

Effects of Caloric Restriction on Intestinal Microbiota

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Abstract. The goal of a calorie-restricted diet is to improve one's health, extend one's lifespan, slow down the aging process, and ward off metabolic and age-related diseases. Changes in food can have a substantial impact on the balance of good bacteria and harmful bacteria in the gut, as research has revealed that gut microorganisms play a key role in health, aging, and illness. Calorie control is one of the most common dietary interventions, and its effect is mainly achieved by the microbial regulation in the intestinal tract. Different calories also affect the composition and metabolism of intestinal microbiota. This paper discusses probiotics, proinflammatory microorganisms, butyrate producing bacteria, amino acid producing microbes and so on, and points out the effect of food composition on intestinal microbiota and the metabolism of intestinal microbiota.

Keywords: Calorie Restriction, Intestinal Microflora, Food Components, Metabolism

1. Introduction

Calorie restriction (CR) is defined as a reduction in calorie consumption of 20% to 40% without sacrificing critical nutrients. It was first reported by McCay et al. in 1935 to extend the life of rats. Over the past 70 years, many trials have shown that CR is the most effective means of slowing aging, with the exception of genetic manipulation.

CR is an important model for the study of aging mechanisms and interventions in humans, and there are many studies on the application of CR to humans. There are three ways to control calories. The first is a calorie restricted diet (CRD), which is 20% protein, 30% fat, and 50% carbohydrate. Second, intermittent fasting (IF) is the way a person eats for a period of time (e.g., 16 to 48 hours) during which they eat normally. The third is the fasting simulated diet (FMD). This phase simulates a fast (usually 4-5 days), which contains low-calorie, low-calorie, carbohydrate and protein intake, but also a high amount of fat. Usually, there are two primary methods for achieving the required calories (% CR). The first way is to reduce the daily diet or intermittent fasting, followed by a strictly controlled diet to lose muscle weight. Overall, adhering to a particular dietary pattern is more effective [1].

Chronic caloric restriction (CR) has been shown to extend lifespan in nonhuman primates, but its long-term effects in humans have yet to be confirmed. This paper focuses on calorie-restricted food components, the role of these components on the intestinal flora and the metabolism of these substances by intestinal microorganisms, and the role of calorie restriction on the intestinal flora.

2. Major Intestinal Microflora

The intestinal flora is composed of microorganisms parasitic in the small and large intestine of the host, with a population of about 30+ trillion. These bacteria affect the digestion and absorption of the organism, and can influence the immunity of the organism as well as the therapeutic response to tumors. Bifidobacteria, lactobacilli, thick-walled bacteria, anaphylactic bacteria, actinomycetes and amoebae are the main representative bacteria of the intestinal flora. In the jejunum, it is mainly Gram-negative aerobic bacteria and contains small amounts of Streptococcus, Lactobacillus, Staphylococcus, Enterococcus, and Escherichia coli [2]. According to the characteristics of the intestinal environment, strictly anaerobic bacteria are much more abundant in the adult intestine than parthenogenic anaerobes and aerobes. Flora, fauna and parasites are interdependent and interact with

each other, creating a balance of three dynamics. The intestinal flora can be divided into three main categories: commensal, pathogenic and neutral bacteria.

2.1. Symbiotic Bacteria

Commonly known as "probiotics", its role is to help digestion, decomposition, metabolism, absorption, digestion, and promote the body's immune function. Resistant starch, also known as resistant starch or indigestible starch, cannot be enzymatically hydrolyzed in the small intestine, but can be fermented with volatile fatty acids in the colon of human gastrointestinal tract [3]. The term "resistant starch" refers to the fermentation of starch that can evade digestion in the small intestine and reach the colon. These resistant starches are generally classified into four grades, RS2,RS3,RS3,RS4 (Figure 1). beneficial bacteria like are able to break down the RS3,RS4 grades in the most difficult digestive system. These microorganisms are an important part of the human body, capable of synthesizing many vitamins, participating in the digestion of food, promoting intestinal peristalsis, inhibiting the growth of harmful bacteria, breaking down harmful substances, etc. [4].

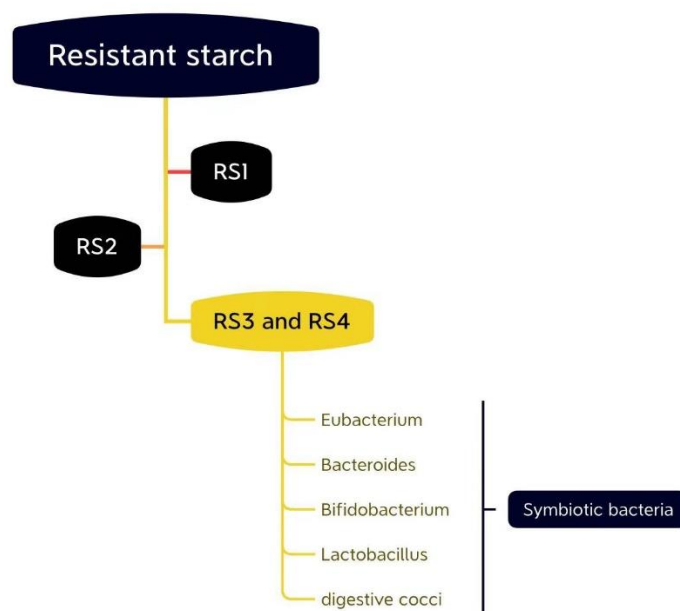


Figure 1. Resistant starch that can be decompose by beneficial bacteria

2.2. Pathogenic Bacteria

This is what we generally understand as "harmful bacteria". This kind of flora is able to directly cause the occurrence of digestive tract diseases, In a healthy state, this kind of flora is able to directly cause the occurrence of digestive tract diseases, in a healthy state, this kind of flora in our intestinal tract is comparatively small compared to other flora, so will not show pathogenicity, but under certain conditions, the intake of harmful bacteria over the standard food, will lead to the occurrence of disease [5]. This kind of flora mainly includes Clostridium perfringens, Aspergillus, Staphylococcus and Pseudomonas. However, under certain conditions, for example, reduced body resistance or unclean diet, when ingesting food with excessive harmful bacteria, it will lead to the occurrence of disease, such flora mainly include Clostridium perfringens, Aspergillus, Staphylococcus and Pseudomonas, etc. The normal flora and the host maintain a good balance of life. It is possible for the equilibrium connection to be upset under certain circumstances, the bacteria in the original non-pathogenic normal flora can become pathogenic bacteria, called opportunistic pathogens, also known as conditionally pathogenic bacteria. For example, some E. coli. There are three main prerequisites for conditionally pathogenic bacteria: 1. Settlement site change certain bacteria leave their normal host site and enter

other sites, grow and multiply without original restrictions, and then infect and cause disease; 2. Immunocompromised normal flora enter tissues or spread in the blood; 3. Dysbiosis.

2.3. Neutral Bacteria

bacteria as the name implies, its action between the symbiotic bacteria and pathogenic bacteria, under normal circumstances, a certain number of neutral bacteria is good for intestinal digestion and metabolism, but under certain conditions, neutral bacteria may accumulate in the intestinal tract, make some of the intestinal toxins, corruption or carcinogens content increased, leading to the happening of the disease [6]. This group of flora includes *Escherichia coli*, *Lactobacillus*, *Streptococcus* (shiticoccus) and *Veronococcus*. Other bacteria in the intestinal tract, such as *Bacteroides*, *Escherichia coli* and *Akkermansia Muciniphila* (AKK), although these bacteria also consume fat directly, they will increase the appetite for eating high-fat meat after consumption, and consume a large amount of fat, thus falling into a bad cycle.

3. The Main Pathways by Which These Food Components Affect the Gut Microbiome

3.1. Intestinal Flora and Protein Metabolism

Small intestinal bacteria can promote the metabolism of some amino acids, and thus have an effect on the body's amino acids. The main amino acid metabolisms are sulfur amino acid metabolism, aromatic amino acid metabolism and basic amino acid metabolism. On the one hand, nitrogen enters the large intestine, which has a certain influence on the metabolism and community structure of *E. coli*; in addition, *E. coli* can also participate in the metabolism and utilization of nitrogen in large quantities, producing a variety of metabolites, thus having a certain impact on the human body. The epithelial cells of the small intestine do a very efficient job of digesting food proteins and then absorbing them [7]. Highly soluble proteins can escape digestion in the small intestine to some extent, while large amounts of nitrogenous compounds migrate to the large intestine. After the action of intestinal microorganisms and residual pancreatic proteases, many microbial metabolites are generated by protein hydrolysis. Coliforms are more effective in degrading endogenous and exogenous proteins. *Bacillus* spp, *Clostridium* spp, *Propionibacterium* spp, *Clostridium* spp, *Lactobacillus* spp, and *Lactobacillus* spp are the main members of protein hydrolysis. The role of fecal and coliform bacteria in protein hydrolysis varies widely. Only the hydrolysis of spherical proteins leads to higher microbial viability in feces. During the fermentation process, the complex protein is firstly broken down by a variety of bacterial peptidases, proteases and peptidases, producing a large amount of free amino acids and short peptides. The fermentation of the proteins yields 2-methylbutyrate, isobutyrate, isovalerate, organic acids, H₂ and CO₂, and trace amounts of phenols, amines, indoles and ammonia [8]. Some bacterial metabolites are transported to the colon and depending on their concentration in the intestinal lumen, can have favorable or unfavorable effects on them. Certain bacterial metabolites enter the portal vein through the portal blood and perform a variety of physiological functions in the liver and surrounding organs. *Clostridium* spp. in the gut are the main drivers of amino acid fermentation, while their utilization is mainly caused by digestive streptococci. The intestinal microbial community also plays an important role, including biosynthesis from scratch.

3.2. Lipid Metabolism

Fewer than 5% of the entire amount of dietary fat consumed by humans makes it to the colon on a daily basis, amounting to only 4-5 grams. Triglycerides and phospholipids are broken down into polar head groups and free lipids by lipases present in gut flora. Phospholipids, mostly in the form of phosphatidylcholine, are present in tiny levels and are the predominant dietary fat. *Lactobacillus*, *Enterococcus*, and *Clostridium* are gastrointestinal bacteria that may convert lipids to 1,3-propanediol.

Clostridium perfringens can convert choline to trimethylamine. Saturated fatty acids can activate the TLR-mediated pro-inflammatory signaling pathway, while ω -3 unsaturated fatty acids can inhibit this pathway. In addition, lipid levels or BMI have been linked to the presence of specific bacteria in the intestines, for example, Clostridiaceae and Trichophytonaceae bacteria are associated with LDL but not BMI and other lipoproteins; Eggerthella spp. are associated with elevated triglyceride levels and reduced HDL levels, while Butyricimonas spp. was strongly associated with lower triglyceride levels. It has been suggested that a high-fat diet has an effect on the composition of the gut microbiota, but it is not clear whether the effect is due to an increase in fat content or a relative decrease in carbohydrates [9].

3.3. Pyruvate Metabolism

Majority of the carbohydrates in the body are broken down into the end product, pyruvate, which serves as a link in the metabolic chain between sugars, amino acids and lipids. Pyruvate metabolism is shown in Figure 2. Host intestinal microflora can ferment pyruvate to produce succinate, lactate and acetyl coenzyme A. These intermediates can be further metabolized to short chain fatty acids (SCFAs) (Figure 3) [10]. Acetic acid is produced by intestinal flora via the acetyl coenzyme A or Wood-Ljungdahl pathway. Propionic acid is produced from succinate to methylmalonyl coenzyme A via the succinate pathway and can also be synthesized via the acrylic acid pathway and the propylene glycol pathway. Butyric acid is formed by the condensation and subsequent reduction of two acetyl coenzyme A to butyryl coenzyme A. Certain microorganisms in the gut can use both lactic and acetic acids to synthesize butyric acid. SCFAs are the most intensively metabolic end products of the gut flora, with a wide range of physiological functions. In addition to SCFAs, small amounts of alcohols such as ethanol, propanol, and 2,3-butanediol are also created during pyruvate metabolism [11].

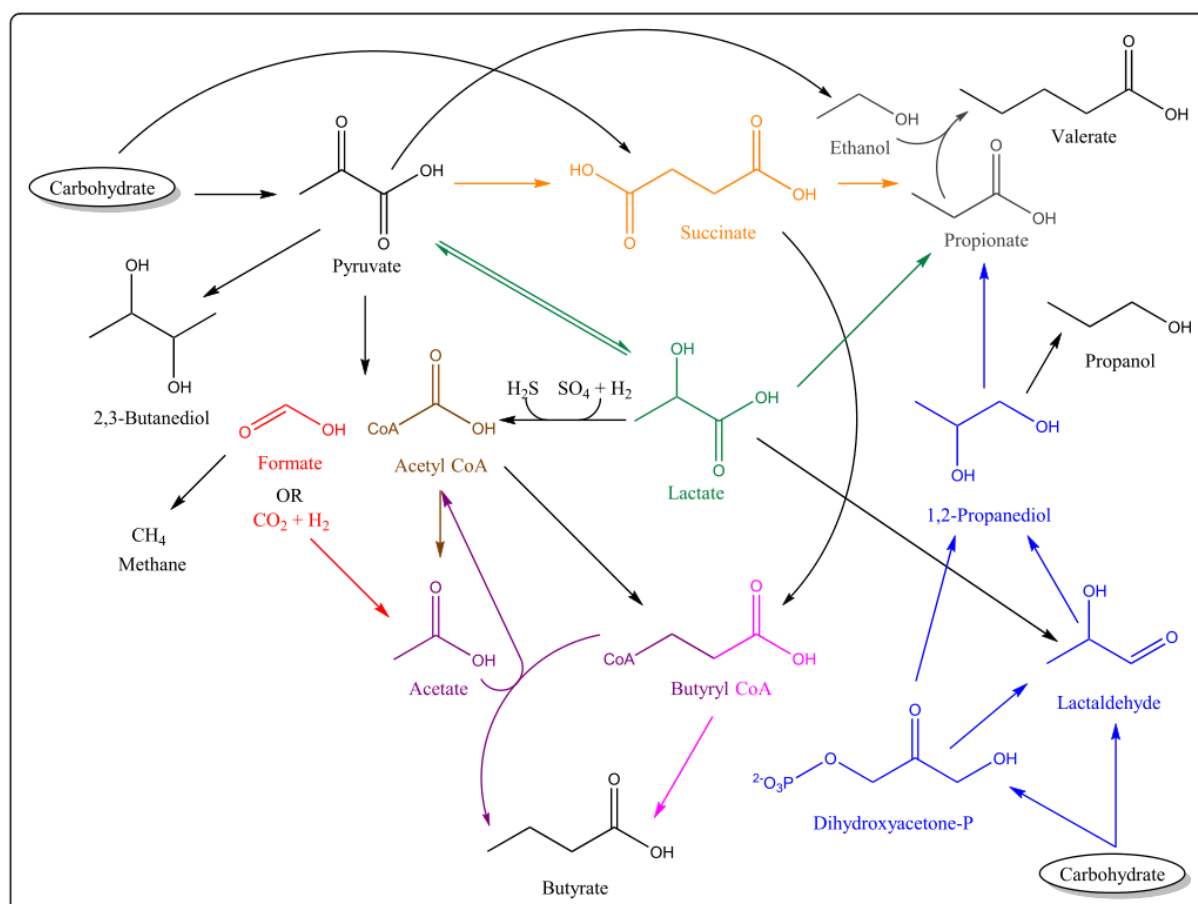


Figure 2. The human gut microbiome's pyruvate catabolism strategies [10]

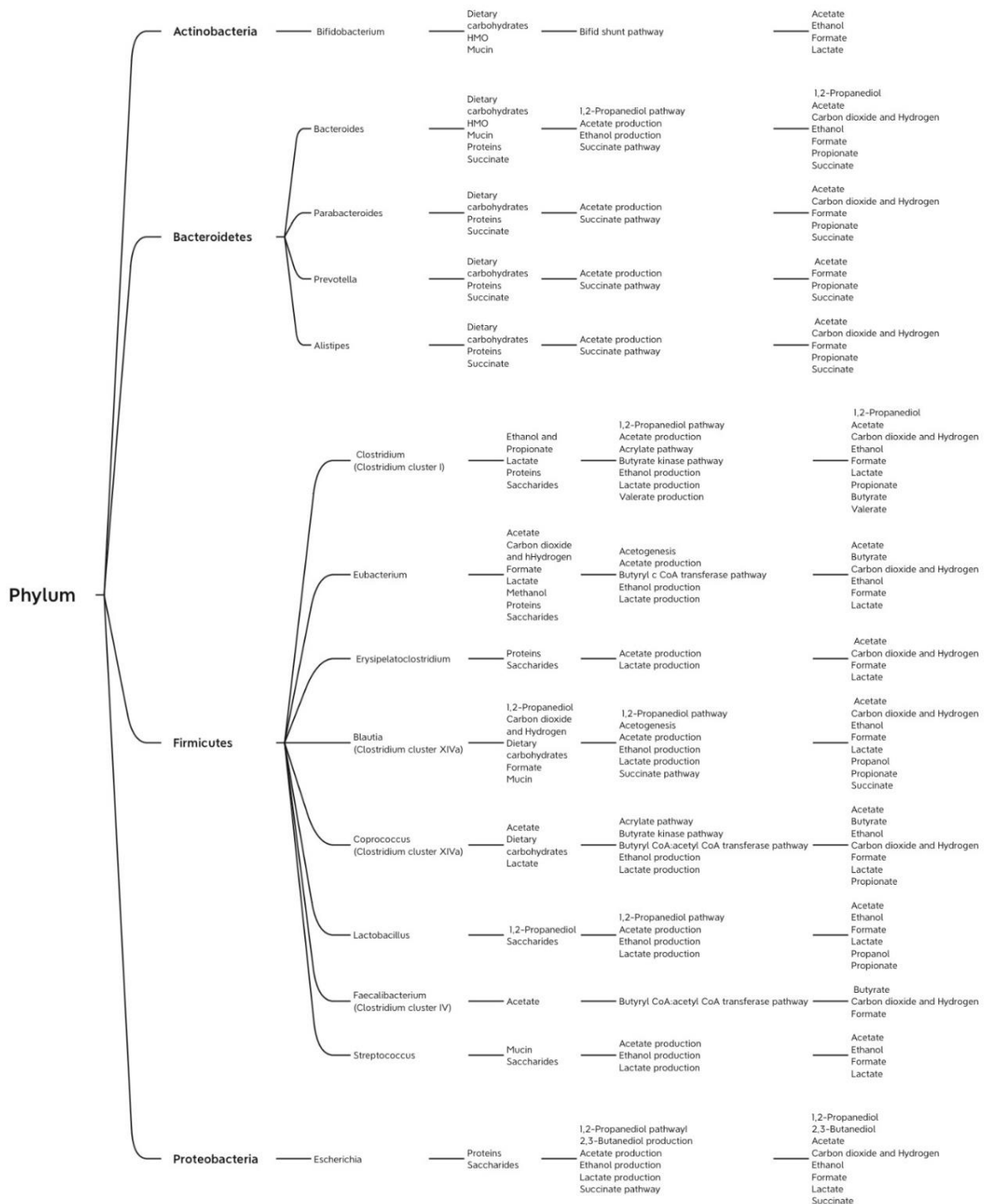


Figure 3. The metabolisms of the major genera found in the human gut microbiome [10]

4. Differences in the Effects of Different Modes of Caloric Restriction on Intestinal Microflora

4.1. Diet

DiETING, reducing the amount of food consumed. During the diet, researchers studying the composition of excrement found that dieting causes an increase in certain microorganisms, such as *Clostridium difficile*, because the increase in antibiotics causes such microorganisms to accumulate, which in turn causes certain gastrointestinal diseases, such as colitis and dysentery, in addition, because these bacteria also affect the body's absorption of nutrients, thus affecting the body's energy

balance [12]. In the trial, similar bacteria were found in both subjects and rats, and the researchers said that the toxins of *C. difficile* were associated with *C. difficile*, which causes weight loss. Overall, the low-calorie diet had some effect on intestinal microflora and on the ability of *C. difficile* to colonize the body. The aforementioned changes can hinder the body's absorption of nutrients and reduce the absorption of nutrients, but they do not cause the corresponding clinical manifestations.

4.2. Intermittent Fasting

In one study, it was found that mice that fasted for 16 or 20 hours had an increase in *Acinetobacter* (Akk), a known bifid species that is one of the highly abundant intestinal microorganisms in healthy humans, which degrades mucin into a variety of metabolites that can serve as a source of energy for other bacteria and the host, as well as modulating the host's immune response and biological functions, among other things. Another bacterium, *Alistipes*, is reduced, and it causes certain diseases and inflammation in the organism. However, this effect on the microbiome ceased after the end of the fasting period. In another study, people who adopted intermittent fasting (16 : 8 patterns, i.e., 16 hours of fasting and 8 hours of free diet) showed no significant differences in gut microbiome composition [13]. It was concluded that the 16-hour daily fasting regimen resulted in a significant increase in the levels of *Akkermansia* and a decrease in the levels of *Alistipes*. It also resulted in more significant correlations and clustering of flora in the network. The effect of indirect fasting on mice was significant, and when the normal diet was resumed, the mice recovered to some extent as the effect disappeared, but there was some difference in the significance of the recovery effect. A comparative analysis of all the data showed that 16 hours of fasting per day was relatively healthy, because the intestinal environment of the mice was always maintained at a healthy level when the normal diet was resumed [14].

5. Effects of Caloric Restriction on The Bacteria with Different Functions

The effect of CR on microorganisms in the intestine of mice has been tested in rats and mice. In animal studies, the dieting program was based on healthy or high-fat diets with 10% to 40% caloric intake, while in human studies, the daily caloric intake was 700 to 1500 kcal/day. Some studies were conducted in the context of non-alcoholic fatty liver disease (NAFLD) or influenza. The main factors affecting the effect of CR are feed composition and feed age. A rat experiment showed that microorganisms that worked on a 30% high-fat diet were not found in rats on a 30% normal diet. Also, CR-induced changes in flora differed in the middle-aged and older groups. Another human experiment showed that the gut microbial composition was very different in lean and obese individuals, but this difference disappeared after long-term CR. The results showed that after the CR intervention, the gut microbial composition of the obese population was significantly different from that before the intervention. Among them, the thick-walled phylum, the phylum *Anthobacteria*, and the phylum *Aspergillus* were the main phylum; however, the relative abundance of these strains was altered differently under the effect of CR. It was found that the promotion of healthy organisms by caloric restriction was closely related to the intestinal flora [15]. Fasting-induced microbial changes differed across species and different parts of the gut. A study on crucian carp showed that IF enhanced the α -diversity of its intestinal flora and also favored ecosystem stability, with increased relative abundance of *Bacillus anthracis*, *Daniostoma* spp. and mucinophilic *Ackermannia* (AKK). The abundance of *Lactobacillus*, which has immunomodulatory and lipid metabolism-altering functions, was inversely correlated with the inflammatory response. The thick-walled phylum and the methanobacterium phylum account for more than 90% of the total intestinal microorganisms, with the thick-walled phylum involved in host absorption of dietary energy and the methanobacterium phylum responsible for metabolism of sugars and lipids. Figure 4 summarizes the changes in microorganisms of different taxonomic classes in different studies.

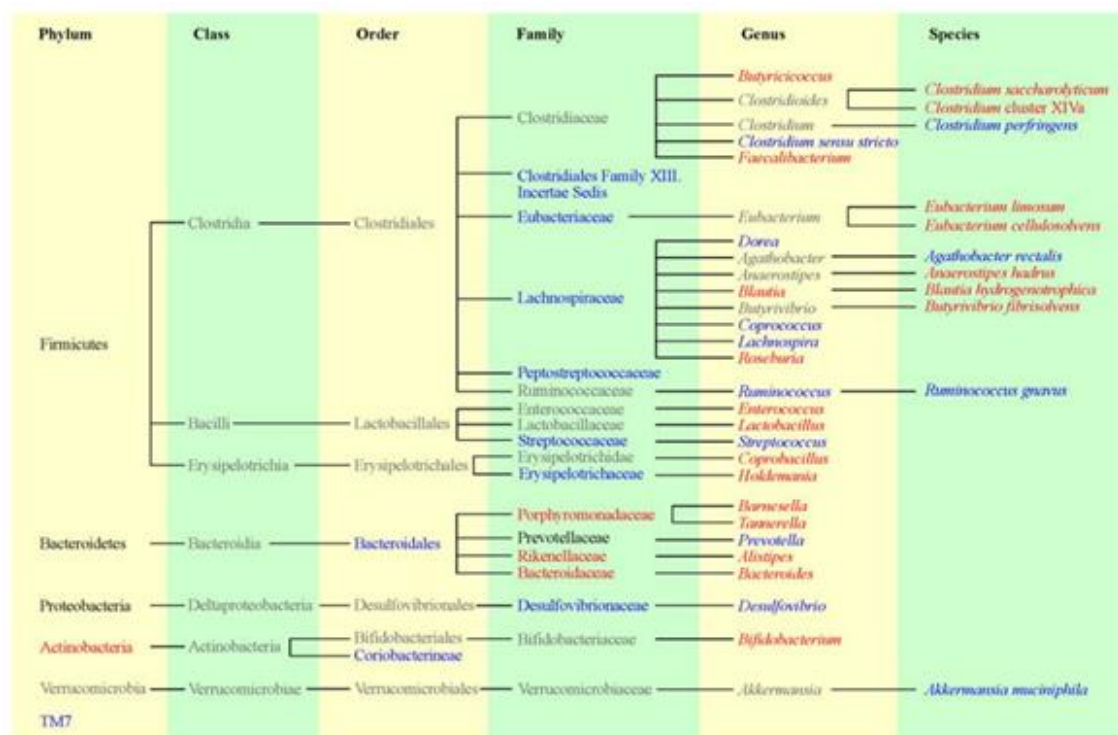


Figure 4. Phylogenetic tree of reported taxa [16]

5.1. Probiotic Strains

There was a correlation between increased levels of probiotics and decreased cholesterol, triglyceride levels, and Lactobacillus and Bifidobacterium levels, which suggests that Lactobacillus growth is linked to its role in lipid metabolism [17].

5.2. Microorganisms That Promote Inflammation

Some bacteria are inhibited by CR. Desulfovibrionaceae, Streptococcaceae, and TM7 all induce obesity, diabetes, and the inflammatory mucosal inflammatory response in inflammatory bowel diseases, which all generate a mild inflammatory reaction. A key biomarker capable of binding antigens generated by Gram-positive bacteria, circulating lipopolysaccharide-binding protein (LBP), was lowered following intervention (45 days in rats with 25% diet compared to 800 kcal/day in people for 28 days); according to the findings, LBP levels are a reflection of the antigen load in the blood and the inflammatory response of the body. CR therapy significantly reduces the rate of antigen translocation from the intestine to the blood because of a decrease in the number of Gram-negative bacteria in the gut [18].

5.3. Butyrate Producing Bacteria

Intestinal bacteria release butyric acid, a short-chain fatty acid, which is created in the large intestine from fermented fiber by fermenting food. By lowering intestinal permeability, its position as a key energy source for intestinal cells has been demonstrated to have anti-inflammatory effects. CR has a promotive effect on certain over-producing butyric acid bacteria, such as Bacillus coelicolor, Holdemania, cellulose-solventing fungi, and Clostridium glycoenes [19]. In addition, studies on rat models showed that butyric acid promotes glucagon-like protein 1 (GLP-1) in enteroendocrine cells, thereby improving blood glucose and insulin response in humans.

5.4. Microorganisms That Are Capable of Degrading Amino Acids

After CR treatment, the metabolic function of intestinal microorganisms is also altered, and the bacteria associated with the metabolism of amino acids are also altered. During amino acid

metabolism by microorganisms, the biosynthesis of lysine is significantly increased, while the synthesis of phenylalanine and tryptophan is decreased, the degradation of branched-chain amino acids is significantly decreased [20].

It was concluded that the phylum Firmicutes, Bacteroidetes and Proteobacteria are the main phylum of intestinal microorganisms; However, changes in the relative abundance of these microorganisms caused by CR had distinct results. Several studies found that dietary treatments favored the thickwalled phylum over the Proteobacteria phylum, whereas long-term (45 days) CR enriched the Proteobacteria phylum in obese participants and dramatically lowered the ratio of thick-walled phylum: Proteobacteria phylum. Some of the inconsistencies may be due to changes in the relative abundance of low-grade taxa due to changes in their diets, while others may be due to changes in the overall microbial diversity. In general, CR treatment reduced the relative abundance of Clostridium bacteria, resulting in a drop in the families Ducheneidae, Lachnospiraceae, Peptostreptococcaceae, and Daniostreptococcaceae. This resulted in a drop in Bacteroidales abundance, while Porphyromonadaceae, Rikenellaceae, and Bacteroidaceae, all of which belong to this order, showed a rising tendency [16].

6. Food Components Directed by CR and Population Targeted by CR

Reducing the body's production of reactive oxygen species by caloric restriction and good nutrition appears to help lengthen longevity and delay the onset of age-related illnesses. Studies have shown that calories can limit changes in the levels of hormones and lipid metabolites and alter energy metabolism. Weight loss, improved glucose homeostasis, and greater energy expenditure are all benefits of dietary protein restriction (PR). Analysis of the lung and spleen proteomes showed that caloric restriction inhibited antigen processing and presentation proteins and reduced levels of proteins involved in glycogen metabolism and protein glycosylation. As a result, a wide spectrum of proteins was upregulated, including keratin and laminin, which play a role in ligament support, Mfap4, which helps maintain elastic fiber stability, Serpinb3a, a key player in the beginning of acute inflammatory reactions and in maintaining the integrity of the epidermal barrier, serpinb12, a cellular barrier that protects epithelial tissues such as the lung from external stimulation. In addition, a number of proteins are involved in the protection of tissues against oxidative stress [21].

Caloric restriction (CR) for longevity and health advantages has been well documented in many species, including nonhuman primates, but its long-term consequences in humans have yet to be determined.

Caloric restriction is also indicated in patients with depression. Caloric restriction has received increasing attention because of its apparent effects on the neuroendocrine system and mood states. Both basic and clinical studies have shown that caloric restriction can trigger metabolism, including stress responses and neurogenesis. Caloric restriction can also be beneficial for patients with CVD. Caloric restriction has emerged as a non-pharmacological therapy for the prevention of cardiovascular disease (CVD). Recent findings are that calorie restriction may be a way to reduce the development of atherosclerosis. Endothelial eNOS regulates endothelial function and operates as a fundamental regulator of vascular tone and dynamic homeostasis by producing the gas nitric oxide (NO) [22]. SIRT1 (Sir2) is a NAD(+)-dependent deacetylase that plays a key role in metabolism, immune response and aging. A key role in a range of biological events including metabolism, immune response and aging. CR promotes endothelial-type nitric oxide synthase (eNOS) activity and SIRT1 expression, resulting in improved vasodilation and thus better regulation of blood pressure and blood flow. Moderate CR improves cardiometabolic risk in non-obese youth and middle-aged adults. In recent years, there is growing evidence for CR in the prevention of cardiovascular disease.

7. Conclusion

An effective calorie-restrictive diet (CR) is one that does not cause starvation or nutrient deficiencies. For centuries, it has been widely accepted as a natural method for boosting health and extending life. Caloric restriction can affect the composition of the intestinal flora, and this paper explores this with respect to probiotic strains, proinflammatory microbes, butyrate-producing bacteria, and microbes with amino acid degradation, showing that the relative abundance of probiotics and butyrate-producing microbes increased, while pro-inflammatory strains exhibited suppressed relative abundance. Different forms of caloric restriction also affect the composition and metabolism of the intestinal flora. Dieting and restrictive diets show differences and similarities in the metabolism of the intestinal flora, with dieting allowing some degree of *Clostridium difficile* colonization resistance, while during fasting, mice show an increase in *Ackermania* (Akk bacteria) and a decrease in *Alistipes*. Beneficial and harmful bacteria in different intestinal tracts may also be affected to different degrees. However, the dysbiosis of intestinal flora induced by caloric restriction has not been clearly investigated and this would be an interesting area of research.

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