the application of KD in epileptic children of different ages (from 3.5 to 10 years) a significant decrease in the amount of IGF-1 in the blood was observed [24]. Inhibition of the IGF-1 receptor (IGF-1R) with denbinobin (natural tumor-reducing substance of plant origin) resulted in the impairment of IGF-1 activity and its downstream signaling pathways, leading to the regression of lung adenocarcinoma and associated angiogenesis in mice [68]. Nevertheless, the therapeutic significance of IGF-1 reduction in TSC should be viewed somewhat skeptically, because in the regulation of mTOR, the growth factors exert their activity by inhibiting the TSC1/TSC2 complex, whose activity in TSC is already disabled.

TSC2 activity has a positive effect on the regulation of p27, an inhibitor of cyclin-dependent kinases (CDKs) [69]. In phase G0 and early phase G1 of the cell cycle, p27 is localized in the nucleus, where it inhibits cell growth and proliferation [58]. p27 is a primary regulator in the development of mammalian cells and plays a role of tumor suppressor [70]. The expression of genes TSC1 or TSC2 leads to a rise in p27 protein levels. In a normal state, tuberin binds to p27 preventing its degradation and increasing its content in the cell [59]. Thus, one can suspect that the impaired functioning of hamartin and/or tuberin in TSC will result in degradation of the p27 protein and its low level in the nucleus which in turn will result in unrestricted cell growth. *In vitro* studies on human kidney cell cultures have shown that β-hydroxybutyrate stimulated expression of the p27 protein, leading to a decrease in cell proliferation and halting them in the G0/G1 phase [71]. Such a correlation indicates the utility of a ketogenic diet in TSC treatment and in the inhibition of tumor development, however, further research is indispensable.

ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is a progressive disease associated with emerging cognitive impairment [72]. The pathology of Alzheimer's disease is associated with the appearance of senile plaques in the brain, abnormal neurites and tauopathies. Senile plaques contain large amounts of β-amyloid (Aβ) peptide formed by the decay of the amyloid precursor protein (APP). A mutation in APP results in increased production of particular forms of Aβ (Aβ42 iso-

forms mainly). Thus, despite the still unclear role of Aβ in Alzheimer's disease, it is considered as the main neuropathological phenotype of this disease [73].

The role of KD in regulating and reducing insulin levels in the blood has also been noted. Insulin receptors are highly expressed in the brain, mainly in the cortex and hippocampus, where they have an impact on memory and learning processes [74]. Here, the effect of insulin relies on stimulating the secretion of β-amyloid, leading to an increase in its concentration. KD, by reducing insulin signaling, may contribute to a reduction of β-amyloid protein levels in the brain. The decrease in the level of insulin, which has a signaling function similar to growth factor IGF-1, may have an inhibitory effect on protein synthesis and promote the degradation of existing ones. This can lead to the loss of proteins that are sensitive to degradation, such as amyloid peptides [75].

Additionally, reducing the supply of carbohydrates leads to a reduction in glycation (the process of glucose binding to free amino groups of proteins, escalating their aging) and the related oxidative stress, factors that enhance the symptoms of AD [76].

When discussing the impact of KD on the brain of AD patients, attention should be paid to the adenosine and adenosine receptors that are one of the targets in the treatment of AD [45]. Studies of AD therapy are extended to the blocking of adenosine receptors (A1, A2). This could exclude the use of KD in AD, because KD increases the amount of adenosine, capable of activating membrane receptors (please refer to the above discussed anticonvulsant action of KD). Besides, one of the putative causes in the pathogenesis of AD is the excitotoxic effect of glutamate [77], whose secretion is impaired as a consequence of adenosine activity [78]; this could favor including KD in AD therapy.

PARKINSON'S DISEASE

Parkinson's disease is the second (after Alzheimer's disease) most common degenerative disease of the nervous system [79]. It is characterized by progressive motor symptoms like limb tremor, muscular rigidity, bradykinesis and posture instability. The etiology of the disease is uncertain and largely unclear, however, many researchers highlight the role of the reduction of the mitochondrial respiratory chain complex I, which results in a defect in the functioning of oxidative phosphorylation [80]. The use of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropirydin) in animals causes the development of symptoms characteristic of Parkinson's disease, which is caused by progressive degeneration of dopaminergic neurons in midbrain substantia nigra [81]. MPTP is a neurotoxin which easily passes through the blood-brain barrier and is converted by astrocytes into MPP+ (1-methyl-4-fenylopirydin). MPP+ is selectively absorbed by dopaminergic neurons where it inhibits the mitochondrial respiratory chain complex I [82].

A ketogenic diet alone has not been used in the animal model of Parkinson's disease, however, a positive effect of calorie restriction in its treatment was observed. In the MPTP animal model, the reduction of calories by 30–40% resulted in a decrease in degradation of dopamine neurons when compared to mice on a normal diet. A similar effect of a limited caloric supply on the symptoms of the disease was found in macaque monkeys. In this model, a low-calorie diet initiated the development of resistance to MPTP neurotoxicity [3].

Studies in mice have shown that β-hydroxybutyrate administered to the brain displays protective properties against MPTP-induced damage, such as degeneration of the dopaminergic neurons of the substantia nigra, loss of dopamine in the striatum and the appearance of motor deficits [80].

A study carried out on a small group of patients (5 persons) with Parkinson's disease, who were treated with a ketogenic diet, showed improvement in the health status of the patients after 28 days. The results based on the UPDRS (Unified Parkinson's Disease Rating Scale) score showed an improvement of 21%, 46% or even 81% from the initial scoring [83]. With such a small sample of patients and the relatively short time, the role of the diet results may appear doubtful, however, the direction of research seems to be justified and should be further pursued, since it can provide effective therapy with a negligible risk.

CONCLUSION

The metabolic effects associated with the presence of ketone bodies during the application of the ketogenic diet are an important aim of research for many scientists around the world. Analysis of the alternative metabolic path-

ways activated by nutritional deficiency proves the incredible survival ability of the human body, especially for the protection of our "big brains". The ketogenic diet, modern therapy based on these mechanisms, in its simplicity reveals the complexity of interactions that might be used in the treatment of central nervous system diseases.

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