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Gut Microbiota and Obesity

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Abstract

The current obesity epidemic clearly has many causes, including the impact of our modern world on both our diet and our lifestyle/physical activity. Although many interventions have been recommended, the prevalence of obesity continues to rise and has forced a re-evaluation of the potential interventions that could have an impact. In recent years it has been definitively shown that microbiota in the gastrointestinal tract are altered in obese individuals. Recent data provide a potential mechanistic understanding of the interactions between microbiota and obesity and allow potential new interventions to the control of obesity to be proposed.

Keywords

Microbiota; Obesity; Gastrointestinal; Inflammation; Energy; Gut

Introduction

There is currently an epidemic of obesity occurring in the United States, with the most recent study showing a prevalence of 32.2% among adult men and 35.5% among adult women [1]. Significant factors in this epidemic are our diets, which are increasingly high in carbohydrates and fats, and our lack of physical activity [2]. Although critical, these factors clearly are not the whole story; in 2004, Bäckhed et al. [3] proposed an additional mechanism that implicated gastrointestinal (GI) microbiota.

The resident population of microbiota is an essential part of the development and maturity of the host intestinal track and immune system and has therefore come to be considered by some a virtual organ known as the microbiome [4]. The gut microbiome is the totality of microbes (bacteria, viruses, etc.), their genetic elements (genomes), and environmental interactions within the GI track. This microbiome contains over 10 times more organisms than the number of cells in a human body, but unlike other organs its composition is somewhat unstable. The resident populations of bacteria can be altered within 24 hours of a dietary change; therefore, obtaining a unified picture of the microbiome can be a challenging proposition [5].

The involvement of the gut microbiota in the obesity epidemic was first suggested by the fact that adult germ-free (GF) (ie, bacteria-free) C57BL/6 mice had a 60% increase in body

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fat content when they were conventionalized (ie, colonized) with cecal microbiota from a healthy, normal C57BL/6 mouse [3]. The mechanism for this increase in body fat content was hypothesized to include the fact that the microbiota would have the ability to regulate energy harvest from food components and therefore alter energy storage in the host. Since that original publication in 2004, there have now been 138 primary data publications and 60 reviews that are found by a PubMed search for obesity and microbiota. These publications have led to the proposal of three unique mechanisms through which microbiota may impact host obesity, and these are discussed in this review.

Experimental Approaches to the Study of the Microbiome

The study of the gut microbiome is unique among organ systems, as the microbiome can be shed and replenished and there is the unique opportunity to study this "organ" over long periods of time by obtaining fecal samples from a single individual. This type of analysis has led to the concept of "enterotypes" of the gut microbiome and recent data from 22 individuals have indicated a limited number of host-microbial symbiotic states that might respond differently to diets [6]. However, data from fecal samples have to be interpreted with caution, as several groups have indicated that fecal microbiota communities differ from mucosal-associated bacteria in the GI tract [7, 8]. As the techniques to study, measure, and modify the microbiome are somewhat unique to the field and sometimes are not within the usual repertoire of skills other biologists would utilize, we have detailed some experimental approaches in this review.

Bacterial Culture and Identification

Bacterial culture and identification have been extensively used to identify pathogenic or residential bacterial components of feces or tissue [9]. This method utilizes long-standing phenotypic identification practices such as motility, shape, colony structure, and sugar/metabolite utilization. However, many species remain undefined because there currently is no known method to culture these groups outside of the intestinal tract, and for this reason more advanced methods have been developed using nucleotide amplification.

Fluorescence In Situ Hybridization

Fluorescence in situ hybridization (microscopy-FISH) has historically been utilized to identify bacteria present in tissue sections without nucleic acid purification. Briefly, radioactive or fluorescent-tagged nucleic acid–based probes targeting 16S ribosomal RNA are used to permeate preserved histologic samples and allow for visualization of specific organisms [10]. This procedure has the advantage of precise localization of the bacteria, but does not give quantitative results. A newer method that combines FISH with flow-cytometry (FCM-FISH) no longer allows for tissue localization, but when combined with DNA stains is a rapid, reliable, and quantitative method for the analysis of mixed bacterial samples in feces [11].

Quantitative Real-Time Polymerase Chain Reaction

Quantitative real-time polymerase chain reaction (qRT-PCR) is a second method for enumerating the numbers of bacteria present in feces (or tissue samples), but it relies on nucleic acid extraction from the samples. qRT-PCR has very high sensitivity and reproducibility and is very rapid to perform [12]. As with FISH, specific microorganisms are detected based on sequence-specific probes, but only organisms with known sequences can be quantified.

Denaturing Gradient Gel Electrophoresis and 454 Pyrosequencing

There are two nucleic acid–based methods that can identify unknown and nonculturable organisms. Denaturing gradient gel electrophoresis (DGGE) is a method of creating a physical picture of bacterial diversity through a two-dimensional (2D) denaturing gel. DNA is amplified and separated on the 2D gel, where the amplified products migrate according to G:C content and are visualized as unique bands on the gel [13]. Bacteria can be identified through a combination of purification of DNA from the gel and Sanger sequencing methods [14]. Although Sanger sequencing methods can be used to identify numerous bacterial sequences in GI samples, the new high-throughput pyrosequencing technology offers a more rapid and cost-effective method for total microbiome analysis. 454 Pyrosequencing is a method that differs from traditional sequencing in that it does not measure chain termination, but instead relies on the detection of pyrophosphate release upon nucleotide incorporation. This method has now been combined with a novel barcoding approach, which allows simultaneous sequencing of multiple individual samples [15, 16].

Metatranscriptomic Approach and Nuclear Magnetic Resonance

The use of these rapid and extensive sequencing techniques has revealed the enormous diversity of the GI microbiota and its rapidly changing nature [5, 17]. Therefore, recent studies have combined these methods with bacterial gene expression analysis. This metatranscriptomic approach has identified a “core microbiome” at the gene expression, rather than at the organismal lineage, that is associated with obesity [17, 18]. A second method to look at the function of this “core microbiome” is via metabolomics. Nuclear magnetic resonance (NMR) can be used to measure very small molecules, such as individual amino acids, carbohydrates, and lipids/fatty acids. By utilizing the unique magnetic properties from each molecule, NMR measures the magnetic radiation from a sample and is able to measure hundreds of molecules. This is optimal when attempting to measure small molecules from either serum or even feces [19]. Using this type of technique, microbial metabolites generated during colonic fermentation of food stuffs can be determined and their subsequent impact on blood and tissue metabolites determined [20–22].

Germ-Free Models

The concept of altering commensal populations to enhance the health of humans has been long studied, but has only recently been utilized for manipulation of the obese phenotype. Through the use of mouse models, we are able to extract information about how each individual group of bacteria contributes to the microbiome and to the host. GF models are mice or rats that are completely bacteria free. These mice are optimal as negative controls and also invaluable as a “clean source” when looking to mono-colonize an individual with single bacteria to understand how they impact the host [23–25]. One of the landmark experiments indicating the role of the microbiota in obesity utilized GF mice, which were colonized with an “obese microbiota” or a “lean microbiota.” The “obese microbiota” transfer resulted in mice with a greater increase in total body fat and clearly identified the gut microbiota as a contributing factor in the obesity story [26]. Mice that are colonized with a specific known bacteria are termed gnotobiotic (or “known life”) and can help us understand the role of specific bacteria in inflammation and disease course [24].

Mechanisms Linking Microbiota and Obesity

The earliest observations indicated that mouse models of obesity (ie, the *ob/ob* mouse) had an alteration in the overall proportions of two major divisions of bacteria. Normal humans and mice have 60% to 80% *Firmicutes* (which are primarily nonculturable, butyrate-producing *Clostridium* cluster XIVa) and 20% *Bacteroidetes* (*Cytophaga-Flavobacterium-Bacteroides*) [27]. However, the obese mouse model (*ob/ob*) had a 50% reduction in

Bacteroidetes and an increase in *Firmicutes* [27, 28]. A similar decrease in *Bacteroidetes* and increase in *Firmicutes* is also seen when C57BL/6 mice are fed a high-fat (HF) diet [28, 29, 30•, 31, 32•]. The reciprocal result is seen in caloric reduction studies [30•]. To determine if these alterations in microbiota contributed to HF diet-induced obesity and insulin resistance, several groups have now fed a HF diet to GF mice. Two groups used GF C57BL/6 mice and both determined that GF animals were protected against both obesity and insulin resistance after HF diet, therefore implying that gut microbiota clearly influence the effects of diet on the host [33, 34]. However, a third group utilized C3H mice and concluded that the absence of intestinal microbiota did not protect mice from diet-induced obesity [35]. Although the reasons for this difference in results are not known, one possibility is that some strains of C3H mice are resistant to the gram-negative bacterial product, lipopolysaccharide (LPS). As increased levels of and response to systemic LPS have been proposed as one of the potential mechanisms of microbiota-influenced obesity (see below), if these mice cannot respond to LPS, then this might explain the discrepancy.

Altered Energy Intake

The resident bacteria within the GI tract are responsible for a significant portion of our energy intake, allowing us access to energy sources that may have otherwise been indigestible. The *Firmicutes* that are increased in obese mice and humans have been shown to be more adept at breaking down otherwise indigestible carbohydrates and converting them into absorbable energy products [5, 17, 36, 37]. If the microbiota were to shift between lean and obese individuals, it would seem likely that this change would affect the efficiency of energy production/absorption in the GI tract and may either facilitate or inhibit progression toward obesity. When analyzed via gene chips, it was observed that bacteria from obese individuals have increased expression in gene sets specific to motility, transcription, and saccharide metabolism [26].

Taking all of this into account, you can begin to piece together a picture of the path toward obesity. Westernized diets push the commensal populations toward a *Firmicutes*-friendly environment, ending with an overall increase in *Clostridia* populations. The increased *Clostridia* populations, acting as more efficient carbohydrate metabolizers, extract greater energy from the caloric intake, allowing for higher energy utilization. That extra energy, if not spent, will ultimately be stored as fat deposits. To better understand the disposition obese individuals have toward increased energy consumption, colonic/cecal health was examined as well as GI metabolites. Upon examination, cecal contents of both mouse and human studies revealed that obese individuals had significantly increased levels of short-chain fatty acids (SCFAs) [36, 38]. SCFAs such as acetate, propionate, and butyrate were in greater abundance in obese individuals. SCFAs are common byproducts of carbohydrate metabolism [39]. It should not be surprising that most SCFAs (specifically butyrate) producing bacteria belong to *Clostridia* cluster XIVa and IV [40]. Concentrations of SCFAs were measured in lean and obese mice via NMR. Overall, SCFAs were increased in the urine of obese mice compared with lean [41]. Although acetate has been primarily researched as a factor in high cholesterol, butyrate is highly regarded as an integral component to colonic health [42, 43].

Increased Fatty Acid Metabolism

One of the first publications that implicated the gut microbiota as an environmental factor that regulated fat storage observed that GF C57BL/6 mice conventionalized with normal microbiota had a suppressed expression of intestinal fasting-induced adipose factor/angiopoietin-like protein 4 (Fiaf/Angptl4) [3]. Fiaf/Angptl4 is a target of the nuclear receptor PPAR-α in the liver, but is also expressed in white adipose tissue, skeletal muscle, and intestine [44]. One function of Fiaf/Angptl4 appears to be its ability to raise plasma

triglycerides via its ability to inhibit lipoprotein lipase activity. Through the use of *Fiaf* knockout mice it was established that the suppression of Fiaf/Angptl4 is essential for the microbiota-induced deposition of triglycerides in adipocytes seen after conventionalization of GF mice [3, 33]. It has also recently been shown that the Chinese supplement Rhizoma coptidis can lower body adipose weight and that one potential mechanism for this finding is an inhibition of gut bacterial growth and a subsequent increase in Fiaf/Angptl4 expression in the intestine [45].

Microbiota-Associated Inflammation

For more than 15 years, it has been clear that adipose tissue in obese models has an elevated expression of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α). This has been reported for multiple rodent models of obesity, including the diabetes (*db/db*), obese (*ob/ob*), and tubby (*tub/tub*) mice and the Zucker (*fa/fa*) rat, as well as obese female patients [46, 47]. This TNF-α is primarily made by adipose tissue macrophages and it mediates insulin resistance through its ability to decrease the tyrosine kinase activity of the insulin receptor [48, 49]. Diets known to induce obesity and insulin resistance, such as the HF diet, can increase expression of TNF-α [50]. However, the induction of obesity and insulin resistance are ameliorated if mice are deficient in either TNF-α or the TNF-αR [51, 52].

But why does a HF diet and/or obesity lead to a chronic inflammatory state? Initially, the hypothesis was that increased nutritional fatty acids could lead to activation of the toll-like receptors (specifically TLR4) and subsequent inflammation [53]. However, as discussed above, a HF diet shifts the intestinal microbiome very quickly to a decrease in *Bacteroidetes* and an increase in both *Firmicutes* and *Proteobacteria* [5, 29]. One proposal is that this alteration in intestinal microbiota could lead to increased activation of TLR4, and therefore be partially responsible for the chronic inflammatory state seen in obese individuals.

To address this question, Cani et al. [54] initially asked if a HF diet would increase plasma concentrations of LPS, a TLR4 ligand made by gram-negative bacteria. This low level of LPS in the plasma has been termed “metabolic endotoxemia.” The data indicated that a HF diet in C57BL/6 mice did increase levels of plasma LPS and that direct infusion of LPS mimicked the physiologic effects of a HF diet [54]. Moreover, the effects of the HF diet were ameliorated in mice lacking a component of the TLR4 receptor complex—CD14. This same group went on to implicate intestinal bacteria in the increased plasma concentrations of LPS through the use of oral broad-spectrum antibiotics, which significantly decreased the levels of intestinal microbiota and the levels of plasma LPS [55]. Additionally, the administration of a prebiotic (oligofructose) resulted in an increase in gram-positive intestinal bacteria (including *Bificobacteria*) and a decrease in plasma LPS [56].

These observations allow the consideration that plasma LPS might be a biomarker of the status of obesity-prone individuals or the impact of therapeutic probiotics on obesity-associated intestinal microbiota. Several recent studies indicate that the answer may be yes. The first study investigated serum LPS activity in more than 7000 subjects with a 10-year follow-up. This study concluded that both previously diagnosed diabetic patients, as well as patients with newly diagnosed diabetes (incident diabetes) had higher LPS levels than nondiabetes individuals [57]. In addition, therapeutics such as oral probiotics (*Lactobacillus casei*), when given to mice with diet-induced obesity, can improve not only insulin resistance, but can also reduce plasma levels of LPS-binding protein (a marker of endotoxemia) [58].

This involvement of TLR activation was confirmed in a Sprague-Dawley rat model fed a HF diet, which can exhibit either an obesity-prone or an obesity-resistant phenotype. All the obesity-prone rats, but none of the obesity-resistant rats, had increased TLR4 activation [37].

Additional support comes from an experiment utilizing gnotobiotic and conventional Swiss Webster mice, which demonstrated that conventionally raised mice on the HF diet had increased hepatic levels of the inflammatory marker serum amyloid A, but that this effect of the HF diet was ameliorated in MyD88-deficient mice (MyD88 is a component of the TLR signaling pathway) [59]. Although TLR4 has been the receptor most implicated in this mechanism, it has also been recently shown that mice lacking TLR5 have metabolic syndrome [60]. This is at least in part due to an altered intestinal microbiota, as transfer of the microbiota from a TLR5-deficient mouse to a wild-type gnotobiotic mouse conferred metabolic syndrome to the recipients [60]. Intriguingly, a recent study on insects has also demonstrated a metabolic syndrome that is induced by a protozoan intestinal infection [61].

The mechanism for this increased plasma LPS from intestinal microbiota is probably increased intestinal permeability. C57BL/6 mice fed a HF diet have increased permeability to small molecules, such as FITC-dextran and also have decreased or altered expression of the tight junctional proteins occludin and zonulin-1 [55]. Similar findings were seen in HF diet–raised obesity-prone Sprague-Dawley rats, but not in obesity-resistant rats [37]. The impact of intestinal microbiota on permeability has recently been shown to involve glucagon-like peptide-2 (GLP-2) [62]. If *ob/ob* mice are given a GLP-2 agonist, then intestinal permeability is lowered, and tight-junction integrity and the systemic inflammatory phenotype is improved. As GLP-2 has receptors not only in the intestine but also in the brain, it is an intriguing possibility that there is a gut-brain axis that might potentially link intestinal microbiota to feeding behaviors [63]. Intracerebroventricular infusion of GLP-2 can inhibit food intake and, consequently, alterations in intestinal microbiota may have long-term effects on the gut-brain axis and body weight homeostasis [64].

Establishment of the Microbiome

It appears clear that the microbiota can impact energy metabolism and be associated with obesity and metabolic endotoxemia. If so, then the questions arise of: How do we acquire our microbiota? What is known to influence the microbiota present? Can we modify our microbiota in a predetermined fashion? Many studies have shown that the initial bacterial colonization of the intestine is at birth, primarily from the mother and/or other caregivers [65, 66]. However, newer work has now focused on the impact of microbiota on weight gain during pregnancy and on whether this impacts the subsequent weight of the child later in life.

The majority of this work has been published by a group from Finland, who have primarily utilized the techniques of FCM-FISH and qRT-PCR to show that if a mother was overweight before pregnancy, then she had significant increases in the numbers of fecal *Bacteroides-Prevotella* group (FCM-FISH) and *Staphylococcus aureus* (qRT-PCR) from the first to the third trimester [67]. The impact of this alteration in maternal microbiota on the microbiota of 1- and 6-month-old infant stool samples indicated that the infant fecal microbial composition was influenced by the maternal weight gain during pregnancy and by maternal body mass index (BMI) during early pregnancy [68]. Six-month-old infants from mothers with a BMI greater than 25 kg/m^2 had more fecal *Clostridium histolyticum* (FCM-FISH) and *Clostridium leptum* (qRT-PCR), but less *Bifidobacterium* genus (qPCR). However, in contrast to the findings in the mothers, the levels of *Bacteroides-Prevotella* decreased in 6-month old infants from mothers with high BMI or who had excessive weight gain. In support of this decrease in *Bacteroides-Prevotella* in offspring of overweight mothers, the offspring of rats fed a HF diet also had fewer *Bacteroides-Prevotella* in the jejunum [69]. Additionally, rats that were exposed to pre-weaning overnutrition also had lower numbers of *Bacteroides-Prevotella* [70].

To determine if this altered microbiotal composition actually has any correlation with weight in children, this same group of children was followed until age 7 years [71]. None of the bacterial groups found to be significant in overweight mothers or their offspring were correlated with increased weight gain in childhood; however, increased levels of *S. aureus* during infancy did correlate with a child being overweight at age 7 years. A second study also investigated whether factors known to alter intestinal microbiota have an effect on body weight at age 7 years [72]. Factors investigated included delivery mode, maternal pre-pregnancy BMI, and early exposure to antibiotics (< 6 months of age). Children from mothers with a high pre-pregnancy BMI were more likely to have overweight children at age 7 years; however, this study did not correlate these findings with the intestinal microbiota composition.

Although these studies appear to indicate that our microbiota is established very early in life, there are also studies that indicate that microbiota can be manipulated by various weight loss techniques. In adolescents subjected to an obesity treatment program including both calorie restriction and increased physical activity, there was an increase in the *Bacteroides-Prevotella* group and a decrease in *Clostridium* spp [73, 74]. In adults who have undergone Roux-en-Y gastric bypass the *Bacteroides-Prevotella* and *Escherichia coli* species increased 3 months after surgery, whereas lactic acid bacteria (including *Lactobacillus/Leuconostoc/Pedicoccus* group and *Bifidobacterium* genus) decreased [5, 76].

If weight loss is associated with altered microbiota and if the obesity phenotype can be transferred by fecal microbiota, then it could be proposed that a bacteria might exist that could induce a lean phenotype. This concept has been most extensively tested through the administration of probiotics. Probiotics are live microorganisms that are thought to be beneficial to the host. The most common types are lactic acid bacteria and bifidobacteria and are often found in yogurt or dietary supplements. Studies using *Lactobacillus rhamnosus* GG, *Lactobacillus plantarum* strain 14, *Lactobacillus paracasei* ssp paracasei F19, and *Bifidobacterium breve* B-3 all demonstrated that probiotic intervention appears to have a beneficial effect on obesity [77–80]. The probiotics appear to work by reducing mean adipocyte size, inhibiting lipoprotein lipase, and improving insulin sensitivity [77–79].

Conclusions

Obese individuals and models all show a propensity for a dysbiosis that includes an increased ratio of *Firmicutes:Bacteroidetes*. This alteration in the proportion of bacteria in the lumen of the GI track affects not only the ability of the microbiome to generate energy sources from indigestible carbohydrates, but also the deposition of triglycerides in adipocytes. This altered bacteria also appears to have an increased exposure to the host immune system due to a leaky intestinal barrier and induces a constant state of chronic inflammation. This impact of the microbiota on obesity has led to multiple preliminary studies on the use of “good” probiotic bacteria to alter the obese phenotype. These studies have all shown that probiotic intervention has a beneficial effect and may lead to novel interventions for overweight or obese human patients.

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