

Opinion

Clostridium butyricum Strain MIYAIRI 588 (CBM588) as a Precision Probiotic Therapy in the Ketogenic Diet: A Possible Application?

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Abstract: The ketogenic diet has proven to be effective in many recent studies not only as a weight-losing strategy but also as a valuable add-on therapy in medical conditions such as diabetes and epilepsy. Additionally, frequent conditions such as autism spectrum disorders and Alzheimer disease could have a benefit derived from ketogenic diet metabolic changes. Many of these benefits could be driven by an intestinal microbiota change. While the effects of a ketogenic diet on microbiota should still be thoroughly clarified, as most studies observe an increase in bacterial strains considered neuroprotective such as *Akkermansia muciniphila*, with a concomitant reduction in some pathogenic strains such as *Salmonella* spp. it is important to highlight how many studies show a reduction in butyrate-producing strains, leading to a colonic proinflammatory state with increased intestinal permeability and an increase in pathogenic bacterial strains. The *Clostridium butyricum* strain MIYAIRI 588 (CBM588) is a butyrate-producing strain that was recently approved for human use in Europe due to its safety and effectiveness. The beneficial effect of CBM588 on the human colon could derive from a mucosal layer thickness increase and mucosal immune cell regulation, leading to a reduction in diarrhea and mucosal damage. Additionally, CBM588 could improve systemic insulin sensitivity and reduce the splanchnic organ inflammatory state. Therefore, CBM588 is a bacterial strain that should be considered an add-on when following a ketogenic diet, leading to a reduction in some of the potential gastrointestinal side effects and improving weight management through increased insulin sensitivity and the optimization of the lipid metabolism.

Keywords: ketogenic diet; probiotic; *Clostridium butyricum* CBM588; *Clostridium butyricum* MIYAIRI 588; CBM588; weight loss; butyrate



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1. Introduction

The ketogenic diet is a nutritional approach which has been used since the 1920s in the management of epilepsy refractory to other therapeutic approaches [1]. In recent decades, the ketogenic diet has been considered in the management of other morbid conditions, for example, the management of obesity and other metabolic and neurodegenerative disorders [2]. Unlike the Mediterranean diet, which is based on a significant consumption of vegetables, fruit, nuts, whole grains, and extra virgin olive oil, the modest consumption of fish and poultry and the limited consumption of sweets, red meat, and dairy products, and considered by some authors to be functional for the management of obesity and the prevention of metabolic and chronic degenerative diseases [3,4], the ketogenic diet is characterized by a very low carbohydrate consumption (between 5% and 10% of one's total caloric intake, for a total, in any case, of less than 50 g per day). This is used as a means of increasing the production of endogenous ketones [5] which are capable of constituting

an alternative energy substrate for various organs and tissues. These characteristics have led us to investigate the use of the ketogenic diet in the management of autism spectrum disorder (ASD), Alzheimer's disease [6], glucose transporter 1 deficiency syndrome [6], and autoimmune multiple sclerosis (AIMS) [7]. Due to the particular characteristics of the ketogenic dietary model, the impact on the intestinal microbiota and, consequently, the implications this could have for health are the subject of much previous research [8–11], as is the use of potential solutions for microbiota management when following a ketogenic diet.

2. Gut Microbiota and Nutrition: An Ecosystem View

The intestinal microbiota is considered, by some authors, to be a real organ [12] capable of modulating the health of the organism at different levels by intervening with the production of bioactive compounds, providing protection from pathogens, intervening in energy homeostasis, in the metabolism of nutrients, and in immune modulation, which constitutes an extremely dynamic entity constantly influenced by environmental and nutritional factors [13]. Numerous nutritive and non-nutritive substances, such as dietary fibers, are introduced into the body through food. Substances not considered nutrients for the human body can be considered nutrients for the microbiota. Many substances classified as “dietary fiber”, for example, can be considered “microbiota-accessible carbohydrates” (MACs), as they can be metabolized by various microorganisms, including those capable of producing short-chain fatty acids (SCFAs) [14]. SCFAs produced by bacteria constitute the main source of energy for human colonocytes, they carry out a key signaling role between the intestinal microbiota and the host [14], and they have the ability to influence the resolution of the inflammatory process [15]. Among the main SCFAs produced by bacterial fermentation processes, we can identify butyrate mainly produced by *Firmicutes*, propionate mainly produced by *Bacteroidetes*, and acetate mainly produced by anaerobes [14,16]. Some microorganisms show the ability to produce various other physiologically essential substances. Some show the ability to produce vitamins, including B12 and K, functionally integrating what is taken with the common diet [17,18]. Recently and interestingly, it has been highlighted how intestinal microbiota can provide a deep interaction with the neuroendocrine system by producing neuroactive molecules; several strains of *Lactobacillus brevis*, *Bifidobacterium dentium*, *Bifidobacterium adolescentis*, and *Bifidobacterium infantis* have been identified as effective producers of γ -aminobutyric acid (GABA), the main inhibitory neurotransmitter of the central nervous system of mammals [19–22]. Given these interactions, it is tempting to speculate how microbiota patterns and its composition could affect not only organic and functional bowel diseases such as inflammatory bowel diseases and irritable bowel syndrome, but also how it could affect central nervous system conditions and diseases such as epilepsy. Considering these aspects, it is evident how the intestinal microbiota in the context of the human organism represents an ecological community closely related to the health of the host organism. This potentially offers numerous possibilities for intervention on human health [23–25] in a positive way, bringing about advantageous nutritional measures for the host and for the microbiota; however, we should not overlook which nutritional interventions could have negative effects on the host. The availability of a large variety of foods composed by a huge combination of nutrients in different quantities and proportions constitutes an element capable of exerting significant perturbations on the structure and function of the microbiota [26]. Therefore, host health could be endangered when microbiota perturbations are uncontrolled; however, when these perturbations are functionally managed, it should be considered an opportunity.

3. Ketogenic Diet and Microbiota

A previous study on an epilepsy animal model showed how a ketogenic nutritional approach leads to significant changes at the microbiota level, favoring an increase in *Akkermansia* and *Parabacteroides* [11], strains which, according to some authors, are capable of acting on the metabolism of γ -amino acids glutamate (GG) [27–29], shifting the GABA/glutamate balance towards a less epileptogenic picture, with an increase in GABA [11]. A similar

evaluation of an animal model of Alzheimer's disease showed that a ketogenic diet rich in short fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) is correlated to an increase in *Akkermansia muciniphila* and *Lactobacillus* spp. levels, with a decrease in pro-inflammatory microorganisms such as *Desulfovibrio* and *Turicibacter* [9], and a reduction in overall α microbial diversity [10], effects which are related to neurovascular improvements correlated with a lower risk of developing the disease. Analogous studies carried out on an animal model of autism have shown how a ketogenic nutritional approach is correlated to the biphasic modification of the overall microbial richness (decreased in the 1st week, normalized after 12 weeks, and increased at 24 weeks), to the improvement in the Firmicutes/Bacteroides ratio (generally related to the improvement of behavioral symptoms), but contrary to previous works, a reduction in *Akkermansia muciniphila* was observed [30]. The analysis of the human model showed that the ketogenic diet is associated with a decrease in pathogenic proteobacteria (*Escherichia*, *Salmonella*, and *Vibrio*), together with the increase in *Bacteroides* spp. in epileptic pediatric subjects [31], with positive effects on the symptomatic manifestations [32], the authors describe an increase in *Bacteroides* spp. also in healthy subjects. A further analysis describes, in a sample of epileptic pediatric subjects treated with a ketogenic diet, a reduction in *Bifidobacteria*, *Escherichia rectale*, and *Dialister*, important species in the prevention of pathologies such as colorectal cancer, irritable bowel syndrome (IBS), and necrotizing enterocolitis, with a simultaneous increase in the relative abundance of Actinobacteria and *Escherichia coli*, describing a partially dissonant picture from that described by the previous study, especially in regard to Proteobacteria [33]. Attempting to clarify the differences found between epileptic pediatric subjects responding and not responding to the ketogenic diet, Zhang et al. showed in the responders in their study a greater quantity of Bacteroides and a decrease in Firmicutes and Actinobacteria, while in non-responders, an increase in Clostridia, *Ruminococcus*, and *Lachnospiraceae* (phylum Firmicutes) was demonstrated [34]. Noteworthy is the finding in patients with glucose transporter 1 deficiency syndrome, where the ketogenic diet leads to an increase in *Desulfovibrio* [8], a genus belonging to a heterogeneous group of sulphate-reducing, motile, anaerobic bacteria related to the inflammatory state of the mucosa of the intestinal layer [35] with potentially negative effects, suggesting that the use of pre- or probiotics constitutes an element of considerable importance in maintaining the intestinal ecosystem. Rondanelli et al. described in their review of the literature relating to the effects of the ketogenic diet on the microbiota an increase in *Akkermansia*, *Christensenella*, and Bacteroides, together with a transient reduction in *Bifidobacteria* and *Lactobacilli* (reversible in the subsequent dietary phases where reintegration was expected of carbohydrates and the increase in energy) [36]. Duncan et al. described, following a ketogenic diet, a significant reduction in Firmicutes, including *Roseburia* and *Eubacterium rectale*, some of which represent some of the major producers of butyrate [37]. Similarly, Russell et al. described how *Roseburia* and *Eubacterium rectale* also decrease in the case of a normocaloric diet with a high protein content and low carbohydrate content, while *Faecalibacterium prausnitzii* and other related bacteria seem to be less affected [38]. Aleman et al. described how increased lipolysis during a ketogenic diet leads to an increase in the production of b-hydroxybutyrate (BHB), finding a negative correlation with *Faecalibacterium* and *Roseburia* [39]. Therefore, a ketogenic diet could cause perturbations on the microbiota with effects that could be opposite on host health, both potentially positive and negative, given that the considered studies could have significant differences to each other in terms of the patient selection and type of intervention; we should not overlook these potential negative effects of the ketogenic diet and eventually consider how to correct them.

3.1. The Effect of Nutrients during the Ketogenic Diet

Nutrient composition also plays a significant role in the microbiota. Wolters et al. described in their systematic review how dietary profiles extremely rich in saturated and monounsaturated fatty acids exert negative effects on the microbiota, in particular, a high consumption of monounsaturated acids correlates with a decrease in bacterial richness [40].

In describing the data from a randomized controlled trial, Wan et al. reported how a high fat consumption correlates with the increase in Bacteroides and the reduction in butyrate producers such as *Faecalibacterium* and *Blautia* [41]. The amount and type of protein consumed have also been correlated with changes in the microbiota; high protein consumption has been associated with reductions in microbial abundance and changes in the composition [42–46]. Interestingly, mung-bean-derived proteins were correlated with an increase in Bacteroidetes and a decrease in Firmicutes, while pea-derived proteins favored an increase in *Bifidobacterium* and *Lactobacillus* [42]. In a similar way, Basciani et al. described how the use of whey or animal proteins correlates with the increase in Bacteroidetes and the reduction in Firmicutes, while for vegetable proteins, a statistically significant change is not reached at the level of Bacteroidetes [47]. Similarly, the use of non-nutritive sweeteners (NNSs) and low-calorie sweeteners (LCSs), widely used during ketogenic diets, can also cause changes in the microbiota. Their bacteriostatic and/or prebiotics effects can induce qualitative–quantitative changes [48]; this argument, according to some authors, cannot be extended to stevia-based sweeteners for which there is currently not enough information available [49]. The effects generally described for NNSs and LCSs are summarized in Table 1.

Table 1. Effects generally described for NNSs and LCSs. (↑ Increased, ↓ Decreased).

Substance	Effects Described	Comments
Nonnutritive sweeteners (NNSs)		
Aspartame	<i>Akkermansia muciniphila</i> ↑ <i>Enterobacteriaceae</i> ↑ <i>Enterococcaceae</i> ↓ <i>Enterococcus</i> ↓ <i>Parasutterella</i> ↓ <i>Clostridium cluster IV</i> ↑ <i>Escherichia coli</i> ↓	The observed effect may be due to the animals stopping eating rather than aspartame intake.
Acesulfame-K	<i>Escherichia coli</i> ↓ Firmicutes ↑ <i>Akkermansia muciniphila</i> ↓	The inhibition of the growth of <i>Escherichia coli</i> is greater at intermediate doses, decreasing to zero at the higher doses tested. In combination with sucralose.
Cyclamate	<i>Bifidobacterium</i> ↑ SCFAs ↓ Microbial count ↓ Fermentation profile ↓	
Sucralose	Firmicutes ↓↑ Bacteroidetes ↓↑ <i>Turicibacter</i> ↑ <i>Escherichia coli</i> ↑ Proteobacteria ↑ Valeric acid ↑	Opposite results are reported by several authors.
Saccharin	<i>Bifidobacterium</i> ↑ Firmicutes ↓ SCFAs ↓	
Steviol glycosides	<i>Bifidobacterium</i> ↓ Fermentation profile ↓ <i>Bacillus</i> ↑ <i>Streptococcus salivoxodontae</i> ↑ <i>Staphylococcus lugdunensis</i> ↑	
Low-calorie sweeteners (LCSs)		
Erythritol	SCFAs ↑	

Table 1. Cont.

Substance	Effects Described	Comments
Isomalt	<i>Bifidobacterium</i> ↑ β-glucosidase ↓	
Lactitol	<i>Bifidobacterium</i> ↑ <i>Lactobacillus</i> ↑ <i>Akkermansia muciniphila</i> ↑ <i>Bacteroidetes</i> ↓ <i>Clostridium</i> ↓ Coliforms ↓ <i>Eubacterium</i> ↓ SCFAs ↑	
Maltitol	<i>Bifidobacterium</i> ↑	
Mannitol	SCFAs ↑	
Sorbitol	Osmotic/laxative ↑	
Xylitol	<i>Firmicutes</i> ↑ <i>Bacteroidetes</i> ↓ <i>Lactobacilli</i> ↓ <i>Clostridium difficile</i> ↓	

3.2. The Possible Consequences on Intestinal Health

Exploring the possible consequences of a ketogenic diet on an animal model of inflammatory bowel disease (IBD), Li et al. described how the ketogenic diet worsens colitis, reporting altered anatomic-histological parameters, greater levels of serum inflammatory cytokines (IL-1 α , IL-6, TNF- α , IL-17, GM-CSF, and IL-10), increased intestinal permeability, and greater weight loss [50]. From the point of view of the microbiota, there is an increase in pathogenic strains such as *Proteobacteria*, *Enterobacteriaceae*, *Helicobacter*, and *Escherichia-Shigella* and a decrease in potentially favorable taxa such as *Erysipelotrichaceae* [50]. The authors also report an increase in the secretion of biliary metabolites and a significant decrease in the metabolites involved in the production of unsaturated fatty acids, including stearic acid, arachidic acid, erucic acid, and docosanoic acid. Overall, these data suggest a worsening of colitis with increased intestinal and systemic inflammation, an impaired intestinal barrier, and impaired gut microbiota [50]. The effects described in these paragraphs are summarized in Figure 1.

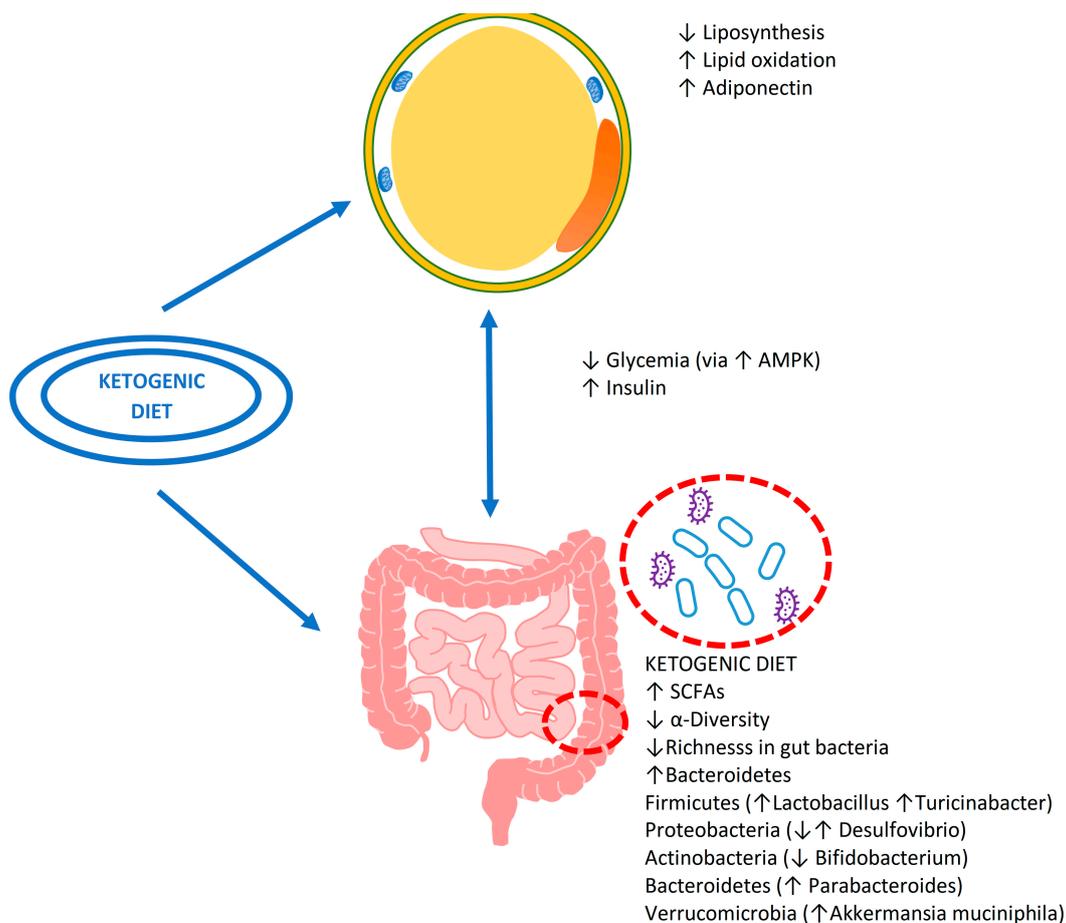


Figure 1. Effects of the ketogenic diet on the intestinal microbiota and main systemic implications (see text). (↑ Increased, ↓ Decreased).

4. *Clostridium butyricum* CBM588

Clostridium butyricum is an anaerobic, spore-forming microorganism capable of producing short-chain fatty acids (SCFAs) starting from undigested dietary fiber (in particular, butyric acid and acetic acid) to being widely spread in nature (soil, vegetables, dairy products) and normally found in the human intestine [51,52], where it is considered a symbiote [53]. The *Clostridium butyricum* strain MIYAIRI 588 (CBM588) was isolated by Dr Chikaji Miyairi in 1933 from human fecal material and it was used in various Asian countries (Japan, Korea, and China) for decades [54] for the treatment of diarrhea, particularly if associated with the use of antibiotics [55–57]. Its use for this application has been documented in adults and children [55–57]. The safety profile of CBM588 is extremely high, the strain has not been shown to be pathogenic or capable of influencing toxic effects, and it is antibiotic sensitive, devoid of pathogenicity factors and of the genes necessary for the production of clostridial toxin [58]. Its use in Europe has been authorized as a novel food ingredient [59]. In particular, the ability to produce SCFAs is considered a feature of considerable importance on the health of the host organism, including the implementation of the gastrointestinal barrier, the modulation of inflammatory processes, and the modulation of the intestinal immune response [60]. Evaluations carried out on the animal model have demonstrated a high colonizing power and the ability to produce significant levels of butyric acid [61,62]. This ability makes the CBM588 of considerable interest in the management of various problems.

5. Functional Characteristics of *Clostridium butyricum* CBM588

Several studies on animal models have investigated the effects of CBM588 by analyzing its mechanisms of action. Hagihara et al. described how following the administration of CMB588, a significant increase in the expression of MUC2 occurs in an animal model of diarrhea induced by antibiotics (clindamycin), which leads to an increase in the production of mucin, with a significant reduction in epithelial damage to the level of the colon [63]. This is believed to be attributable to butyrate production, consistent with what has been observed in several in vitro studies, where the exposure of goblet cells to butyrate correlated with an increase in the MUC gene expression and in the production of mucin [64,65]. A further mechanism described in the literature for CBM588 is that relating to the implementation of the barrier function by acting on the tight junctions (TJs). By analyzing a mouse model of antibiotic-induced diarrhea, the use of CMB588 was found to increase the expression of occludin, claudin-4, and ZO-1, structural proteins of TJs [63,66]. Similarly, in a mouse model of severe acute pancreatitis, the administration of CMB588 together with another strain of *C. butyricum* (CGMCC0313.1) was correlated with a recovery in the expression of ZO-1, ZO-2, and occludin and lower pancreatic levels of *E. coli* and *Enterococcus*, demonstrating an improved barrier function [67,68]. Analyzing this mechanism in more depth, Hagihara et al. described how the administration of CMB588 is related to an increase in the production of IL-17 by $\gamma\delta$ intraepithelial T lymphocytes, which act as a first line of defense at the level of the lamina propria in the colon [63]. In this context, the action of IL-17 manifests itself by favoring the integrity of the intestinal barrier thanks to the increased production of the structural proteins of the TJs [68], exerting a protective effect and favoring the repair processes. Furthermore, modulation effects on inflammatory processes due to the increased production of lipid metabolites with anti-inflammatory effects, such as palmitoleic acids, prostaglandin metabolites, and pro-resolving mediators, are also described for CMB588 in the mouse model. These mediators include protectin D1, which contributes to promoting the secretion of IL-10 by colonic T cells [63,69,70]. The sum of these effects exerted by CMB588 leads to a reduced permeability to pathogens and to lipopolysaccharide, with all the benefits that can derive from it. The functional characteristics of *Clostridium butyricum* CBM588 are summarized in Figure 2.

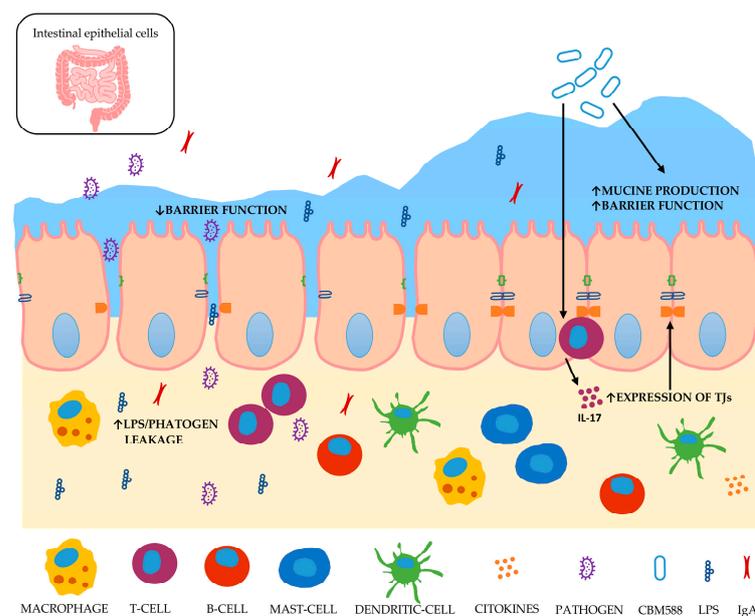


Figure 2. *Clostridium butyricum* CBM588 promotes the thickness of the mucous layer and the integrity of the tight junctions thanks to the stimulation of the release of IL-17 exerted on the intraepithelial T cells, with a reduced permeability of pathogens and lipopolysaccharide. (↑ Increased, ↓ Decreased).

5.1. Effects of CBM588 on Digestive Disease Models

Numerous authors have investigated the use of CBM588 in the treatment of digestive diseases such as irritable bowel syndrome (IBS), ulcerative colitis (UC), gastric ulcer, and intestinal dysbiosis. Araki et al. described in the mouse model how the administration of CBM588 is related to a reduction in diarrhea and mucosal damage induced by dextran sulfate sodium (DSS) [71]. Similarly, Hayashi et al. described how the administration of CBM588 prevents experimentally induced colitis in the mouse model [62]. Additionally, according to what was reported by Okamoto et al., the administration of CBM588 started one week before the induction of colitis in an experimental model, with DSS correlating with a significantly lower ulceration index and myeloperoxidase (MPO) activity, showing at the same time a higher content of proliferating cells (PCNA) around the lesions, with the simultaneous finding of higher levels of Lactobacillus and Eubacterium, and higher levels of butyrate, propionate, and acetate at the cecal level [72]. Describing conceptually similar results in a mouse model of experimentally induced IBS, Zhao et al. reported that the administration of *C. butyricum* is related to a reduction in intestinal visceral hypersensitivity and mucosal inflammation [73]. Analyzing the effects related to the administration of CBM588 in three different models of gastric ulcer (from alcohol, from stress related to the reflux, and from pyloric ligation), it was highlighted that there was a reduction in the mucosal damage and an improvement of the inflammatory state, with a reduction in the oxidative stress, consistent with the mechanisms previously described [74]. Consistent with the previous results, studies carried out on the human model demonstrate how the administration of CBM588 is correlated with the increase in Bifidobacterium and Lactobacillus, the decrease in Enterococcus and Enterobacteriaceae, and the recovery of fecal anaerobes following an antibiotic treatment [55,57,75] in the absence of changes in the overall gut microbiota diversity.

5.2. Accessory Metabolic Effects of *Clostridium butyricum* Potentially Functional to Diet Therapy

Various data in the literature also report potentially functional effects to diet therapy, obtainable with the administration of *Clostridium butyricum*. In a mouse model of diabetes, it has been described how the administration of *Clostridium butyricum* or butyrate correlates with an increase in intestinal and serum GLP-1 levels [76]. This led to the activation of the IRS-1/Akt pathway, with an improvement of glycemic homeostasis, of the metabolic processes related to the adipose organ, also showing an improvement in the picture of pancreatic inflammation. The authors describe a decrease in hepatic gluconeogenesis levels with the downregulation of glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase 1 (PCK1) and the upregulation of GLUT4 receptor-mediated glucose uptake and the action of the uncoupling protein UCP1. Butyrate is considered the crucial element in improving insulin sensitivity [76]. Analyzing different mechanisms of action, the work of Li et al. considered how the intake of *Clostridium butyricum* correlates in the mouse model with a decrease in adipose deposition contextually with an increase in the number of resident T-Reg lymphocytes (aT-Reg), the expression of activated Wnt10b in adipose tissue, and of increased serum butyrate levels [77]. Furthermore, the authors report increased levels of TGF β [76] already described as a potential promoter of Wnt10b expression [78]. The Wnt10b-induced activation of Wnt signaling promotes the inhibition of lipogenesis [79,80], as confirmed by the absence of effects in Wnt10b-KO mice. The sum of these effects suggests a scenario of potential interaction between aTregs, the Wnt signaling pathway, and the lipid metabolism [77]. The authors conclude that the administration of butyrate and consequently that of *Clostridium butyricum* (as an endogenous producer of butyrate) are able to alleviate experimentally induced obesity in the animal model, with an improvement in the level of hepatic steatosis and the glucose metabolism [81], coherently with what was described by previous studies [81–85], as well as for the CBM588 strain. As reported by Stoeva et al. experimentally, the CBM588 strain has demonstrated efficacy in the control of *Clostridium difficile* infection, dyslipidemia, dysglycemia and intestinal inflammation caused by the inoculum of dextran-sulphate, with dynamics related to its ability to produce butyrate. The

authors also describe how other strains of *Clostridium butyricum* have demonstrated clinical action in IBS-D, again due to the production of butyrate [51].

5.3. Clinical Evaluation of *Clostridium butyricum* CBM588

As previously discussed, CBM588 has demonstrated a good safety profile, good colonization capabilities, and the ability to promote a eubiotic intestinal environment due to its ability to produce butyric acid. Based on these characteristics, different authors have analyzed different clinical applications. Analyzing the prevention of antibiotic-associated diarrhea Seki et al. describe how the concomitant administration of CBM588 in children with upper respiratory tract infection or gastroenteritis treated with antibiotics is related to a drastic reduction in diarrhea episodes and in the reduction of anaerobic bacteria and Bifidobacteria. In subjects who received antibiotic only diarrhea occurred in 59% of cases, in subjects who received CBM588 halfway through the antibiotic treatment in 5% of cases, while in subjects who received it from the beginning in 9% of cases [55], suggesting the utility of its administration in the prevention of antibiotic-induced diarrhea in children. A similar evaluation in adult subjects carried out by Imase et al. in the context of *Helicobacter pylori* eradication therapy reported that the administration of CBM588 is related to an incidence of diarrhea or soft stools of 14% against 43% of subjects who followed exclusively antibiotic therapy. When doubling the administered dose of CBM588, none of the subjects analyzed reported diarrhea or soft stools [57]. Yasueda et al. analyzing the onset of pouchitis in subjects undergoing total proctocolectomy with anal anastomosis of the ileal pouch (IPAA) reported how the administration of CBM588 is related to only one episode in the nine subjects observed, while among the eight subjects who took a placebo four developed pouchitis [86]. Analyzing the possibility of reducing symptoms after gastric bypass surgeries, Chen et al. report a significant improvement in symptom scores after taking CBM588 for two weeks [87]. Studying the effects of CBM588 add-on administration in subjects with treatment-resistant major depressive disorder (TRD), Miyaoka et al. reported that 70% of the subjects evaluated responded to the treatment with a remission rate of 35.0%, suggesting that the use of CBM588 as an add-on to the therapies employed in these patients could be a very promising avenue [88]. Dizman et al. analyzing the effects of CBM588 ad-on administration in patients with metastatic renal cell carcinoma with clear cell and/or sarcomatoid histology and intermediate or low-risk disease receiving nivolumab and ipilimumab reported progression-free survival (PFS) longer in nivolumab-ipilimumab treated with CBM588 compared with those who did not take CBM588 (12.7 months versus 2.5 months, hazard ratio 0.15, 95% confidence interval 0.05–0.47, $p = 0.001$), findings suggesting that CBM588 appears to improve clinical outcome in patients with metastatic renal cell carcinoma treated with nivolumab-ipilimumab [89].

6. Discussion

In this work, we attempted to analyze the potential role of *Clostridium butyricum* CBM588 as an ad-on therapy in ketogenic dietary approaches. The ketogenic diet is currently used in the management of various neurological, neurodegenerative, and metabolic disorders [1,2,6,7]. How to discuss the adoption of a ketogenic diet involves a significant impact on the microbiota level with an increase, according to the majority of the authors analyzed, in the levels of *Akkermansia muciniphila* [9,11], a reduction in the overall α microbial diversity [10], which can be normalized and recovered in time [30], and an improvement in the Firmicutes/Bacteroides ratio [30,32], changes that are described as functional both to the neurological picture and to the metabolic picture. Interestingly, it was possible to note that in neurological patients responding to the ketogenic diet, a higher level of Bacteroides was found at the same time as lower levels of Firmicutes and Actinobacteria [34]. In this context, a notable effect reported by various authors following a ketogenic diet is the reduction in butyrate-producing strains such as *Roseburia*, *Eubacterium*, *Balutia*, and *Faecalibacterium* (which are affected at different levels depending on the study taken into consideration) [37–39,41]. The reduction in butyrate-producing strains, and therefore the

reduction in butyrate, could probably support what was reported by the authors who describe the worsening of the animal model of inflammatory bowel disease (IBD) following a ketogenic diet, with the worsening of colitis, weight loss, histological parameters, and the cytokine picture, together with the increase in intestinal permeability [50]. Precisely, the impairment of intestinal permeability could represent the crucial element in this context; TJs form a link between epithelial cells, participating in the regulation of paracellular transport [90], a reduced expression of constituent proteins, such as occludin, claudin, JAM, and ZO-1, which is correlated with an increase in intestinal permeability. The increase in permeability allows bacteria, components such as LPS and/or bacterial metabolites and other molecules with antigenic action, to reach the lamina propria, promoting an inflammatory response. Experimental animal models KO for occludin in fact show a histopathological picture of chronic inflammation and hyperplasia of the gastric epithelium [91]. In this context, the CMB588 strain is of great potential interest, especially considering its potential as a butyrate producer. As discussed, its administration in the animal model has been correlated with an increase in the MUC gene expression and mucin production [64,65] and with the implementation of TJs and the related structural proteins occludin, claudin-4, and ZO-1 [60,63] by an IL-17-dependent mechanism mediated by lymphocytes $\gamma\delta$ intraepithelial T lymphocytes [63,68]. These effects are also combined with anti-inflammatory ones due to the increased production of lipid metabolites, which promote the secretion of IL-10 by colon T cells [63,69,70]. Overall, these effects can contribute in a functional way to controlling the alterations induced by the ketogenic diet. Additionally, the butyrate-mediated increase in the intestinal and serum GLP-1 levels could potentiate and have beneficial dietary effects, as, notoriously, GLP-1 delays gastric emptying and increases gastric volumes, also enhancing insulin secretion and inhibiting glucagon release, alongside reduced lipogenesis and consequent visceral adiposity reduction [76,77]. The use of CBM588 could potentially also be integrated by other behavioral, nutritional, and nutraceutical strategies. The introduction of an active lifestyle, complemented by structured exercise, as indicated by Zeppa et al., can promote the overall adoption of healthier eating habits, potentially useful not only for the management of the metabolic framework but also for a better compliance and dietary management [92]. Even the use of some nutraceutical principles normally used as an add-on in the management of various clinical problems [93–95], such as Boswellia, quercetin, and berberine, could prove to be a useful strategy in integrating the effects exerted by CMB588 taken together with the ketogenic diet. As described in the literature, Boswellia has proven to be able to strongly favor the relative abundance of Bacteroidaceae, potentially favoring the achievement of a more functional Firmicutes/Bacteroides ratio for a good response to the ketogenic diet [34]. For berberine, in addition to documented anti-inflammatory effects, the ability (similarly to Boswellia) to favor the reduction in the Firmicutes/Bacteroides ratio, a functional factor for a good response to the ketogenic diet, is also described. Furthermore, berberine, by favoring the development of *Akkermansia muciniphila*, acts synergistically with CMB588 in promoting mucus production and colonic epithelial barrier integrity [96,97]. Anti-inflammatory/senolytic effects are also described for quercetin [98,99], but above all, prebiotics are functional to the reduction in the Firmicutes/Bacteroides ratio in animal models of non-alcoholic fatty liver disease (NFLD) [100]. Furthermore, for the management of conditions such as IBD and IBS, where the fermentation processes necessary for the production of butyrate should complicate the clinical management, the use of α and β galactosidase could prove useful in making these approaches more tolerable [101]. The use of CMB588 can prove to be a useful add-on in the functional management of a ketogenic diet, which can potentially be completed and optimized through the use of specific prebiotic and enzymatic tools.

7. Conclusions

Clostridium butyricum CBM588 is a bacterial strain with functional characteristics for the optimal management of a ketogenic diet, capable of acting by modulating some of the potential gastrointestinal side effects. Furthermore, for CMB588, functional effects are also

described for weight management through the implementation of insulin sensitivity and for the modulation of body composition through the optimization of the lipid metabolism. All these effects can be further optimized through the functional use of prebiotic and/or enzymatic nutraceutical tools with synergistic effects. Future studies should focus on evaluating the clinical impact of these effects in the management of ketogenic diets, with the aim of clarifying the concrete applicability of these possibilities.

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