

## KETOGENIC DIET AND GUT MICROBIOTA

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### Abstract

Over the last years numerous studies suggest that gut microbiota plays an important role in many aspects of human health and diseases. The intestinal microbiota is a very dynamic community of microbial cells that are influenced by external factors like nutrition. In fact gut microbes use ingested nutrients for fundamental biological processes, producing metabolics outputs that have significant impact of human physiology. Considering that microbial community has multiple mechanisms of action such as the production of bioactive compounds, pathogen protection, energy homeostasis, nutrient metabolism and regulation of immunity, establishing the influence of diet is very important. The ketogenic diet (KD), which has become very popular in recent years, is characterized by high levels of fat, adequate levels of protein and low levels of carbohydrate and is a dietary approach used for weight loss and neurological diseases.

The purpose of this minireview is to analyze the interactions between the ketogenic diet and gut microbiota and how this could affect human health.

**Keywords:** *microbiota, gut microbiota, ketogenic diet.*

## Introduction

The study of the microbiota and everything that revolves around it has multiplied in recent years and the discoveries and information deriving from this increase in work has led the scientific community to review the perception they had on the huge amount of microorganisms present in the tract human gastrointestinal (GI) [1].

The human microbiota is considered an endocrine organ because it exerts a direct action on the intestinal mucosa and the enteric nervous system and is also able to influence the function of other organs and systems besides the GI. It is very extensive and heterogeneous both for its numerous morphological and biochemical constituents. Studies show that the biochemical complexity of the intestinal microbiota exceeds that of the brain [1]. The microbiota includes trillions of microbial cells and thousands of bacterial species [2]. Most of the intestinal flora is composed of microorganisms defined as strict anaerobes, which exceed the amount of facultative anaerobes and other anaerobes by two or three orders of magnitude. Over 50 bacterial phyla have been described, the human gut microbiota is dominated by only two phyla, the Bacteroidetes and the Firmicutes [3].

The microbiota influences the host through the production of bioactive metabolites, which regulate many biological pathways involved in immunity and energy. Microorganism produces a great amount of B12 and K vitamins [4-5], short chain fatty acids (SCFAs) (butyrate by Firmicutes, propionate by Bacteroidetes and acetate by anaerobes) which represent the greatest source of energy for intestinal absorptive cells [6-7], and  $\gamma$  amino butyric acid (GABA), the major inhibitory neurotransmitter of mammalian central nervous system [8].

Numerous metagenomic data produced by the comparison of the feces of sick and healthy subjects have shown that the profile of the intestinal microbiota changes considerably if the subject is healthy or suffering from some pathology. Numerous data and observational studies have described a significant correlation between an imbalance of the microbiotic profile, or dysbiosis, and the development of some very serious diseases. In fact, changes in the gut microbiota have been

observed in individuals with obesity, diabetes, liver disease, cardiometabolic disorders, inflammatory bowel disease, autoimmune conditions, cancer, and even nervous system disorders. From these considerations it can be deduced that the intestinal microbiota can be a potential source of innovative therapies. Both environmental and genetic factors represent the two great stimuli capable of influencing the composition of the intestinal microbiota. It is not surprising if elements such as human genetics, mode of delivery, different types of diets or prolonged fasting, any medications, stress, infections, harmful habits such as smoking, physical inactivity or any interventions such as bariatric surgery can modify the human gut microbiota [9].

A study evaluated the impact of dietary fibers on the composition of the microbiota: it showed that a diet composed of non-refined foods and rich in "microbiota accessible carbohydrate" (MACs) is able to support the growth of microbes that produce short chain fatty acids (SCFAs), which are important signalling molecules between the intestinal microbiota and the host [10]. Moreover, specific type of fatty acids affect the gut microbiota in different way [11], in particular monounsaturated fatty acids (MUFA's) and polyunsaturated fatty acids (PUFA's) omega 3 may be the control key of low-grad systemic inflammation, gut inflammation and as well as obesity [12]. Studies conducted on humans have shown that a diet rich in fats induces an increase in anaerobic bacteria and Bacteroidetes while a diet rich in monounsaturated and polyunsaturated fats does not alter the intestinal microbiota [13]. Instead, the Western diet, high fat – high sugar and low fibers, reduces the production of SCFAs and produces detrimental metabolites, favouring the proliferation of bacteria related to chronic inflammation [14]. A diet consisting of fructose, glucose and sucrose in the form of a lot of fruit administered to people showed, after careful analysis of the intestinal microbiota, an abundance of Bifidobacterium and a reduction of Bacteroides. The addition of lactose to the diet confirmed these changes, also reducing Clostridium [13]. A diet rich in proteins allows the development of proteolytic bacteria such as Bacillus, Clostridium perfringens, Streptococcus, Staphylococcus and Bacteroides

fragilis. The proteins undergo a process called fermentation and takes place in the distal colon where the carbohydrates are now exhausted and the pH is close to neutral. Excessive consumption of protein in a diet can increase fecal pH [15]. Some strains of bacteria present in the human microbiota are involved in the formation of compounds such as phenol and indole from the deamination of aromatic amino acids. Among those responsible we have the Bacteroidetes, the Clostridium, the Bifidobacterium, the Enterobacterium and the Lactobacillus. The precursors of nitrosamine, a known carcinogen, originate from amines, produced by the decarboxylation of amino acids by Clostridium, Bacteroidetes and Bifidobacterium [15-16].

Specialized and restricted dietary approach adopted as a treatment of some diseases, such as ketogenic diet for refractory epilepsy, by reducing or excluding certain type of foods, may influences the microbiota composition and then the host physiology [17-19]. That is the case of very low carbohydrate ketogenic diet (VLCKD) that, by the drastic reduction of the carbohydrate intake, showed an impairment both on the diversity and richness of gut microbiota [20]. Ketogenic diet permits a very low carbohydrate consumption (around 5% to 10% of total caloric intake or below 50g per day) to enhance ketone production (3 hydroxybutyrate, acetate and acetoacetate) [21]. Originally, VLCKD has been used as a treatment for epileptic patients that non respond to anticonvulsant medication [22], but currently it has become popular for its benefits extended to metabolic diseases, obesity and neurodegenerative diseases such as autism spectrum disorder (ASD), Alzheimer's diseases [23], glucose transporter 1 deficiency syndrome [24] and auto immune multiple sclerosis (AIMS) [25].

## Methods

Narrative review with experimental animal model studies and population studies on humans in which authors investigated the effect of ketogenic diet on gut microbiota.

## Results

Olson et al. [26] found that two species of bacteria (Akkermansia and Parabacteriodes) were significantly increased in mice that were fed ketogenic diet, and colonization with these species revealed an anti-seizure effect in germ-free mice or treated with antibiotics.

Ma and colleagues [27] studied mice treated with ketogenic diet rich in short fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), and found an increase in beneficial bacteria (Akkermansia Muciniphyla and Lactobacillus) and a reduction in pro-inflammatory microbes (Desulfovibrio and Turicibacter). Furthermore mice showed neurovascular improvements related to a lower risk of developing Alzheimer's disease.

In a 2016 study on murine models of autism, the ketogenic diet led to the improvement of the Firmicutes to Bacteroides ratio and to an enhancement in ASD behavioural symptoms [28]. In animal models, a ketogenic diet with 75% fat and maintained for 10-14 days led to a significant reduction in Methanobrevibacter, A. muciniphila, Enterobacterium, Lactobacillus and Roseburia [29]. A further study conducted on mice showed that following a ketogenic diet with 75% fat and maintained for 10-14 days, C. coccoides is the only component of the gut microbiota that does not undergo modifications. C. leptum correlates negatively with serum ketones and positively with blood glucose levels and, therefore, is highly sensitive and its concentrations fluctuate widely in response to diet. Bacteroides and Prevotella are linked to  $\alpha$ -hydroxybutyrate and have increased, contrasting the data of numerous experimental studies [30].

In multiple sclerosis (MS) the dysbiotic colonic flora ferments foods into dangerous compounds affecting the organism. The KD restored the microbial bio-fermentative mass and normalizing the concentration of the colonic microbiome. The study was conducted on subjects suffering from multiple sclerosis and subjected to a ketogenic diet for 6 months with a quantity of carbohydrates less

than 50 g / day and demonstrated a variation in the composition of the intestinal microbiota. The study showed that *E. rectale*, *Akkermansia* and *C. cocoides* do not vary during the entire period of the ketogenic diet. *Bacteroides*, *Ruminococcus*, *Verrucomicrobium* and *F. prausnitzii* are reduced already after 2 weeks of ketogenic diet and then gradually increase and return to the initial levels or exceed them in the following weeks. Colonies of *Enterobacteriaceae* and *B. longum* are drastically reduced after 12 weeks on a ketogenic diet, while *C. difficile*, *C. histolyticum*, *Lactobacillus*, *Prevotella* and *B. bifidum* after only 2 weeks. [31]

A study in children [32] investigated the connection between microbiome and refractory epilepsy. Patients with epilepsy have a higher amount of pathogenic proteobacteria (*Escherichia*, *Salmonella* and *Vibrio*), which decreased after KD treatment. Moreover, the authors found an increase of *Bacteroidetes* that digest and metabolize high-fat nutrients and regulate secretion of 6-17 interleukins in dendritic cells, which is connected with the seizure effect on epileptic patients [33].

In other studies, the KD has led to further results: increase of *Bacteroides* and decrease of *Firmicutes* and *Actinobacteria* in responders pediatric patients (with reduced or stopped seizure frequency) and increase of *Clostridia*, *Ruminococcus* and *Lachnospiraceae* in non-responders [34]; increase in *Desulfovibrio* spp. in patients affected by Glucose Transporter 1 Deficiency Syndrome [35]; reduction of *Bifidobacteria* in epileptic children that are health benefits (prevention of colorectal cancer, irritable bowel syndrome and necrotizing enterocolitis) [36].

At the moment, very few data are available on the action of ketone bodies on the intestinal microbiota. Crawford et al. in 2009 [37] showed that the microbiota of mice, following a 24-hour fast, did not undergo a significant change in bacterial diversity, but nevertheless gave useful indications: fasting was associated with a significant increase in *Bacteroidetes* and a significant reduction in *Firmicutes*. These changes are contrary to those seen when mice switch from a low-fat diet to a Western-style diet.

## Discussion

All the studies examined, revealed supposed connection between gut microbiota, ketogenic diets and systemic effects.

Bacteria taxa, richness and diversity are strictly influenced by ketogenic diet. A few human and animal studies have shown different results demonstrating positive effects on reshaping bacterial architecture and gut biological functions, while others reporting negative effects as a lowered diversity and an increased amount of pro-inflammatory bacteria.

Nevertheless, short period studies and with specific disease conditions have been carried out [28, 31-32, 35], limiting generalization to the overall population. Additionally, the microbiota may be highly variable and its plasticity could be dependent on past and specific dietary pattern [38]. In agreement with these considerations, Healey and colleagues concluded that because of the high variability among people of microbiome composition, it is actually difficult to identify how microbiota may change the diversity in relation to a specific dietary pattern [39].

Nonetheless, it is essential to point out that the modified microbiota composition, changed by KD, play an important role on itself activity of KD [26, 28, 32]; the changes have been demonstrated to be necessary in order to provide positive effects such as the anti-seizure effect and amelioration of neurovascular function [26, 34].

The observation that a KD can modulate and reshape gut microbiota represent a potential and promising future therapeutic approach. Ketogenic diet is a powerful tool and needs to be further refined and well formulated considering its impact on gut health. In conclusion, further research with long-term clinical trials has to be performed in order to establish safer and healthier specific dietary intervention for patients.

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