Diet, Gut Microbiota and Obesity

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Abstract

Increasing evidence suggests that alteration of gut microbiota ('dysbiosis') can lead to a number of diseases, including obesity, which affects a large population in the world and is now a global health issue. The mechanisms of gut microbiota-mediated obesity are just being explored and characterized in recent years. It has been suggested that dysbiosis of gut microbiota contributes to obesity development mainly in three ways: affecting energy harvest, altering host gene expression, and triggering chronic inflammation. Among the factors that determine and influence gut microbiota composition, diet is one of the best characterized in human and animal studies, and has been long linked with weight gain or loss. In this review, we will discuss recent advances of mechanisms through which gut microbiota dysbiosis leads to obesity. We will further discuss the underlying causes of obesity-related gut microbiota, highlighting dietary effects.

Keywords: Diet; Gut microbiota dysbiosis; Obesity

Introduction

Obesity is a medical condition with excess body weight, increasing the risk of a number of diseases, such as Type 2 Diabetes (T2D), heart disease, liver disease, and stroke. In clinic, obesity is described as Body Mass Index (BMI) [1] higher than 30kg/m², which is a general standard for defining obese individuals. It is believed that consumption of high-calorie diet and reduced physical activity are the causes of high prevalence of obesity in high-income countries, where sedentary lifestyles are becoming predominant [2]. Emerging evidence now suggests that gut microbiota plays a key role in mediating diet- and lifestyle-caused obesity (Figure 1) [3].

The human Gastrointestinal (GI) tract is estimated to be colonized by over 100 trillion (10¹⁴) microbes, ten times more than the number of human cells in the body [4]. The term gut microbiota describes all the commensal microbial species living in the intestine. It is becoming evident that gut microbiota plays critical roles in maintaining human health on many aspects,

including fermenting unused energy substrates, training the immune system, maintaining epithelial integrity, regulating gut development, and preventing the invasion of pathogenic bacteria [4,5]. Recent studies based on 16S ribosomal-RNA gene sequencing and metagenomic analysis have started to explore the species diversity of gut microbiota within and between individuals [6,7]. It is estimated that about 1000 species can colonize the human gut. Although there is a big range of variation in the species level from different individuals, one recent study showed that the gut microbiota of most individuals can be classified into one of three clusters ('enterotypes') based on the three dominant bacterial genera, Bacteroides, Prevotella, and Ruminococcus [8].

The composition of the gut microbiota is determined and influenced by a number of factors, such as genetics, age, geographic origin, diet and the use of antibiotics [4,5]. One study has compared bacterial species of fecal samples from 531 individuals of different ages (0-70 years) and geographic origins (three populations from US and Malawi) [9]. It was found that the diversity of the gut microbiota within individuals is much higher in adults than in children, but that the interpersonal differences are significantly higher in children. The composition of the bacterial community converges towards an adult-like microbiota by the end of the first 3-5 years of life. These features are shared in all three populations.

Gut Microbiota and Obesity

Studies from the past decade have provided strong evidence of the association between obesity and gut microbiota [10-14]. For example, by transplanting human whole fecal microbiota from obese (Ob) and lean (Ln) twins to germ free mice, one recent study showed that the gut microbiota modulates host metabolism to regulate body mass [13]. Mice that received fecal microbiota from the Ob twins had increased total and fat mass and showed obesity-associated metabolic disorders, phenotypes that were not observed in mice receiving fecal microbiota from

the Ln twins. The mechanisms of microbiota dysbiosis on obesity development are just being explored, and we will discuss them as below (Figure 1).

Energy harvest

Gut microbiota could promote host energy harvest from the diet, which is supported by the observation that conventionally raised mice show more body weight gain than germ free mice. Fecal microbiota transplantation experiments show that transfer of the gut microbiota from obese mice ('obese microbiota') to germ free and wild type recipients led to an increase in body weight in the recipients, supporting the proposal that obese microbiota is more efficient in extracting energy from the diet than that of lean individuals [15]. Furthermore, in obese microbiota, the relative abundance of two dominant bacterial phyla Firmicutes and Bacteroidetes shifted to a status with high metabolic potential, i.e., fewer Bacteroidetes and more Firmicutes, compared to normal microbiota [15,16], resulting in increased fermentation to enhance the hydrolysis of indigestible food and production of Short-Chain Fatty Acids (SCFAs), such as butyrate, acetate and propionate [17-19]. Particularly, butyrate is the main energy substrate for cellular metabolism in colonic epithelium; acetate and propionate are utilized by liver and act as substrates for hepatic lipogenesis and gluconeogenesis. SCFAs, especially butyrate and propionate, also activate gut gluconeogenesis [20]. In germ free mice, the colon epithelium is energy deprived and undergoes autophagy due to lack of butyrate produced by gut microbiota [21]. Studies showed that the fecal concentration of SCFAs was higher in obese compared with obese and normal-weight adult and children [17,22]. Additionally, diet induced mice obesity models have revealed the role of SCFA receptors in obesity. For example, mice with the null mutation of free fatty acid receptor 2 (FFAR2), FFAR2^{-/-} mice, fed a high fat diet had less weight gain than FFAR2 wild type mice [23], while there is no difference in weight gain in FFAR3^{-/-} mice compared with WT

mice maintained on either a normal food or high fat diet over a 5-month time period [24]. Furthermore, several studies indicated that SCFAs supplementation treatment may protect against diet-induced obesity, as observed that mice fed a high fat diet along with SCFAs supplementation gained less body weight than the control high fat diet-fed mice [24-26]. Thus, these data suggest that differences in the metabolic capacity of an individual's gut microbiota may contribute to the development of obesity at least in certain conditions.

Roux-en-Y Gastric Bypass (RYGB) is one of the most common and effective bariatric surgeries for treatment of severe obese patients [27] and the mechanisms of RYGB-mediated weight loss include reduced food ingestion, change of appetite, release of satiety-promoting hormones and shift in bile acid metabolism [28]. Recent studies from RYGB patients [29] and RYGB-mouse model [30] have begun to characterize the gut microbiota as a mediator for RYGB-induced metabolism effects. Both studies have shown not only that the gut microbiota composition shifted to a different status after RYGB surgery, correlating with a sustained weight loss, but also that transplantation of RYGB-microbiota to germ-free mice can induce significant weight loss of the recipients compared to mice receiving control microbiota. It was observed in one study [29] that the concentrations of SCFAs (acetate, propionate and butyrate) were decreased in RYGB patients, and thus the reduced energy harvest was proposed to contribute to weight loss. In the other study [30], however, it was found that the proportions of SCFAs in RYGB mice were changed (acetate decreased, propionate increased), but the net energy intake was not significantly different. With previous findings [31], the authors proposed that RYGB-microbiota induces weight loss through increasing energy expenditure [30]. In summary, it is evident that gut microbiota plays a pivotal role on RYGB-induced weight loss, but the mechanisms are needed to be clarified from future studies.

Figure 1: Specific diets, such as Western diet, can cause dysbiosis of gut microbiota, from 'normal microbiota' to 'obese microbiota', which leads to obesity development by altering the host gene expression, influencing the energy harvest from diet, and triggering low-grade chronic inflammation.

Host gene expression

Gut microbiota not only directs the fermentation of indigestible dietary polysaccharides into absorbable monosaccharides in gut lumen, but also shapes the host metabolism status by affecting the expression of host genes that regulate energy storage and expenditure. It has been revealed that gut microbiota instructs the host to increase hepatic lipid accumulation, which is associated with the development of insulin resistance and T2D [18]. The expression of fasting-induced adipose factor, Fiaf, a circulating Lipoprotein Lipase (LPL) inhibitor, is suppressed by conventionalized microbiota. Through suppressing Fiaf, the gut microbiota increases lipoprotein lipase activity, thus enhancing the uptake of fatty acids in gut epithelial cell and deposition of lipids in adipose [18,32]. Consistently, germ free Fiaf^{-/-} mice show 67% higher LPL activity in epididymal fat pad than germ free littermates harboring the wild type Fiaf allele. SCFAs, a family of fermentation end-products by gut microbiota, as mentioned above, may influence host susceptibility to T2D through epigenetic regulation of gene expression as a histone deacetylase inhibitor [33].

Studies have also shown gut microbiota may promote obesity through preventing host excess energy expenditure. AMP-Activated Protein Kinase (AMPK), a “fuel gauge” that monitors cellular energy status, has been implicated in the regulation of glucose and lipid homeostasis, and its activation leads to maintaining cellular energy stores, mostly by enhancing oxidative metabolism. It has been revealed that AMPK activity is diminished or impaired in skeletal muscle of individuals with obesity and diabetes [34]. Compared to conventionally reared mice, germ free mice show persistent resistance to obesity induced by Western diet with high fat and high sugar, which is linked to its increased AMPK activity in liver and skeleton muscle, suggesting that the presence of gut microbiota suppresses host AMPK activity to prevent excess energy expenditure [35]. Thus, gut microbiota and its products interact with molecules expressed by gut epithelium and thereby regulate the balance between energy storage and expenditure at the host side.

Chronic inflammation

Obesity is associated with low-grade chronic systemic inflammation, which has been implicated in insulin resistance and the development of T2D [36]. High Fat Diet (HFD) induced obesity is tightly associated with increased expression of several pro-inflammatory cytokines, such as IL-1, IL-6, TNF- α , and MCP-1 [37-39]. Studies show that obese mice and humans have significantly elevated bacteria-derived plasma lipopolysaccharide (LPS) levels, termed as “metabolic endotoxemia” [40,41], which contribute to the low-grade inflammation through the signal cascade involved in LPS binding to Toll-Like Receptor 4 (TLR4) and subsequent activation of innate immune response. Subcutaneous infusion of LPS could lead to the increase of body fat to a similar extent as in high fat diet-fed mice [40], a phenotype that was not present in LPS unresponsive TLR4^{-/-} or CD14^{-/-} mice [40,42-44]. Similarly, elimination of gram-negative bacteria by antibiotics reduced metabolic endotoxemia and the LPS levels in obese mice, alleviating the obesity phenotype [45]. In addition to LPS, the

circulating fatty acids, other ligands of TLR4 signaling, could also activate host immune response, contributing to obesity-related chronic inflammation [44]. Two other Toll-like receptors, TLR2 and TLR5, have also been implicated in gut microbiota dysbiosis-induced immune activation, although the mechanisms remain elusive [46,47]. Besides, the obese microbiota may also cause chronic inflammation by breaking the gut barrier integrity, leading to leaky gut symptoms [48]. A recent study in HFD-induced rodent obesity model added further insights in the association of gut microbiota dysbiosis, chronic inflammation, and metabolic disorders. Through analyzing the changes of gut immune system in HFD-induced T2D mice, they found that HFD-fed mice impaired the T cell homeostasis in the gut, i.e., a loss of IL-17/ROR γ t CD4 T cells (Th17 cells). Normal food-fed ROR γ t^{-/-} mice, which lack gut mature Th17 cells, spontaneously developed T2D phenotypes, such as glucose intolerant, hyperinsulinemic, and slightly insulin resistant. Further studies revealed that HFD-induced decrease in relative abundance of the *Porphyromonadaceae* and increase of *Bacteroidaceae* and *Comamonadaceae* are responsible for the dysregulated gut immune response through affecting the gene expression profiles of gut Antigen Presenting Cells (APCs) and reducing their ability to induce Th17 cell differentiation. Symbiotic treatment that modulates the abundance of these bacterial species protected mice from HFD-induced metabolic disorders through prevention of the loss of Th17 cells. In turn, the impaired gut immune homeostasis further enhances the deleterious gut microbiota composition, thus preceding the onset of metabolic disorders [49]. These studies suggest a vital role of gut immune system in linking gut microbiota dysbiosis to metabolic disorders.

Diet and Gut Microbiota

Diet is one of the factors that can shape the gut microbiota structure, and certain types of diets have been associated with obese microbiota (Figure 1) [50,51]. For example, the *Bacteroides* genus is highly associated with the consumption of animal proteins, amino acids and saturated fats, which are typical components of Western diet, while the *Prevotella* genus is associated with the consumption of carbohydrates and simple sugars, which are typical for agrarian societies. People with a *Bacteroides*-dominated gut microbiome will gain a *Prevotella*-dominated microbiome by switching from a Western diet to a carbohydrates-based diet for an extended period of time. Consistently, another study found that the European microbiome is dominated by taxa typical of the *Bacteroides*, whereas the African microbiome is dominated by the *Prevotella* [52].

To gain more details how diet calories influence gut microbiota, one human study investigated the dynamic changes of gut microbiota in 12 lean and 9 obese individuals in response to diets with different caloric contents, and found that the nutrient load can rapidly influence the composition of gut microbiota [53]. Meanwhile, by monitoring ingested calories (energy consumption) and stool calories (energy loss) using bomb calorimetry, it was found that stool energy loss in lean individuals was directly correlated with changes of Firmicutes and Bacteroidetes (two dominant bacterial phyla in the distal

gut): an increase in Firmicutes and a corresponding decrease in Bacteroidetes contribute to reduced energy loss, that is to say, elevated energy harvest. In another study on obese and overweight subjects, microbial gene richness, instead of the bacterial composition, was used as readout for gut microbiota status, and it was found that 18 (40%) individuals are with Low Gene Count (LGC) and 27 (60%) are with High Gene Count (HGC) [54]. The LGC group showed more noted systemic dys-metabolism (such as high insulin resistance) and low-grade inflammation compared to the HGC group, and 6-week dietary intervention, which was shown to increase the microbial gene richness in LGC individuals, significantly improved the metabolic status. The low-grade inflammation, however, appeared to be relatively refractory to dietary intervention. To characterize how quickly the human gut microbiota can respond to the changes of diet, David et al. [55], found that it took only one day for the changes to occur after the intervention diet reached distal gut microbiota and that the gut microbiota went back to original structure two days after the intervention ended, suggesting that gut microbiota can be altered by diet in a very acute manner.

Studies from animal models have also provided rich insights into the interaction between diet and changes of gut microbiota. One study took advantage of gnotobiotic mice harboring a community of 10 sequenced human gut bacteria to explore the response of the microbiota to the changes of diet [56]. A series of diets with defined concentrations of four macronutrient gradients (casein for protein, corn oil for fat, starch for polysaccharide, and sucrose for simple sugar) were administered to these mice, and shotgun sequencing of total fecal DNA was performed to determine the absolute abundance of each bacteria. It was shown that total community abundance and the abundance of each species were best associated with casein level: seven out of ten species (such as *Bacteroides caccae*) are positively correlated with casein concentration and three remaining species (such as *Eubacterium rectale*) are negatively correlated with casein concentration. The authors further generated a linear statistical model, which, to some extent, can predict the abundance of each bacterial species from each of the perturbed diet components. Besides extrinsic factors (such as diet), intrinsic factors (such as genetics) can also shape the structure of gut microbiota in mammals, but whether they contribute equally is not clear. A recent study reported that diet can overrule the differences of gut microbiota that are associated with different genotypes, including inbred, transgenic and outbred mice, suggesting that diet plays a dominant role in shaping gut microbial ecology [57].

Obesity is strongly associated with specific diets (e.g., Western diet), which, as discussed above, can influence the composition of gut microbiota. Thus, an association between changes in the diet induced-gut microbiota and the development of obesity has been proposed [58]. Accordingly, an epidemiological study shows that yogurt consumption can prevent age-associated weight gain, which may be due to the effects of probiotics in the yogurt [59]. Indeed, one study with 10-year follow up records after child birth have tested the impact of the probiotic intervention on the development of obesity, and the results suggest that modulation

of gut microbiota can modify the growth pattern of child by preventing weight gain during the first year of life [60].

Conclusion and Outlook

More and more findings have pointed to the critical roles of gut microbiota on obesity development and its related complications, leading to the idea of targeting gut microbiota for obesity treatment. Although it has become evident that certain types of microbiota dysbiosis can lead to obesity, it is still not well known whether this impact is due to the change in the amount of single or multiple bacterial species, or it is due to the ratio changes of certain species. Thus, future studies should put efforts on the more accurate quantitative analysis of each species in one bacterial community, but also on the exploration of effects from individual bacterial species (like mono-association studies). In addition, identification of gut bacteria-derived molecules which trigger the downstream events leading to the development of obesity will greatly facilitate strategies for interventions.

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