

A Review of the Role of Gut microbiome in Obesity

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Abstract. Obesity has become a global epidemic during the last several years. In addition to genes, lifestyle, socioeconomic status, and other factors that mainly give rise to obesity, gut microbiome recently has aroused great concern for its pivotal role in obesity and host metabolism. A great number of studies have done to uncover the inner associations between gut microbiota and obesity. Among the commonly reported findings, the phylum of Firmicutes and Bacteroidetes are highly related to excessive weight gain, with a higher ratio of F/B in obese subjects. In this review, we summarized some important studies focusing on the alteration and possible role of different bacterial taxa affecting obesity. We also discussed the diet effect on intestinal microbial community and potential molecular mechanisms of energy metabolism involved by gut microbiota.

1 Introduction

An increasing number of people have been suffering from obesity over the world in recent decades. Obesity is a risk factor for many related diseases, including diabetes, fatty liver, and cardiovascular diseases. Previous studies have demonstrated that excess accumulation of adipose tissue which mainly depends on energy intake and expenditure results in obesity [1]. However, recent researches have shown that gut microbiome is tightly associated with obesity, providing new ideas in the potential use of microbiota in clinical applications for addressing and preventing obesity. Humans' gut is the most important habitat for microbiota, estimated to harbor 99% of total microorganisms in the human host [2]. Gut microbiota involves in food digestion [3], development of the immune system, host metabolism, and functions in mucosal barrier [4], benefiting mutually with the host. The disorder of gut microbiota can trigger many chronic diseases such as inflammatory bowel disease [5], type 2 diabetes [6]. And obesity is no exception to this rule.

Modern molecular biology methods such as pyrosequencing of 16S rRNA, fluorescent in situ hybridization (FISH), and next-generation sequencing (NGS), have brought great convenience to the investigation of the gut microbiome [7, 8]. Ruth E. Ley's team found a significant increase of Firmicutes and a decrease of Bacteroidetes in obese mice gut by 16SrRNA sequencing [9] and Fredrik Bäckhed and his co-workers' findings showed a rapid increase in germ-free mice body fat after colonization of a gut microbiota [10]. Besides, more studies have focused on the relationship between obesity and alterations in the composition of gut microbiome including the number of related species and their genetic coding products by comparing microbiome in the obese and lean gut and microbiota transplantation.

Furthermore, other studies have revealed various mechanisms of the interaction between gut microbiota and host in energy balance. In this review, we researched some important literature regarding the microbiome in obesity and summarize the most reliable findings.

2 Obesity

Obesity has become a serious health problem worldwide in recent decades, an increasing number of people are suffering anguish brought by the weight gain including unsightly appearance, disease torture, inferiority complex. BMI (Body Mass Index) is an important international standard to measure the degree of obesity and health. BMI ranging from 18.5 to 24.9 is defined as normal, BMI beyond 25 is defined as overweight, BMI beyond 30 is considered as obesity.

A survey published in 2014 showed that the proportion of adult men over the world who are overweight or obese (BMI>25) from 28.8% in 1980 to 36.9% in 2013 while the figure for women increased by more than 10% in 2013 compared to 1980. This growth trend was also seen in children and adolescents in both developed and developing countries, increasing to 23.8% in boys and 22.6% in girls in developed countries and to 12.9% in boys and 13.4% in girls in developing countries in 2013 [11]. It is predicted that the percentage of the overweight or obese population will up to 57.8% in adults in 2030 [12].

Obesity is considered as an inflammation caused by the adipocyte hypertrophy and hyperplasia initiates crosstalk between adipocyte and resident macrophage (M2) in white adipose tissue (WAT) [13]. This physiological process can be triggered by many factors. For example, some results showed that energy balance plays a pivotal role in fat accumulation. D.A. Díaz-Rizzolo et al [14] investigated 182 prediabetes subjects aged over 65. They found obese individuals' total daily

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energy expenditure is lower than non-obese individuals while they intake similar Kcal per day, and almost all the obese subjects formed a sedentary lifestyle. In addition, other studies reported that associated genes, lifestyle, unhealthy diets like high-fat diet [15], and socioeconomic status [16] are account for weight gain as well. This epidemic disease (obesity) can bring about many serious diseases including gallstone disease [17], fatty liver disease, insulin resistance (the main reason for diabetes) [6], hyperlipidemia, hypertension, cardiovascular diseases [15]. Complications of obesity and obesity itself are highly likely to limit lifespan and increase mortality, especially for older people. In these years, many researchers and scientists have linked gut microbiome to obesity, paying their attention to the influences triggered by alteration of gut microbial community and the mechanisms of host-bacteria interaction in the gut.

3 Gut microbiome

An incredible number of microorganisms are in the human body or on the body's surfaces, playing an important role in body development and disease resistance. However, human digestive system, especially for the gastrointestinal tract is a huge habitat for a large number of microorganisms harboring about 10^{14} bacteria, and most of them are colonized in the large intestine, with the total number much bigger than that of human body cells [18, 19]. The gut microbiota is also demonstrated to have about 500 times genes than that human own [20], encoding products that involve in the food digestion.

People obtain their original gut microbiome from their mother after they were born [21], and then the community composition and function change with growth stages, different environmental factors such as diet, antibiotics, pathogens as well as geography [22-24]. Research indicated that a highly similar structure and diversity of the gut microbial community is shown between family members, which illustrated the importance of environmental factors in shaping the intestinal microbiota. The genotype is regarded as another reason which is responsible for the microbiota differences. [9, 22]. Although the gut community for each person is unique and varies between individuals, a core functional microbiome contains genes which is tightly associated with the synthesis of signal matters and involved in metabolism [25]. This regulation was demonstrated by Peter J. and his colleagues by studying 154 volunteers' fecal microbiotas.

Researches have illustrated that more than 500 phylotypes inhabit our gut [26] and these species include fungi, protozoa and viruses, and bacteria (Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Fusobacteria, Verrucomicrobia, and Cyanobacteria) which is the most dominant in the colon. Most of the bacteria are strictly anaerobic (97 %) [27]. Bacteroidetes (B. thetaiotaomicron is very common in different individuals) and Firmicutes (95% of the Firmicutes were Clostridia class) are the most abundant phylum of gut bacteria [9, 26]. The Bacteroidetes can degrade a wide range of substrates and produce acetate and propionate while the Firmicutes are specialized in indigestible polysaccharides

fermentation and generate butyrate [28].

Gut microbiota is tightly associated with human health particularly in host metabolism by regulating energy homeostasis, chronic-low grade inflammation, and function of preventing the damage caused by endogenous microorganisms and toxins. The adverse alteration of gut microflora may pose a threat to human physical health [29]. Intestinal disorders can trigger inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), asthma, obesity, and impair the immune system [24].

4 Gut microbiome in Obesity

4.1 Firmicutes and Bacteroidetes in obesity

The top four rich families in the gut microbiota are the Bacteroidetes, Proteobacteria, Actinobacteria, and Firmicutes [30]. However, Bacteroidetes and Firmicutes are the most abundant phylum among them. An early study in 2005 indicated that the two taxa are dominated in mice gut [9]. Ruth E. et al analyzed human gut microbiota in 12 obese volunteers' stool samples by 16S rRNA genes sequencing and observed that Firmicutes and Bacteroidetes are the major phyla [31]. Husen Zhang et al had the same observation [32].

The change in the abundance of the two phyla is the main manifestation in obese patients' gut microbiota. Ruth E. Ley and his team analyzed the distal gut microbiota from genetically obese ob/ob mice, lean ob/+, wild-type siblings, and their ob/+ mothers. Findings were that compared to lean mice, there is a dramatic increase in Firmicutes and a reduction in Bacteroidetes taxa in ob/ob mice gut [9]. This phylum-wide compositional pattern associated with body mass is also seen in some human experiments. Researches show that the ratio of F/B (Firmicutes/Bacteroidetes) in the human gut microbial community is determined by human body weight, which means that obese people have a higher ratio of F/B in contrast to non-obese people [31, 32]. However, some scientists did not obtain this ratio pattern. SH Duncan et al didn't observe any differences in the abundance of Bacteroidetes between obese and lean groups [33]. Andreas Schwiertz et al acquired a lower ratio of F/B in obese and overweight feces [34]. Collado, MC and his co-workers found a higher proportion of Bacteroides in obese pregnant women [35]. Despite these different findings, A well-known paper published in 2006 reported that the gut microbiota transplantation from obese mice to germ-free mice cause a dramatical total body fat increase in contrast to those who received microbiota from lean donors, which may imply that microbiome from obese mice (a high ratio of F/B) can absorb energy more efficiently from food substrates [36]. This research achievement showed a significant relevance of the two phyla in the study of obesity.

Some studies also reported other species including a subgroup of Firmicutes and Bacteroidetes altering in obese people or mice intestine. Prevotellaceae, the member of Bacteroidetes family, were observed to be much richer in overweight individuals [32] than people with normal weight while Bifidobacteria which belongs to

Firmicutes and Bacteroides decreased in the overweight pregnant gut [37]. Ruixin Liu et al. [38] reported a significant drop in Bacteroides thetaiotaomicron abundance in obese young Chinese participants and found that the diet-induced body-weight gain in mice can be mitigated by feeding this species. The amount of Faecalibacterium prausnitzii and Lactobacillus are associated with obesity as well [36, 39, 40]. Methanobacteriales which can degrade organic matter into CO₂ and CH₄ are found more in obese mice than their lean siblings [36].

The prevalence of obesity has become more severe in children. Maternal obesity which can result in children's gut microbial community alteration [41] might be account for overweight in offspring. It is worth mentioning that children who remained normal weight had a higher amount of Bifidobacteria in their infancy's stool than children who became fat later and Staphylococcus aureus is contrarily lower for normal-weight children [42]. These results bring the possibility to forecast infant future body mass through microbiota in their early feces.

4.2 Diet-induced alteration of the gut microbiome in obesity

Diet pattern is considered as one of the main reasons for causing obesity. Lots of studies have observed that diet is high fat, low fiber, high carbohydrate can lead to weight gain [43, 44]. During these decades, many scientists have associated this phenomenon with the alteration in the gut microbiome community resulting from the diet. It has been reported that changes in the diet can account for 57 % of the variations in microbiota compared to genetic variations in a host that can only account for 12 % [43]. In addition, the Gut microbiome community can responses quickly to the diet change. For example, the microbiota structure shifts within a single day when changing to "Western" diet from a low-fat diet rich in plant polysaccharide [44].

Lots of studies have done in obese and non-obese subjects to explore the relationship between the gut microbiome and diet patterns. The western diet is known for its high fat, sugar, and low fiber composition and easily causes obesity. Mediterranean Diet is a healthy, simple, light and nutritious diet benefiting a lot to human health. Vegetarian Diet is a plant-based diet with low fat and rich fiber. Distinct microbiota alterations in the intestine are

correlated with different diet patterns. Similarly, we focus on the change of Firmicutes and Bacteroidetes first. Obese mice who eat a western-diet are found to have a higher ratio of Firmicutes / Bacteroidetes in their gut and a decrease of micro-diversity because of a bloom of the Mollicutes belonging to Firmicutes [43]. An increased calorie diet raises the amount of Firmicutes and cause a reduction in Bacteroidetes, as well as feces energy loss in lean subjects which may suggest an energy harvest increase [45]. In contrast, an opposite change in the two phylum results from a low-calorie diet was demonstrated in another report [31]. The abundance alteration of other species is observed as well. Sugar is found to decrease the proportion of Anaerofilum, Gemellaceae, Streptococcaceae, Rikenellaceae taxa in people with obesity [46]. A high-fat diet can lead to a reduction in Clostridium leptum and Enterobacter spp and a rise in Lactobacillus spp., Bifidobacterium spp., Bacteroides spp., and Enterococcus spp. [47]. Besides these, we also summarized other findings in the chart below (Table 1). Moreover, a transplantation experiment illustrated that gut microbiome functions as a bridge between diet and obesity [43]. Lean germ-free mice harboring microbiota from obese mice (induced by western diet rich in fat/sugar) occurred greater fat accumulation than recipients who received microbiota from lean donors, which means the "diet-induced" alteration of gut microbiota with a stronger ability of energy harvest may be responsible for obesity.

Some scientists have made new discoveries by studying the dynamics of body weight and the gut microbiome. In Peter J. Turnbaugh et al study [43], through analyzing stool samples from 12 unrelated obese humans who consumed a one-year lasting fat-restricted or carbohydrate-restricted diet with low calorie, they found the amount of Mollicutes class decreased with weight loss. I Nadal et al's work revealed that the calorie-restricted diet and increased physical activity can induce a decrease in the abundance of Clostridium histolyticum and E. rectale-C. coccoides in overweight and obese adolescents correlated with BMI z-score reductions [48]. It is also found that a group of Firmicutes producing butyrate (a kind of gut bacteria metabolites absorbed by colonic epithelium as the energy source [49]) decreased as obese individuals lost weight with a carbohydrate-restricted diet [33]. Their results illustrated a possible relation between intervening diet, body mass, and microbiome change, but mechanisms behind this connection are still unclear.

Table1. Effect of diet pattern on gut microbiota

Diet pattern	Effect on gut microbiota
Western Diet (high in saturated fat, carbohydrate, salt, and low in fiber)	Non-human Primates: a higher Firmicutes-Bacteroides ratio and increase in Clostridiaceae and Lactobacillaceae [50] Mice: Increase in Firmicutes and Erysipelotrichaceae [51] Mice: Increase in Dorea and decrease in Bifidobacterium and Akkermansia [52]
Mediterranean Diet (more intake of olive oil, vegetables, fruit, fish, seafood, and beans)	Human: Increase in Faecalibacterium prausnitzii (fiber consumption) and reduce in Ruthenibacterium lactatiformans, Flavonifractor plautii, Parabacteroides merdae, Ruminococcus torques and Ruminococcus gnavus [53] Human: increase in Christensenellaceae and decrease in Firmicutes-Bacteroidetes ratio [54]
Vegetarian Diet (rich in dietary fiber, plant-polysaccharide, and low in fat, calories)	Human: increase in Bacteroidetes and decrease in Firmicutes [55] Human: decrease in the abundance of Enterobacteriaceae [56] Human: increase in Prevotella and decrease in Bacteroides [57]

5 Mechanisms in energy metabolism influenced by gut microbiota

5.1 SCFAs associated metabolism

Digestion of food in the human gut requires a variety of enzymes encoded by microorganisms' genes to make up for the lack of human gene functions [58]. Consequently, Diet fiber such as complex plant carbohydrates and resistant starch cannot be completely degraded by host hydrolase independently. Therefore, the absorption of these indigestible polysaccharides relies on the gut microbiota fermentation which breaks macromolecular to short-chain fatty acids (SCFAs) as the products [59]. SCFAs contain three main substances including acetate, propionate, and butyrate [60]. Butyrate is mainly absorbed by colonic epithelium and oxidized there with energy generation while propionate and acetate serve as substrates in other metabolisms in the liver or other tissues to modulate energy homeostasis [61]. It was estimated that about 5%-10% of the daily calories that humans require provided by SCFAs [62]. In addition, scientists found that uptake of SCFAs from sugar in large intestine acquires much less calorie than direct sugar uptake does in the small intestine. This finding may suggest that having a diet with fermentable, indigestible fiber can achieve less energy uptake in contrast to digestible diet, bringing the possibility to control body weight in obesity [63].

Intestine microbiota structures and microbiome can be influenced by lots of factors such as Colonic milieu. pH is one of the most important environmental elements that make a difference to SCFAs production. And in turn the production of these acids triggers a pH decrease in the gut environment which alters the composition of microbiota such as creating a better condition for the growth of butyrate-producing Firmicutes [64].

SCFAs also play a role in signal transduction. Until now, some mechanism studies have revealed no less than

one metabolism pathways that SCFAs regulate such as Fatty acid metabolism which is tightly associated with fat accumulation. Three main signaling pathways are involved in the regulation: 1) SCFAs activate AMPK and then stimulate the expression of PGC-1 α resulting in an increase of fatty acid oxidation and decrease of synthesis in liver and muscle tissue. 2) SCFAs directly promote the production of PGC-1 α and UCP-1 proteins and in turn increase the fatty acid oxidation in brown adipose tissue. 3) SCFAs increase the concentration of leptin (a hormone that can reduce appetite and increase energy release) in white adipose tissue, which is mediated by FFA2[65-67]. FFA2 is called GPR43 (mostly expressed in immune cells) as well. It is the G protein-coupled receptors of SCFAs together with GPR41 (FFA3) (highest expression in adipose tissues) [68]. They are also involved in regulating appetite through stimulating the release of gut hormones, glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) by L cells such as inhibition of food intake by propionate [69].

Some studies pay attention to the relationship between body weight, gut microbiota and, cecal or fecal SCFAs concentrations. In Fernandes, J et al research, more abundant short-chain fatty acids were found in obese and overweight individuals' stool samples in comparison to lean subjects' and Firmicutes to Bacteroides ratio rose as fecal total SCFAs increased [70]. An increased concentration of butyrate and acetate in obese mice caecum with a higher ratio of F/B was reported [36]. Other evidence also showed that higher fecal SCFAs is correlated with gut dysbiosis and obesity [34, 61, 71]. However, Barczynska, R and his colleagues observed lower fecal SCFAs in obese children with a higher proportion of Firmicutes than non-obese children [72]. Therefore, these findings suggest that correlations between microbiota, SCFAs remain unclear.

In addition to the mechanism of SCFAs produced by indigestible polysaccharides fermentation, other mechanisms have been demonstrated and reported as well. Therefore, we summarized some valuable previous studies on possible mechanisms in the table below (Table 2).

Table2. Mechanisms in energy metabolism influenced by gut microbiota

mechanisms	contents
SCFAs involved mechanism	Short-chain fatty acids (SCFAs) derive from fiber fermentation by gut microbiota and absorbed by colonic epithelium as an energy source or act as signaling molecular in lipid and glucose metabolism [61].
Fiaf involved mechanism	Fasting induced adipocytokines (Fiaf), a circulating lipoprotein lipase (LPL) inhibitor, is suppressed in Intestinal epithelium by gut microbiota, which in turn activate LPL and accelerate the accumulation of triglycerides in adipocytes while reducing the consumption of fatty acid by inhibiting the expression of genes encoding the peroxisomal proliferator-activated receptor coactivator (Pgc-1 α) and enzymes involved in fatty acid oxidation [10, 73].
AMPK involved mechanism	Adenosine monophosphate-activated protein kinase (AMPK) is involved in regulating cellular energy status. The release of AMPK is suppressed by gut microbiota and then inhibit the fatty acid oxidation and promote the triglycerides and lipo synthesis [74, 75].

Bile acids involved mechanism	Gut microbiota mediate lipid metabolism in the liver and diet-induced obesity through farnesoid X receptor (FXR) activated by bile acids. Bile acids also combine with G-protein-coupled bile acid receptor 1 (TGR5) as a signaling molecular and in turn stimulate GLP-1 release in the gut to involve in glucose metabolism [76, 77].
Choline involved mechanism	Very - low - density lipoproteins (VLDL) which consists of choline derived Phosphatidylcholine can export triglycerides to the organs and in turn reduce its accumulation. Gut microbiota regulates the storage of triglycerides in the liver by producing trimethylamine from choline which indirectly control VLDL [75, 78].

6 Conclusion

Obesity is of increasing concern for its expanding infected population and associated metabolic diseases. In addition to diet, lifestyle, host genes, and other factors, intestinal flora has become a hot topic in obesity research in recent years. Scientists have made great progress in this field in combination with modern molecular biology research methods. Changes in the abundance and community composition of intestinal microbial species associated with obesity have been revealed, which in turn cause alteration in the gene expression and metabolic pathways of gut microbiota. What's more, this tiny ecosystem in the colon may also play a media role in diet pattern and obesity. Although some of the molecular mechanisms that link obesity to intestinal microbiota disorders have been confirmed, a number of conflicting findings and results have added to the complexity of issues and researches. Thus, there are still numerous questions and phenomena waited to be answered and explained. For example, whether the number of samples used to study gut microbiota is sufficient and rigorous enough to provide reliable results? To what extent the changes in Firmicutes and Bacteroidetes are related to body weight and what are the underlying mechanisms? How meaningful are the results of studies on animals such as mice to human studies? How can "certain diet" regulate intestinal microbiome and in turn influence human health? Do viruses, fungi and other community members involve in energy metabolism as bacteria does? Therefore, more human experiments and data are needed to understand the relationship between intestinal microbiota, obesity and energy homeostasis. MicroRNAs are non-protein-coding RNA that participate in the regulation of gene expression by acting on target mRNAs, thereby affecting some important physiological processes and metabolic pathways. Recent studies have shown that miRNA can modulate adipogenesis and play an important role in the resistance of gut microbiota to pathogenic bacteria, and its expression may be regulated by intestinal microbiota. These results suggest that miRNA may become a new direction for the study of the interaction between obesity and gut microbiota.

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