

The Role of Ketogenic Diet in Gut Microbiota

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Abstract. Several studies point to a vital role for gut microbiota (GM) in preventing disease and reducing inflammation in humans. Gut microbiota has an important relationship with the human brain-gut axis, and the biological metabolites they produce are closely linked to the function of nervous system. Ketogenic diet (KD) is thought to be effective on the makeup of GM and thus affecting human health due to its low calorie and fiber consumption. Recent research has found KD can affect GM composition under pathological conditions, such as drug refractory epilepsy (DRE). So as to achieve the purpose of treating DRE. Therefore, this article aims to explore the effect of KD on the human GM and explore whether it has important implications for human health. Finally, we found that KD can modulate human health by affecting gut microbiota richness, increasing some microbes that can produce beneficial metabolites, and reducing some pro-inflammatory microbes to prevent and treat specific diseases.

Keywords: Ketogenic diet, Gut microbiota, Epilepsy

1. Introduction

Human gut microbiota (GM) was confirmed to have essential function in human metabolism, physiology and immune processes, and dietary habits are an important factor in determining the component as well as function of GM, thus largely determining the structure of human GM [1]. Because of its symbiotic interaction with humans, gut microbiota is an essential element in maintaining normal physiological metabolism of human body and avoiding diseases and other physiological processes [2]. The human body's health and the management of some diseases are impacted by the gut microbiota, which is a dynamic community that changes as a result of changes in many circumstances, including dietary patterns. Therefore, it is crucial to determine the impact of a specific dietary pattern, such as ketogenic diet (KD), on the composition, structure, and function of GM. Ketogenic diet can be a popular representative of low-carbohydrate diet, which is characterized by carbohydrate restriction and high-fat diet. It has received widespread attention due to its association with the treatment and prevention for certain diseases like drug refractory epilepsy (DRE) [3].

The purpose of the essay is to look into the potential effects of KD on GM composition, and then to look into the effects of ketogenic diet-induced alteration on human health and specific illnesses.

2. The Connection Between Human Health and Gut Microbiota

2.1. Association Between Symbiotic Effects of Gut Microorganisms and Immune System

The vaginal region, respiratory tract, skin surface, gastrointestinal system, and oral cavity are among the places where microorganisms can be found, among which microorganisms in the gastrointestinal tract system are now a direction of research on human nutrition and microbial effects [1]. Symbiosis refers to the phenomenon that multiple organisms grow together, influence each other, and promote each other's growth. The microbiota significantly affects how the human immunity works and how it is activated. Therefore, the human immune system produces the function of maintaining a symbiotic relationship with a variety of different microorganisms. According to statistics, there are about 10^{14} different kinds of bacteria in the human intestinal tract, and most of them cannot be cultured alone [2]. The genes can affect the physiological activities of organisms, and then directly affect the metabolic pathways of the host. The number of genes in human GM greatly

outnumbers others in human genome, according to studies. Millions of microorganisms and many types of individual bacteria make up this organism. Therefore, gut microbes can produce a large number of life metabolites that the human body cannot synthesize [2]. And these genes can encode several highly specific enzymes capable of digesting and fermenting complex biological macromolecules by hydrolyzing glycosidic bonds [2]. At the same time, they also use their metabolites to provide nutrients to the host, otherwise, some substances cannot be directly absorbed by the host. So the human body allows these microorganisms to exist symbiotically in the gut instead of foreign substances such as pathogens and antigens that cause an immune response and are cleared from the mucosa [2].

The microbiota inhibits pathogen colonization through multiple mechanisms, involving direct rivalry for resources, the creation of antimicrobial compounds, and the control of host immunological responses, which are its primary functions [4]. Numerous inflammatory and autoimmune illnesses, including those of central nervous system(CNS), are caused by microbes in human gastrointestinal tract. Several bacterial species can produce specialized commensal antigens that induce immunological modulation [4]. Gut microbes are part of the "microbe-gut-brain" axis that affects CNS by producing various neuroactive compounds that affect human behavior, and gut microbes can also produce antigens through which they pass to mimic the host's neuropeptides and neurohormones to trigger autoantibody production [4]. And studies have confirmed that gut microbes can affect diseases in CNS by affecting the immunity system and then through the axis of gut-brain such {Smith, 2013 #6} as multiple sclerosis, anorexia nervosa, etc [4].

2.2. Association Between Gut Microbiota Imbalance and Human Disease

These intestinal bacteria are what provide the host its own and distinctive enzymatic and metabolic pathways [5]. According to current research, there are five potential pathways of interaction between GM and the brain. These routes connect the blood-brain barrier to neuronal connections, the neuroendocrine HPA axis, gut bacteria that produce neurotransmitters, the gut immune system, and lastly the gut mucosal barrier [5]. Moreover, microbiota can also help to reduce pathogen colonization. When GM is unbalanced, it will decrease the number of dominant non-pathogenic microbiota and its ability to colonize, allowing opportunistic pathogens to establish the niche, which eventually results in infection [1].

After being processed in the stomach, human dietary components will eventually reach the large intestine to be metabolized and fermented by anaerobic microorganisms. Its fermentation products will eventually show different biological effects according to the different fermentation substrates and fermentation microorganisms. Some of them are systemic protective metabolites that are beneficial to the human body, like short-chain fatty acids (SCFAs), glucose, succinate, and some are carcinogenic destructive metabolites such as phenols, phenyl acetic acid and p-cresol [1]. Additionally, it has been demonstrated that GM bacteria that can digest dietary fiber are capable of producing active SCFAs, including butyric as well as propionic acids. They have positive biological effects and may contribute to protect cancer and inhibiting inflammatory cells, moreover, it has been found that active SCFAs generated in mice through microbial fermentation can control the function and size of the colon's Treg pool and avoid colitis in a way that is dependent on Ffar2 [6].

Rheumatoid arthritis (RA) is an autoimmune disease that causes the body to produce autoantibody, eventually leading to bone destruction in multiple joints. Numerous studies have revealed that during infection, the composition of patients' GM changes. Approximately 33% of RA patients in Japan were found to have an expanded population of *Prevotella copri* in the gut [7]. *Faecalibacterium*, a butyrate-producing bacterium, was shown in a study to protect intestinal epithelial cells and exert good anti-inflammatory properties, thereby reducing the incidence of RA [7].

GM is crucial for regulating and preventing human cancer. And sundry microbes in the gut can also affect certain cancers. Some enteric serotypes of pathogens such as *Typhimurium*, *Helicobacter pylori*, *Streptococcus pneumoniae* produce polyamines in the gut such as spermidine, spermine polyamine, putrescine, and these metabolites can cause cancer and oxidative stress at a certain time

[1]. Therefore, maintaining control over the intestinal microbiota without an imbalance of pathogenic microbes as well as harmful metabolites is essential for the prevention and regulation of several human diseases.

2.3. Influence of Gut Microbial Composition on Drug Refractory Epilepsy (DRE)

Epilepsy is a long-term neurological disease characterized by a persistent tendency to seizures [3]. Pediatric epilepsy is ubiquitous worldwide. Despite the fact that anti-epileptic drugs (AED) as well as surgery are currently the widely prescribed therapies for infantile epilepsy, 30% of epilepsy infants continue to experience therapeutic futility and repeated attacks [3]. Drug refractory epilepsy (DRE) affects 10%-20% of children with epilepsy according to statistics, and its pathogenesis is multifactorial, so the pathogenesis and causes of many patients are still unclear [3]. Current evidence points to gut dysbiosis as a possible causative factor [3]. Numerous reports have implicated GM in the system of enteric nervous, barrier of blood to brain, and developing of glial cells, are critical for controlling behavior and progressing cognition associated with epilepsy [8]. It has been revealed that the gut microorganisms like *Lactobacillus* and *Bifidobacterium* produce the inhibitory neurotransmitter named gamma aminobutyric acid (GABA), which is the primary component of mammalian CNS [3]. And researchers have found that the increase in GABA is related to diet and microbiota-dependent seizure protection [3].

3. Ketogenic Diet

3.1. Definition and Characteristics of KD

Dr. Russell Wilder of the Mayo Clinic developed the first ketogenic diet therapy (KDT), also known as classic KDT, in 1923 to treat epilepsy. The classic KDT is based on a 1:3 or 1:4 ratio of carbohydrate and protein to fat, which represents 90% of energy from fat and only 10% from a mixture of carbohydrates and protein [9]. The ketogenic diet has been recommended by patients and medical staff in recent decades because of its special efficacy in the treatment of epilepsy, and over time this high-fat, low-carbohydrate diet has become more and more mainstream [10]. It is estimated that KD has helped reduce the seizure burden by at least 50% in over 50% of children whose seizures cannot be controlled only by medication, and 10-15% of these children are seizure-free [11]. At present, to properly satisfy the needs of patients, more and more different types of more flexible ketogenic diets have been discovered. There are now five variations of the ketogenic diet that have been successfully used to treat disorders with underlying metabolic dysregulation, including epilepsy, as well as other conditions including cancer and Alzheimer's disease [10].

3.2. The Therapeutic Value of A Ketogenic Diet for Treating Illnesses

According to studies, children with DRE now benefit from the KD in terms of its therapeutic effects the most. Although the exact process is still unclear, it has been proposed that it may be connected to changes in mitochondrial activity, ketone bodies' impact on neurotransmitter release and neuronal function, the antiepileptic effects of fatty acids [12]. Previous studies have shown that the mechanism that controls seizures is related to the production of ketone bodies from fatty acid oxidation [13]. Additionally, the usage of KD in the treatment of several neurological conditions is progressively growing, including metabolic syndrome, cancer, Alzheimer's disease [10]. Fig. 1 shows how very low calorie ketogenic diet (VLCKD) affects human intestinal health [12].

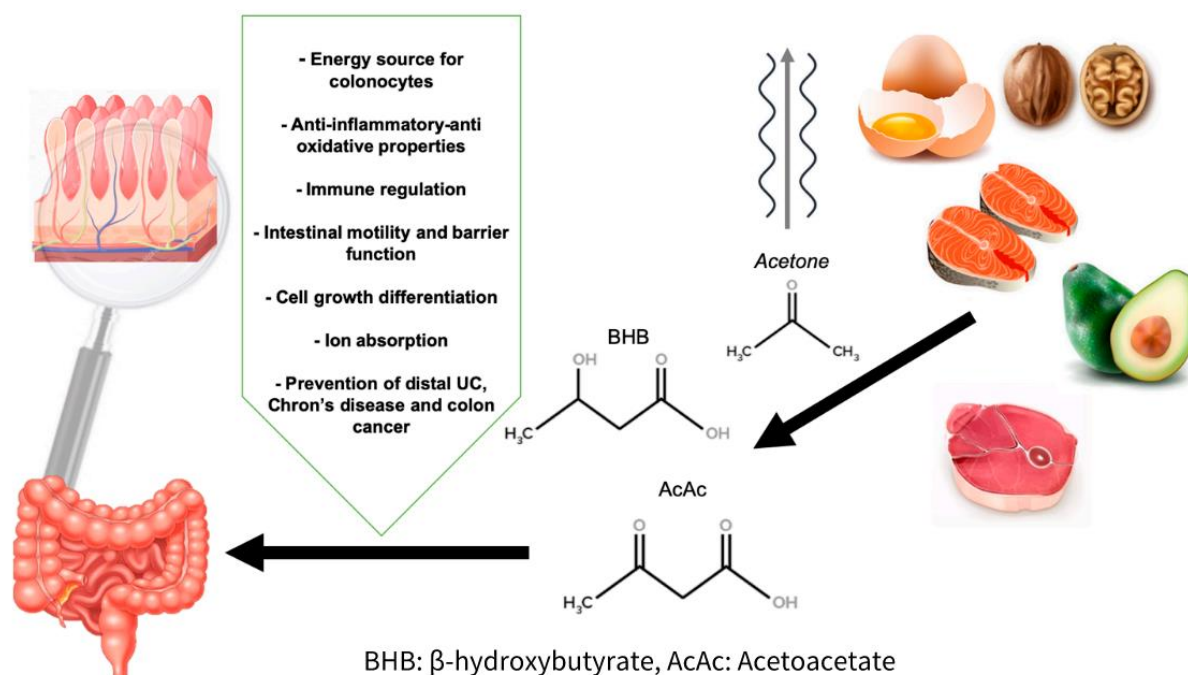


Fig. 1 Influence of ketone bodies and VLCKD on gut health [12].

4. Recent Research Progress on the Effects of KD on Human GM

4.1. Effects of KD to the Makeup of GM

The composition of GM can be modified by exercise and diet, and it has been shown that weight loss induced by VLCKD leads to a reduction in the gut microbiota of *Cryptobacterium* and *Roseobacter*, and an increasing in *Ristensenaceae* and *Akkermansiae* [14]. Furthermore, according to MA et al., KD reduces carbohydrate consumption, which leads to a drop in several gut microbes that produce energy from polysaccharides, leading to a drop in gut microbial diversity [15]. According to Table 1, several research are being conducted to investigate the features and changes in gut microbiota composition during KD [16]. Using qPCR analysis, Newell et al. found that a KD diet consisting of 75% kcal fat had an altered effect on GM in an ASD mouse model. They found a drop in bacterial richness in the cecum and feces, such as *A. muciniphila*, *Methanobrevibacter*, and *Roseburia* [17]. Another study of children with DRE also noted the richness of GM decreased in patients treated by KD [3]. A ketogenic diet consisting of SCFAs, monounsaturated fatty acids and polyunsaturated fatty acids, after an animal study by Ma et al., was shown to increase some beneficial gut microbes like *Akkermansia Muciniphila* and *Lactobacillus* that produce SCFAs. Also they found that after KD treatment, some pro-inflammatory microorganisms like *Turicibacter* and *Desulfovibrio* were reduced, which is believed to be linked to the reduction of Alzheimer's disease as well as neurovascular disease in mice [15].

Table 1. The discoveries concerning the influence of the ketogenic diet (KD) on GM [16]

Subjects/ Animals	N of the Subjects	Ages of the Subjects	Time of Administration	Ketogenic Diet	Findings
Male mice of C57BL/6	9-10 for treated and untreated groups	12 to 14 weeks old	112 days	75.1 % fat(Saturated, monounsaturated, and polyunsaturated fatty acids make up the majority of the fat) + 8.6 % protein, 4.8 %fiber, 3.2 % carbs, 3 % ash, and other nutrients.< 10 %	Microbiota diversity decreases; <i>Adlercreutzia</i> increase, <i>Lactobacillus</i> , <i>Erysipelotrichaceae</i> - <i>Clostridium</i> , <i>A. muciniphila</i> , <i>Turicibacter</i> , <i>Clostridiaceae</i> - <i>Clostridium</i> , <i>Dorea</i> , <i>Desulfovibrio</i> decrease
Mice of SPF C3HeB/FeJ KCNA1 KO + Germ Free wild-type Swiss Webster	Variable for each group	3 to 4 weeks old	3 weeks	6:1 KD	α -diversity decrease; <i>A.muciniphila</i> and <i>Parabacteroides</i> increase

4.2. Effect of Ketogenic Diet on Intestinal Microbial Composition of Drug Refractory Epilepsy (DRE) Patients

KD is expected to alter the formation of the gut microbiome and increase intestinal microbes that produce the beneficial metabolites, which has been observed to improve clinical symptoms of infantile DRE, including lowering the frequency of seizures [18]. Recently, several people have studied the impact of KD for anti-epileptic-related gut microbiota changes in mice. One study found that two microbes, *Akkermansia* and *Parabacteriodes*, increased significantly in mice after four days of VLCKD treatment [19]. In one study, KD was shown to increase the fecal SCFAs concentration of DRE patients. SCFAs may indirectly or directly affect the human nervous system to reduce the incidence of epilepsy in connection by a microbiome-gut-brain axis, according to numerous studies that have confirmed their positive influence on mammals, including immune regulation and energy usage [18]. Another study, shown in Fig. 2, compared infants with epilepsy (P1), infantile epilepsy patients after KD treatment (P2), and healthy infants (Health) in significantly enriched gut microbiota components [3]. These results all demonstrate that there are one or more specific gut microbes that can serve as effective biomarkers and potential therapeutic targets in DRE patients and can be modified by KD diet treatment [18]. In fact, the GM of infantile epilepsy patient differed from healthy controls and was significantly altered after KD treatment, with decreased pathogens, increased beneficial bacteria, increased concentrations of active metabolites that relieve epilepsy symptoms such as SCFAs, and thus decreasing the seizure frequency in infants with epilepsy [3]. In addition, it is not just infants that there is positive evidence that KD has a great treatment effect in adult DRE and refractory status epilepticus, and extends to a variety of neurological and adult non-neurological disorders [3].

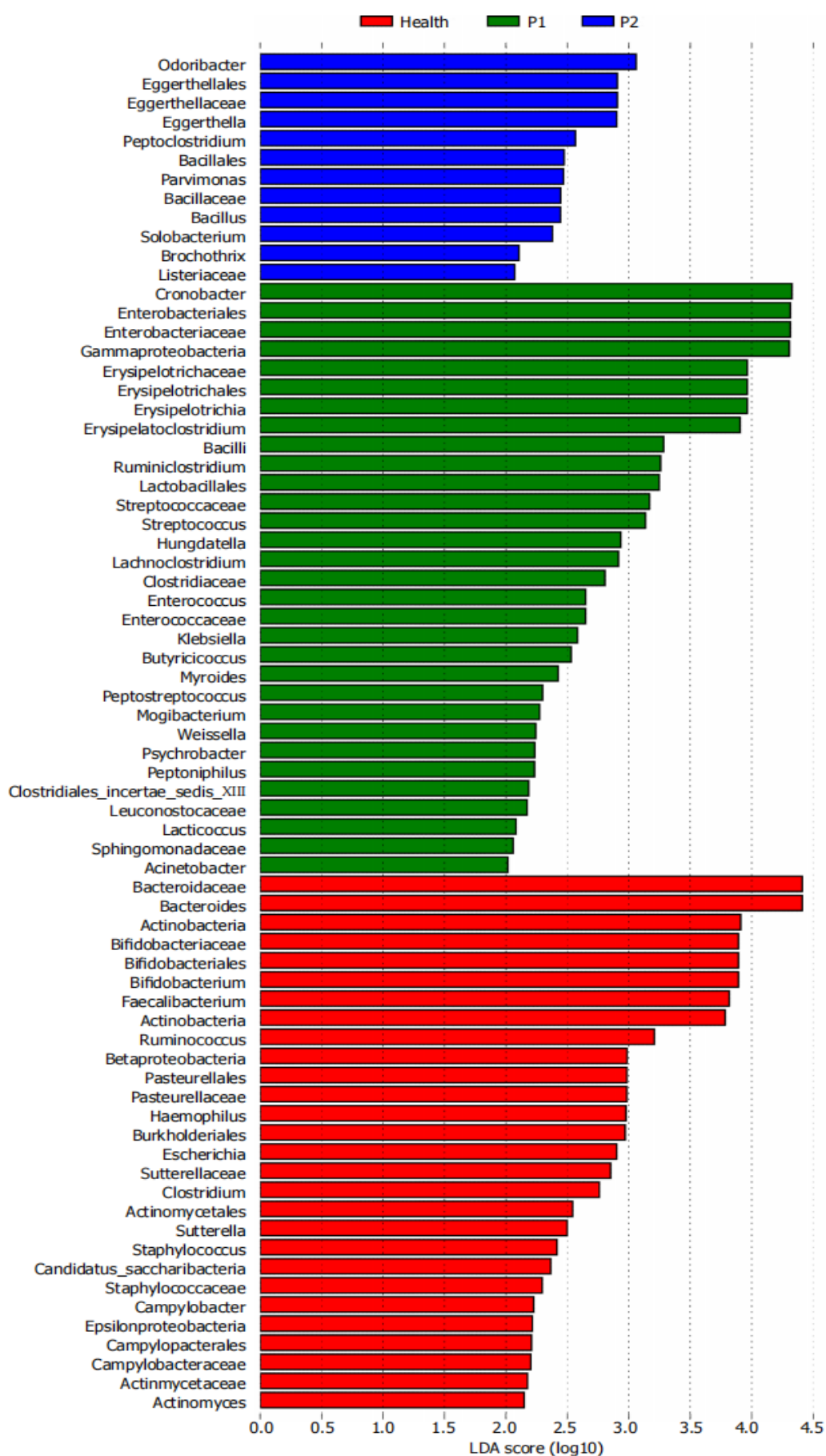


Fig. 2 Significantly enhanced GM components in the P1, P2, Health groups [3].

5. Conclusion

In conclusion, various research has pointed that GM may generate a wide range of metabolites, such as SCFAs, which will make a significant impact on the regulation of human immune system. Therefore, maintaining intestinal microbial homeostasis and controlling the concentration of positive metabolites such as SCFAs within the intestine is a direction for future research on the prevention and treatment of specific illnesses. Secondly, as a dietary modification treatment. KD is capable to affect the abundance of gut microbiota in DRE patients, and increase some beneficial microorganisms such as *Akkermansia muciniphila* and *Lactobacillus* which can produce SCFAs, thus playing a vital role in the treatment of DRE. However, specific treatment mechanism of the ketogenic diet in DRE patients is not yet clear, but it can be confirmed that the ketogenic diet can reduce the incidence of DRE patients to a certain extent. However, there is currently insufficient research on the specific influence of KD on GM. Moreover, there is a lack of experiments in patients with other diseases. Future research can focus on studying the effect of KD in patients with other diseases and disease treatment on GM, and explore the effect of different types of ketogenic diet on GM.

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