sciendo

Review • DOI: 10.2478/ahem-2022-0019 • AHEM • Vol. 76 Issue 1/2022 • 234-242

AHEM

Advances in Hygiene and Experimental Medicine
Postępy Higieny i Medycyny Doświadczalnej

The potential impact of the ketogenic diet on gut microbiota in the context of neurological disorders

Review

Anna Gudan, Ewa Stachowska*

Department of Human Nutrition and Metabolomics, Pomeranian Medical University in Szczecin, Szczecin, Poland

Received 11 January, 2021, Accepted 13 October, 2021

Abstract

One of the most important functional parts of a human intestinal tract is the microscopic intestinal barrier. Its function is to ensure the correct nutrient absorption and to protect against multiple pathogens, xenobiotics, and environmental toxins. Intestinal microbiota is an integral part of the intestinal epithelium. Human microbiota and their host interact with each other, both directly and indirectly, via multiple intermediates and metabolites. Some dietary fat that is not fully digested reaches the distal parts of the intestinal tract, where an interaction with gut microbiota takes place. Studies have shown that an animal-product based diet that provides a greater supply of saturated fat increases the number of bile-resistant microorganisms, including *Bilophila*. The total amount of *Alistipes* and *Bacteroides* is also increased. Long-term consumption of animal-based foods contributes to the formation of the enterotype described as the *Bacteroides* type. The ketogenic diet is mainly based on animal fats. The changes induced by this higher consumption of animal fats are associated with unfavorable metabolic changes. However, more and more research has shown evidence of the therapeutic properties of a ketogenic diet as far as neurodegenerative and metabolic diseases are concerned. Recent reports suggest that the protective effect of a ketogenic diet is highly dependent on the gut microbiota. This review focuses on the correlation between the influence of ketogenic diet on the intestinal microbiota changes observed while analyzing patients with diseases such as epilepsy, Alzheimer's disease, autism spectrum disorder, and multiple sclerosis.

Keywords

ketogenic diet • intestinal microbiota • intestinal barrier • metabolomics

1. Materials and Methods

The literature review was performed in the PubMed database. The keywords used were: ("ketogenic diet" OR "low carbohydrate diet" OR "ketosis" OR "exogenous ketones" OR "keto" OR "high fat diet") AND ("microbiome" OR "microbiota" OR "intestinal microbiome" OR "intestinal microbiota" OR "microflora" OR "intestinal microflora" OR "intestinal barrier" OR "gut barrier" OR "leaky gut"). Scientific data was collected from March 2020 to May 2020. The review of papers included all the papers available so far that included the selected keywords.

2. The microbiome, the intestinal barrier, and transmetabolomics

At the lumen of the intestine, the intestinal barrier meets a diverse ecosystem: the gut microbiota. Recent scientific advances have shown how important the gut microbiota is to the digestive system. It is a complex community of many different species of bacteria, viruses, fungi, and other microorganisms found in the intestinal lumen. It is an ecosystem containing about 1,200 different species, up to 80% of which are variable species. Although the microbiome is a climax community, we can identify many factors that affect its composition. These include internal factors such as the pH of gastric acid, proper bile secretion, and the presence of appropriate digestive enzymes. External factors that contribute to changes in the microflora include diet, physical activity, exposure to stress, environmental toxins, past infections, diseases, and medications and dietary supplements [1]. Adverse changes in the intestinal microbiota resulting in its destabilization significantly deteriorate the functioning of the intestinal barrier and cause inflammation, which markedly increases the risk of irritable bowel syndrome and of compromised well-being. It is also relevant to many diseases, including neurodegenerative diseases, autoimmune diseases, ischemic heart disease, autism spectrum disorders, insulin resistance, diabetes, cancer, and epilepsy. Today, a growing body of research suggests an increasing role for the gut microbiome and its functions in both health and disease [2].

* *E-mail: ewast@pum.edu.pl*

Over the past 15 years, gut microbiota have garnered immense attention from researchers around the world. The development of bioinformatics and research tools has facilitated increasingly precise genetic studies of the gut microbiome with its metabolites. Most focus has been on the bacterial microbiota, but it should be noted that viruses and mycobiomes are no less important, while still insufficiently explored [3]. Nevertheless, the interactions among bacteria, viruses, and fungi are currently vigorously studied because of their potential importance for human health [4]. The gut microbiota interact with their host, both directly through the cellular pathway and indirectly through intermediates and metabolites. The bioactive compounds produced by the gut microbiota are capable of regulating and altering metabolic pathways involved in immunization, energy homoeostasis, and neuronal pathways [5].

The microbiome performs its functions in a state of eubiosis, and disturbances in the quantitative and qualitative composition of the microbiota lead to dysbiosis, which is an important factor in the pathogenesis of irritable bowel syndrome, inflammatory bowel diseases, cancer, neuropsychiatric diseases, or even metabolic syndrome. It seems that dysbiotic shifts in the microbiota are correlated with the Western dietary model, and usually lead to a decrease in the biodiversity of the gut microbiome and in the total number of microorganisms. Nevertheless, little is known about what exactly is meant by dysbiotic microbiota, just as the standard and baseline range of individual microbial species characteristic of the eubiosis state is still not defined. Moreover, researchers are now shifting away from detailed analysis of the taxonomic composition of the gut microbiota to the study of its metabolic functions [5]. It appears that the functional metabolomics of the gut microbiome may play a key role in a wide variety of disorders. The Human Microbiome Project has set the stage for current research by demonstrating that a change in the taxonomic composition of the gut microbiota does not necessarily involve a change in its metabolic functions, and thus does not seem to alter microbiota-host interactions from a signaling threshold, either [3]. Moreover, taxonomic changes in the gut microbiota can occur abruptly, depending on a change in diet, a history of infection, or a sudden introduction of physical exercise; however, as researchers indicate, this does not necessarily involve an equally sudden change in microbiota function [6, 7].

Because of such extensive and considerable interdependencies between the microbiota and the host, the microbiome has come to be viewed as a new organ: the bacterial organ. However, unlike other organs, the gut microbiome exhibits tremendous interspecies variations, which makes it all the more challenging to correctly interpret sophisticated research findings. The composition and function of the intestinal microbiota also vary across life stages. The gut microbiota, next to the human genome, seems to be most implicated in the individual response to specific food components, thus creating handle points for personalized nutritional therapies [8]. This has relevance for athletes who seek to enhance their performance and individual management. However, studies and data on the interactions between the microbiota and the host are still scarce. Meanwhile, it is safe to assume that the gut microbiota interact with the host along bidirectional axes, such as the gut-brain axis, the gut-liver axis, and the gut-muscle axis. The challenge remains to identify specific interdependencies and to precisely distinguish cause from effect [7, 8].

3. Metabolic characteristics of the gut microbiome

The gut microbiome interacts with many nutrients that reach the distal parts of the intestine with undigested food debris. Carbohydrates, proteins, fats, polyphenols, and other bioactive substances have received the most scholarly attention. Indigestible carbohydrates such as dietary fiber provide the main fuel for microorganisms, but not every type of fiber is amenable to bacterial fermentation. Some fractions of insoluble fiber are not metabolized by microorganisms and are excreted. Depending on the composition of the gut microbiome, the quantity and quality of metabolites formed by nutrient biotransformation vary significantly [9, 10].

Short-chain fatty acids (SCFAs) are the most common product of microbial metabolism. They are produced from bacterial fermentation of dietary fiber. They consist mostly of three major acids: acetic acid, propionic acid, and butyric acid, in the ratio of 3:1:1, respectively.

The proportion and types of short-chain fatty acids may vary depending on the dietary pattern, comorbidities, exercise level, or dietary fiber intake alone [10]. The highest concentrations of SCFA are observed in the ascending colon, with somewhat lower levels in the transverse and descending colons. One of the most important properties of short-chain fatty acids is their capacity to trigger specific metabolic effects while acting as signaling molecules in the host. SCFAs have the ability to cross the blood-brain barrier, which is an important consideration for the functioning of the gut-brain axis and for neuropsychiatric and neurological disorders (e.g., epilepsy, autism spectrum disorders) [11]. The ketogenic diet (KD) contributes to a significant increase in betahydroxybutyric acid, which is structurally similar to one of the SCFAs, butyric acid. This fact is probably not insignificant in the context of neurotransmission and the therapeutic effect of KD in neurological disorders.

SCFAs are absorbed in the intestine through monocarboxylate transporters. Once inside the cell, they become involved in metabolic pathways and provide up to 60% of the energy to intestinal cells [7, 9]. The unused acids migrate to the liver, where they can continue to perform metabolic functions. Only a small amount of SCFAs enter the peripheral circulation. The metabolic functions of SCFAs are mainly as follows: reduction of lipopolysaccharide (LPS)–induced inflammation, direct modulation of

systemic immunity, improvement of insulin sensitivity, regulation of appetite, stimulation of white adipose tissue browning, increase in energy expenditure by thermogenesis, and increase in lipolysis [12]. It should be noted that most studies evaluating SCFA production are carried out on stool samples, which may not be fully reliable due to limitations related to intestinal transit time, leaky gut, rate of metabolite transport, or the method of sample collection and storage [11].

SCFAs also exert several beneficial effects locally, such as maintaining the integrity of the intestinal barrier, maintaining an intact mucosal layer, reducing in situ inflammation, and exerting epigenetic effects on histone deacetylase (HDAC). By interacting with GPR receptors, they affect a wide variety of cells, with the final effect depending on the cell type. Recent studies have shown that butyric acid can even improve sleep quality [16]. This is an interesting benefit, given the negligible amount of SCFAs that reach the circulation. SCFA production relies on the composition of the gut microbiota and the dietary regimen, especially the content and type of dietary fiber available for fermentation [13].

Apart from short-chain fatty acids, gut microbiota also produce branched-chain fatty acids (BCFA; branched-chain amino acids). The amount of BCFAs increases significantly within 24 hours of consuming more protein, so BCFAs can be a fairly good indicator of the rate of proteolytic fermentation. Higher protein intake rapidly increases the total pool of isovaleric acid which is present in trace amounts under normoprotein dietary conditions [14]. The role of BCFAs in health and disease is still not well understood. However, it is clear that some of them can be utilized in the process of energy extraction when butyrate is insufficient [15]. SCFAs are also produced by proteolytic fermentation, but their overall pool is much smaller if this fermentation does not come from carbohydrate substrates. The lack of data on the impact of BCFAs, including isovaleric acid, on the host's health means that all analysis is limited to speculation. The process of isovaleric acid formation is associated with increased production of ammonia, which can then be used in the urea cycle or will be excreted from the body due to its toxicity [16]. Ammonia is a toxic byproduct of BCFA metabolism, but the amount of ammonia in the intestinal lumen can be reduced by increased fermentation of dietary fiber. This is possible as a result of growing nitrogen demand as well as preferential saccharolytic fermentation [17]. In addition, the acidic environment in the distal colon may itself result in decreased BCFA synthesis. However, there are some dietary fiber fractions that can cause increased isobutyric acid and isocaproic acid production. Low pH in the colon and availability of dietary fiber are associated with lower bacterial putrefactive fermentation. Starch appears to be important in preventing increased proteolysis, but the process is still dependent on the intestinal lumen pH. BCFA synthesis is 60% lower at pH=5.5 compared to pH=6.820.21 Pieper et al. demonstrated that increasing the amount of dietary fiber from 10g to 30g per day resulted in preferential utilization of fiber over protein and also increased the total amount of SCFA and shortened the intestinal passage of stool [18]. This is very important for understanding the carcinogenic effects of the metabolites of putrefactive microflora found in the feces, and also from the point of view of putrefactive fermentation, which occurs in the terminal sections of the gastrointestinal tract itself. The shorter the intestinal passage, the fewer putrefactive metabolites are likely to be produced [18, 19].

4. Food and the functions of the microbiome

SCFAs are bacterial metabolites with many beneficial metabolic properties. Although most of them have positive effects, there are also studies showing that some SCFAs are linked to obesity or depression [30]. This suggests a variety of effects of SCFAs on overall health, but the main three short-chain fatty acids (acetic acid, propionic acid, and butyric acid) have been associated with general metabolic health. In addition to SCFAs, the gut microbiota also produce a wide range of other bioactive metabolites from the gut contents that reach the distal parts of the intestine. When following a ketogenic diet, it is worth paying particular attention to fat and protein intake, as well as bile acid metabolism within the gut microbiome.

Recent scientific reports have paid considerable attention to methylamines produced within the intestinal microbiome. Their augmented production has been linked to an elevated risk of atherosclerosis, chronic kidney disease, metabolic syndrome, insulin resistance, and steatohepatitis [20]. There are two methylamines in question: trimethylamine (TMA) and trimethylamine N-oxide (TMAO). TMA is the result of microbial metabolism of nutrients such as choline, betaine, lecithin, and carnitine. The main dietary sources of these substances are red meat, eggs, dairy and sea fish. The microbes responsible for the production of methylamines are mainly bacteria from the genus *Prevotellacelleae* and *Enterobacteriaceae*. The metabolism of trimethylamine begins after it crosses the intestinal barrier, enters the bloodstream, and is metabolized by the liver to trimethylamine N-oxide (TMAO) by the enzyme FMO3 (flavin-containing monooxygenase 3). Higher concentrations of TMAO have been associated with many disease entities. However, it is noteworthy that individual differences in FMO3 enzyme activity may vary considerably between people, which is attributed to different levels of TMAO in the bloodstream [21]. The correlation between increased methylamines and metabolic disorders seems to be caused by a person's individual capacity to produce TMAO, rather than by the intake of specific nutrients. Nevertheless, this issue still requires further intervention studies [22].

A higher protein intake is also related to a higher supply of aromatic amino acids, such as tyrosine and phenylalanine. Bacterial metabolism of these two compounds leads to the for-

mation of the uremic toxin p-cresol [23]. Patients with chronic kidney disease are unable to efficiently remove p-cresol from the system, which results in its accumulation. Excess p-cresol is quite strongly correlated with cardiometabolic disorders, severe oxidative stress, destruction of vascular endothelium and vasculopathy, and hypertension. It has been suggested that excess p-cresol in patients with renal impairment may be a major cause of comorbid cardiovascular disease, regardless of existing renal failure [24]. We still know little about the specific groups of microorganisms involved in p-cresol metabolism. In a study by Brinkworth et al., urinary p-cresol was found to be lower in subjects in the low-carbohydrate group, even despite lower dietary fiber content [25]. In contrast, another study by Patel et al. showed disparate results: over 60% less p-cresol production in subjects on a vegetarian diet compared to a control group on a mixed diet. The researchers explained this discrepancy by a significantly higher intake of dietary fiber [26]. Similar findings were obtained by Salmean et al. in a study in patients with chronic kidney disease. Dietary fiber supplementation reduced p-cresol content by 20% relative to the baseline value before the intervention [27]. Because active individuals on ketogenic diets may consume much more dietary protein and less dietary fiber contained in carbohydrate products, consideration should be given to potential changes in the gut microbiota and possible increased production of p-cresol and thus a higher risk of metabolic diseases. Still, this subject needs more scientific data. It is also worth considering the protective effects of physical activity on dysbiotic changes in the gut microbiota with respect to low-carbohydrate diets and toxic metabolites of the microbiota, as well as other protective factors including polyphenol intake and exposure to betahydroxybutyric acid.

Fat as a nutrient is not a homogeneous mixture. Studies investigating the impact of fat intake on the composition and function of gut microbiota initially focused only on diets based on the principles of Western nutrition: highly processed, high-energy, high-carbohydrate, and high-fat foods [10]. It is worth noting that a large percentage of fatty acids in a typical Western diet are trans fats with proven harmful pro-inflammatory effects, increasing the risk of cardiovascular disease, metabolic diseases, and neurodegenerative diseases. The Food and Agriculture Organization of the United Nations (FAO) recommends a daily fat intake threshold of 30% of energy; however, the ketogenic diet goes far beyond the proposed daily intake, in some cases approaching 90% of daily energy intake [15].

Lipids that are not digested reach the distal parts of the intestine, where they interact with the intestinal microbiota. Studies have shown that a diet based on animal products with a higher supply of saturated fat increases the number of bile-resistant microorganisms, including the Bilophila species. The total number of Alistipes and Bacteroides also rises, with long-term consumption of a diet based on animal products contributing to the formation of a Bacteroides enterotype [28, 29]. A completely different enterotype is the Prevotella type, characteristic of low-fat diets with high intakes of plant protein and total plant products, including dietary fiber. In contrast, the Bacteroides enterotype is distinguished by a higher abundance of bile-resistant bacteria, proteolytic bacteria, and putrefactive processes, which is correlated with a fairly low intake of plant products and a comparatively high intake of animal products, including proteins and saturated fats [30]. Microorganisms such as Alistipes have been linked to pain sensations typical of irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD), as well as to depressive disorders [15]. Bacteroides, on the other hand, are implicated in the production of isovaleric acid, isobutyric acid, p-cresol, indole, skatole, endotoxemia, and the production of toxic ammonia [31]. Increased fat intake seems to be concomitantly associated with increased bile in the intestinal lumen and thus with changes in bile acid metabolism via the intestinal microbiota. Bilophila bacteria, which are found to increase with higher fat intake, have been linked to higher amounts of toxic hydrogen sulfide. The adverse changes observed in the gut microbiota are most often connected to the consumption of high amounts of saturated fatty acids extracted from oil palm. This study limitation should be taken into account in further inferencing [29, 32].

As dietary fat is not a homogeneous component, it is worth looking at particular fatty acids and their effects on the gut microbiota. Many researchers have already shown that different fatty acids cause different changes in the microbiota, with a particular focus on omega-3 fatty acids and monounsaturated fatty acids that may even have a prebiotic effect [33]. Animal model studies have demonstrated fundamentally different changes in the gut microbiome after palm oil administration compared to olive oil or safflower oil. Moreover, the amount of palm oil reaching the distal parts of the intestines was much higher than the amount of the other two oils. The changes induced by saturated fatty acid intake appear to be more detrimental, although a high-fat diet is generally seen as having antimicrobial potential, in other words, as reducing the diversity and number of microorganisms in the gut. This seems to be largely related to the antimicrobial effect of bile, which is secreted in greater quantities with high-fat diets [34].

The ketogenic diet is mainly composed of fats, and the changes induced by higher fat intake, including higher amounts of Alistipes, Bacteroides, and Bilophila lead to adverse metabolic changes [31]. Despite this, more and more studies are dealing with the therapeutic properties of KD in neurodegenerative, degenerative, and metabolic diseases. Recent reports suggest that the protective effects of a ketogenic diet may be highly dependent on the gut microbiota. In addition, there are many different nutrients in the diet that may react with each other and with the gut microbiota, thus making it difficult to analyze the effects of individual fats on the composition and function of the gut micro-

biome. Nutrition is the regular intake of a wide variety of nutrients, and selective analysis of specific food elements may be far from the facts in vivo [35].

5. The ketogenic diet and the gut microbiota

Research reports in recent years have largely focused on analyzing the protective and therapeutic effects of KD in many different diseases [36, 37, 38]. The greatest therapeutic potential of the ketogenic diet is currently tied to various types of drug-resistant epilepsy, as well as to genetic metabolic disorders, including GLUT transporter deficiency [39, 40]. No less importance is also attributed to KD in neurological disorders and neurodegenerative diseases. The ketone bodies produced during the ketogenic diet are known to have a therapeutic effect, but the question is whether this is the only factor responsible for the efficacy of KD. It seems that taxonomic changes in the gut microbiota induced by the ketogenic diet, and the accompanying functional changes, are just as important contributors. Information about the effects of the ketogenic diet on the composition and function of the gut microbiota is still scarce, but it is worth discussing recently published studies in this area.

The ketogenic diet triggers an antiepileptic effect in both animals and humans. Such an effect is observed as early as 1–4 days after KD administration, which coincides with the first noticeable and significant changes in the gut microbiota, according to many publications. In a study on mice, Olson et al. demonstrated a distinctive increase in the bacteria *Akkermansia* and *Parabacteriodes* associated with an anticonvulsant effect mediated by decreased activity of the enzyme gamma-glutamyltranspeptidase and, consequently, an increased ratio of GABA to glutamate. This ratio is vital in the treatment of epilepsy and in maintaining normal brain function as well as neuronal pathways [46]. Another study by Ma et al. also using an animal model of neurodegenerative diseases showed improved neurovascular metabolism with a concurrent reduction in the risk of Alzheimer's disease. Mice were fed a ketogenic diet based on short-chain fatty acids, mono- and polyunsaturated fats for 16 weeks. The introduction of KD decreased the amount of pathogenic bacteria from the genera < Desulfovibrio and Turibacter. Moreover, there was a concomitant increase in the number of bacteria producing anti-inflammatory and neuroactive short-chain fatty acids: *Akkermansia mucunifila* and Lactobacillus. These changes were

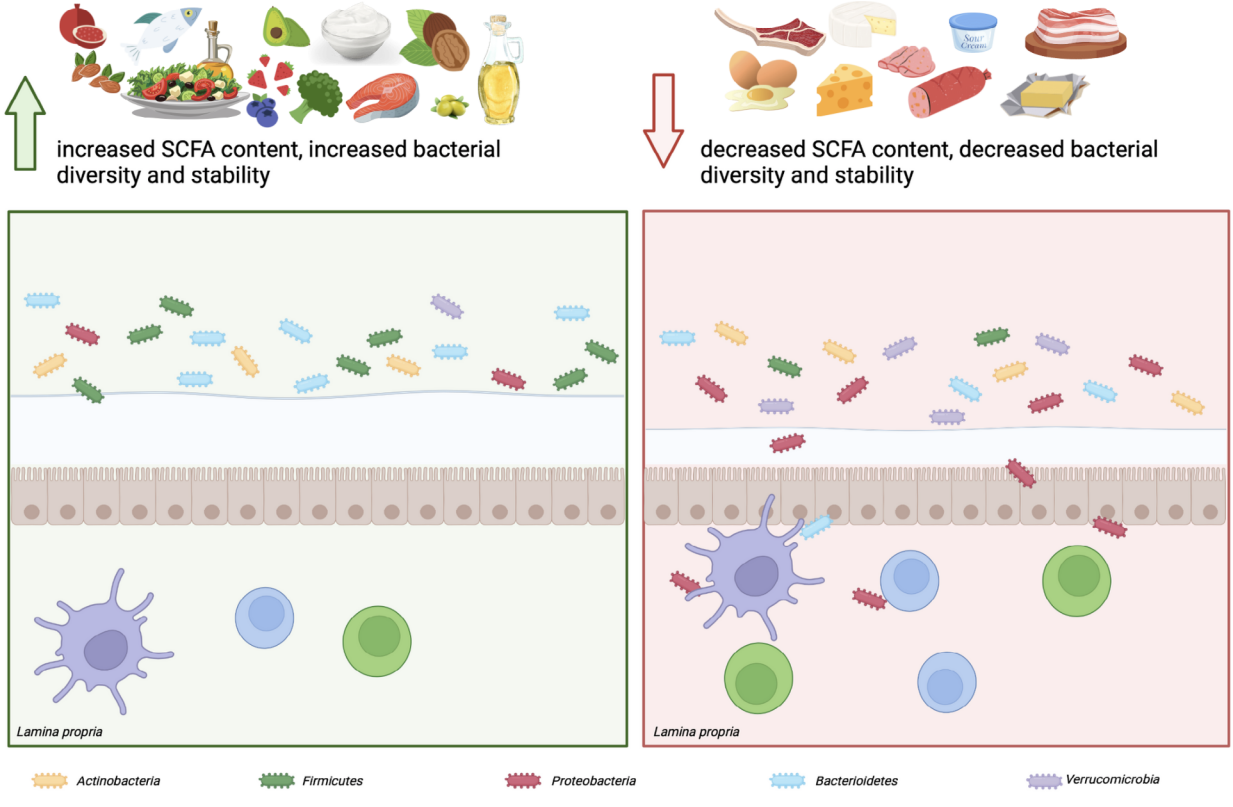

Fig. 1. Impact of diet composition on intestinal microbiome and SCFA synthesis

further correlated with an overall reduction in alpha diversity, which was also confirmed in studies by other authors [41].

The use of the ketogenic diet in autism spectrum disorder (ASD) seems to be a relatively controversial example. However, experimental studies in a mouse model have shown that despite the antimicrobial effect of KD on the gut microbiota, it may carry some benefits in the area of microbiota modulation [31]. After two weeks of KD treatment in a mouse model, researchers observed a statistically significant decrease in total microbiota, a decrease in the commensal bacterium *Akkermansia mucinifila*, and an improvement in the ratio of Fimicutes to Bacteroidetes, all of which appear to be severely impaired in individuals with ASD [42, 43].

A study involving people affected by multiple sclerosis (MS) is an interesting example of the effects of KD on the gut microbiota. The ketogenic diet may be one of the nutritional therapies for multiple sclerosis, as may the modified paleo diet [44]. A recent study by Wahls et al. showed a smaller effect of a modified MCT-based ketogenic diet compared to a modified paleo diet in improving patients' quality of life; nevertheless, the ketogenic diet still shows encouraging therapeutic potential in MS patients [45]. Individuals with MS quite often exhibit significantly reduced numbers and biodiversity of microorganisms in the gut, as well as impaired normal fermentation and impaired short-chain fatty acid production. Dysbiotic overgrowth exacerbates the concentration of neurotoxins in the circulation, which negatively affects the overall well-being of patients and may aggravate and accelerate the onset of MS symptoms. The introduction of the ketogenic diet in patients with multiple sclerosis had a twofold effect. During the first 12 weeks of KD, a strong antimicrobial effect was observed, as well as a sudden reduction in the number and biodiversity of microorganisms. Interestingly, during the following six months, with continuation of the ketogenic diet, the intestinal microbiota underwent further changes, and formed a much more favorable structure than at baseline, before the start of the study. Such a phenomenon is described by researchers as biphasic, and its occurrence alone may have important clinical implications in terms of both nutritional and pharmacological therapy for patients affected by MS [38, 44, 45].

Zhang et al.'s study looked at the effects of the ketogenic diet on the composition and function of the gut microbiota among children with epilepsy [46]. However, in an effort to find a specific indicator of the efficacy of KD, the researchers decided to test for differences between groups that responded to the treatment and those that did not. The application of the ketogenic diet in subjects who positively responded to KD induced a markedly different modification of the gut microbiota than in subjects who did not respond or responded adversely to the ketogenic diet. Individuals who experienced a positive effect of KD showed simultaneously increased amounts of Bacteroides and decreased amounts of Firmicutes and Actinobacteria. In contrast to these changes, subjects who reacted negatively to KD showed significant increases in Clostridia, Ruminococcus, and Lachinospiraceae. These findings should be considered especially when personalizing dietary recommendations and searching for the best therapeutic approach. In the future, gut microbiota may be a potential indicator suggesting the response, or lack thereof, to nutritional treatment with a ketogenic diet [46].

In addition to clinical case studies, it is also useful to review studies on the effects of KD on the gut microbiota in healthy athletic individuals. Such research is currently scarce, but we can single out the work of Murtaza et al., which describes the effects of the ketogenic diet on the composition and function of the gut microbiota of trained elite walkers [47]. The study included professional athletes assigned to three different groups: a ketogenic group, a high-carbohydrate group, and a high-carbohydrate cyclical group. Gut microbiota testing was performed prior to intervention and after three weeks of following the dietary models. Two enterotypes, Prevotella and Bacteroides, were observed in all zero samples collected, with the latter being significantly predominant (20 vs. 7 samples). The carbohydrate groups only slightly changed their gut microbiota profile after the three-week intervention, whereas the ketogenic group experienced a large change in gut microbiota, bumaintaining alpha diversity. The observed changes in the gut microbiota mostly included decreases in Faecalibacterium spp. and Bifidobacterium, increases in *Dorea* spp., *Enterobacteriaceae*, and increases in some species of the genus Bacteroides. Compared to the carbohydrate groups, these changes were rapid and extensive. The ketogenic group experienced both a significant decrease in exercise economy and a decrease in athletic performance. Statistical analysis showed negative correlations between fatty substrate utilization and Bacteroides amount and between *Dorea* spp. amount and exercise economy. In the ketogenic group, the different intestinal enterotypes responded differently to the intervention. Both revealed a significant reduction in Prevotella and an increase in Bacteroides, but this change was not extensive enough to redefine the overall enterotype of the subjects. A similar phenomenon occurred for the bacteria of the genus Faecalibacterium, which decreased for both enterotypes. On the other hand, subjects with the Prevotella enterotype experienced a larger increase in Clostridiales bacteria, and subjects with the Bacteroides enterotype had a considerably greater loss of Bifidobacteri*a* and an increase in Sutterella [47].

6. Summary

- The gut microbiota may mediate the antiepileptic effects of the ketogenic diet through a mechanism dependent on reduced gamma-glutamyltranspeptidase enzyme activity.
- Individuals with multiple sclerosis may exhibit a biphasic microbiota response to the introduction of a ketogenic diet, which relies on the duration of the dietary intervention.

- The efficacy of the ketogenic diet in neurological disease may be at least partially contingent on the baseline composition of the gut microbiota.
- The effects of the ketogenic diet on gut microbiota composition, function, and athletic performance may be at least partially dependent on the baseline enterotype of the subjects.
- The ketogenic diet does not show a uniform effect on the gut microbiota. The exact effect of the ketogenic diet on the composition and function of the gut microbiota requires further study.

Such large discrepancies in the study results suggest that this issue is multifactorial. The effect of the ketogenic diet on the gut microbiota and its functions is a very broad problem, requiring very detailed analysis of large amounts of data. Because of the wide range of factors interfering with reliable interpretation of results, as well as the high risk of many pre-analytical errors, studies of the gut microbiome and its functions should be approached with a degree of skepticism until detailed research methods are able to minimize the risk of research errors. Nevertheless, the knowledge gained to date in this area provides interesting and useful data that should be further explored to fully understand the importance of the gut microbiota and changes in its function in health and disease.

Authors' Contribution

A.G.: research concept and design, acquisition of data, data analysis and interpretation, writing: original draft preparation, literature review; **E.S.**: supervising the project, writing: review and editing, visualization, final proofreading and approval of the version for publication, funding acquisition.

ORCID

Ewa Stachowska https://orcid.org/0000-0002-4009-1977
Anna Gudan https://orcid.org/0000-0002-7052-0189

Funding

PUM - 01/19/RID/11

Conflict of Interest

The authors have no potential conflicts of interest to declare.

References

[1] Fasano A. Zonulin and its regulation of intestinal barrier function: The biological door to inflammation. Autoimmunity, and Cancer. Physiol Rev. 2011; 91: 151-175.

[2] Fiorentino M, Sapone A, Senger S, Camhi SS, Kadzielski SM, Buie TM, Kelly DL, Cascella N, Fasano A. Blood-brain barrier and intestinal epithelial barrier alterations in autism spectrum disorders. Mol Autism. 2016; 7: 49.

[3] The Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature. 2012; 486: 207-214.

[4] Chin VK, Yong VC, Chong PP, Amin Nordin S, Basir R, Abdullah M. Mycobiome in the gut: A multiperspective review. Mediators Inflamm. 2020; 2020: 9560684.

[5] Heintz-Buschart A, Wilmes P. Human gut microbiome: Function matters. Trends Microbiol. 2018; 26: 563-574.

[6] Freestone PPE, Sandrini SM, Haigh RD, Lyte M. Microbial endocrinology: How stress influences susceptibility to infection. Trends Microbiol. 2008; 16: 55-64.

[7] Clarke G, Stilling RM, Kennedy PJ, Stanton C, Cryan JF, Dinan TG. Gut microbiota: The neglected endocrine organ. Mol Endocrinol. 2014; 28: 1221-1238.

[8] Grosicki GJ, Fielding RA, Lustgarten MS. Gut microbiota contribute to age-related changes in skeletal muscle size, composition, and function: Biological basis for a gut-muscle axis. Calcif Tissue Int. 2018; 102: 433-442.

[9] Abdul Rahim MBH, Chilloux J, Martinez-Gili L, Neves AL, Myridakis A, Gooderham N, Dumas ME. Diet-induced metabolic changes of the human gut microbiome: importance of short-chain fatty acids, methylamines and indoles. Acta Diabetol. 2019; 56: 493-500.

[10] Mills S, Stanton C, Lane J, Smith G, Ross R. Precision nutrition and the microbiome, Part I: Current state of the science. Nutrients. 2019; 11: 923.

[11] Miller TL, Wolin MJ. Pathways of acetate, propionate, and butyrate formation by the human fecal microbial flora. Appl Environ Microbiol. 1996; 62: 1589-1592.

[12] Mohajeri MH, Brummer RJM, Rastall RA, Weersma RK, Harmsen HJM, Faas M, Eggersdorfer M. The role of the microbiome for human health: From basic science to clinical applications. Eur J Nutr. 2018; 57 (Suppl 1): 1-14.

[13] Silva YP, Bernardi A, Frozza RL. The role of short-chain fatty acids from gut microbiota in gut-brain communication. Front Endocrinol. 2020; 11: 25.

[14] Aguirre M, Eck A, Koenen ME, Savelkoul PHM, Budding AE, Venema K. Diet drives quick changes in the metabolic activity and composition of human gut microbiota in a validated in vitro gut model. Res Microbiol. 2016; 167: 114-125.

[15] David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature. 2014; 505: 559-563.

[16] Blachier F, Mariotti F, Huneau JF, Tomé D. Effects of amino acid-derived luminal metabolites on the colonic epithelium and physiopathological consequences. Amino Acids. 2007; 33: 547-562.

[17] Diether N, Willing B. Microbial fermentation of dietary protein: An important factor in diet–microbe-host interaction. Microorganisms. 2019; 7: 19. [18] Cummings JH, Macfarlane GT. The control and consequences of bacterial fermentation in the human colon. J Appl Bacteriol. 1991; 70: 443-459.

[19] Pieper R, Boudry C, Bindelle J, Vahjen W, Zentek J. Interaction between dietary protein content and the source of carbohydrates along the gastrointestinal tract of weaned piglets. Arch Anim Nutr. 2014; 68: 263-280.

[20] Dumas ME, Barton RH, Toye A, Cloarec O, Blancher C, Rothwell A, Fearnside J, Tatoud R, Blanc V, Lindon JC, et al. Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. Proc Natl Acad Sci USA. 2006; 103: 12511-12516.

[21] Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, Britt EB, Fu X, Chung YM et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature. 2011; 472: 57-63.

[22] Kühn T, Rohrmann S, Sookthai D, Johnson T, Katzke V, Kaaks R, von Eckardstein A, Müller D. Intra-individual variation of plasma trimethylamine-N-oxide (TMAO), betaine and choline over 1 year. Clin Chem Lab Med. 2017; 55: 261-268.

[23] Gross P, Massy ZA, Henaut L, Boudot C, Cagnard J, March C, Kamel S, Drueke TB, Six I. Para-cresyl sulfate acutely impairs vascular reactivity and induces vascular remodeling: Para-cresyl sulfate and vascular dysfunction. J Cell Physiol. 2015; 230: 2927-2935.

[24] Poesen R, Viaene L, Verbeke K, Augustijns P, Bammens B, Claes K, Kuypers D, Evenepoel P, Meijers B. Cardiovascular disease relates to intestinal uptake of p-cresol in patients with chronic kidney disease. BMC Nephrol. 2014; 15: 87.

[25] Brinkworth GD, Noakes M, Clifton PM, Bird AR. Comparative effects of very low-carbohydrate, high-fat and high-carbohydrate, low-fat weight-loss diets on bowel habit and faecal short-chain fatty acids and bacterial populations. Br J Nutr. 2009; 101: 1493.

[26] Patel KP, Luo FJG, Plummer NS, Hostetter TH, Meyer TW. The production of p-cresol sulfate and indoxyl sulfate in vegetarians versus omnivores. Clin J Am Soc Nephrol. 2012; 7: 982-988.

[27] Salmean YA, Segal MS, Palii SP, Dahl WJ. Fiber supplementation lowers plasma p-cresol in chronic kidney disease patients. J Ren Nutr. 2015; 25: 316-320.

[28] Singh J, Metrani R, Shivanagoudra SR, Jayaprakasha GK, Patil BS. Review on bile acids: Effects of the gut microbiome, interactions with dietary fiber, and alterations in the bioaccessibility of bioactive compounds. J Agric Food Chem. 2019; 67: 9124-9138.

[29] Gorvitovskaia A, Holmes SP, Huse SM. Interpreting Prevotella and Bacteroides as biomarkers of diet and lifestyle. Microbiome. 2016; 4:15.

[30] Wu GD, Chen J, Hoffmann C, Bittinger K, ChenYY, Keilbaugh SA, Bewtra M, Knights D, Walters WA, Knight R, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science. 2011; 334: 105-108.

[31] Paoli A, Mancin L, Bianco A, Thomas E, Mota JF, Piccini F. Ketogenic diet and microbiota: Friends or enemies? Genes. 2019; 10: 534.

[32] Ridlon JM, Kang DJ, Hylemon PB, Bajaj JS. Bile acids and the gut microbiome: Curr Opin Gastroenterol. 2014; 30: 332-338.

[33] Hidalgo M, Prieto I, Abriouel H, Cobo A, Benomar N, Gálvez A, Martínez-Cañamero M.. Effect of virgin and refined olive oil consumption on gut microbiota. Comparison to butter. Food Res Int. 2014; 64: 553-559.

[34] de Wit N, Derrien M, Bosch-Vermeulen H, Oosterink E, Keshtkar S, Duval C, de Vogel-van den Bosch J, Kleerebezem M, Müller M, van der Meer R. Saturated fat stimulates obesity and hepatic steatosis and affects gut microbiota composition by an enhanced overflow of dietary fat to the distal intestine. Am J Physiol-Gastrointest Liver Physiol. 2012; 303: G589-G599.

[35] Olson CA, Vuong HE, Yano JM, Liang QY, Nusbaum DJ, Hsiao EY. The Gut microbiota mediates the anti-seizure effects of the ketogenic diet. Cell. 2018; 173: 1728-1741.

[36] Paoli A, Mancin L, Giacona MC, Bianco A, Caprio M. Effects of a ketogenic diet in overweight women with polycystic ovary syndrome. J Transl Med. 2020; 18: 104.

[37] Pérez-Guisado J, Muñoz-Serrano A, Alonso-Moraga Á. Spanish ketogenic mediterranean diet: A healthy cardiovascular diet for weight loss. Nutr J. 2008; 7: 30.

[38] Storoni M, Plant GT. The therapeutic potential of the ketogenic diet in treating progressive multiple sclerosis. Mult Scler Int. 2015; 2015: 681289.

[39] Lindefeldt M, Eng A, Darban H, Bjerkner A, Zetterström CK, Allander T, Andersson B, Borenstein E, Dahlin M, Prast-Nielsen S. The ketogenic diet influences taxonomic and functional composition of the gut microbiota in children with severe epilepsy. NPJ Biofilms Microbiomes. 2019; 5: 5.

[40] Tagliabue A, Ferraris C, Uggeri F, Trentani C, Bertoli S, de Giorgis V, Veggiotti P, Elli M. Short-term impact of a classical ketogenic diet on gut microbiota in GLUT1 deficiency syndrome: A 3-month prospective observational study. Clin Nutr ESPEN. 2017; 17: 33-37.

[41] Ma D, Wang AC, Parikh I, Green SJ, Hoffman JD, Chlipala G, Murphy MP, Sokola BS, Bauer B, Hartz AMS, Lin A-L. Ketogenic diet enhances neurovascular function with altered gut microbiome in young healthy mice. Sci Rep. 2018; 8: 6670.

[42] Lee RWY, Corley MJ, Pang A, Arakaki G, Abbott L, Nishimoto M, Miyamoto R, Lee E, Yamamoto S, Maunakea AK, et al. A modified ketogenic gluten-free diet with MCT improves behavior in children with autism spectrum disorder. Physiol Behav. 2018; 188: 205-211.

[43] Newell C, Bomhof MR, Reimer RA, Hittel DS, Rho JM, Shearer J. Ketogenic diet modifies the gut microbiota in a murine model of autism spectrum disorder. Mol Autism. 2016; 7: 37.

[44] Swidsinski A, Dörffel Y, Loening-Baucke V, Gille C, Göktas Ö, Reißhauer A, Neuhaus J, Weylandt KH, Guschin A, Bock M. Reduced mass and diversity of the colonic microbiome in patients with multiple sclerosis and their improvement with ketogenic diet. Front Microbiol. 2017; 8: 1141.

[45] Lee JE, Titcomb TJ, Bisht B, Rubenstein LM, Louison R, Wahls TL. A Modified MCT-based ketogenic diet increases plasma β-hydroxybutyrate but has less effect on fatigue and quality of life in people with multiple sclerosis compared to a modified paleolithic diet: A waitlist-controlled, randomized pilot study. J Am Coll Nutr. 2021; 40: 13-25.
[46] Zhang Y, Zhou S, Zhou Y, Yu L, Zhang L, Wang Y. Altered gut microbiome composition in children with refractory epilepsy after ketogenic diet. Epilepsy Res. 2018; 145: 163-168.
[47] Murtaza N, Burke L, Vlahovich N, Charlesson B., O' Neill H., Ross ML, Campbell KL, Krause L, Morrison M. The effects of dietary pattern during intensified training on stool microbiota of elite race walkers. Nutrients. 2019; 11: 261.