The Gut Microbiome and Obesity

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Abstract

The composition of the gut microbiome is hypothesized to be an environmental factor that contributes to obesity. Results of several human studies suggest that obesity is associated with differences in the gut microbiota composition, reduced bacterial diversity, and altered representation of bacterial metabolic pathways. The obese phenotype is associated with increased microbial fermentation and energy extraction from non-digestible food components; however, until recently it was not clear how relatively small increases in energy extraction could contribute to the large and rapid weight gain observed in the animal studies. Mechanisms by which the gut microbiome may influence metabolism and energy homeostasis include regulation of energy uptake from diet, interaction with signaling molecules involved in host metabolism, modification of gut permeability, release of gut hormones, and low-grade, chronic inflammation, the latter being a hallmark of obesity-related diseases.

Introduction

Environmental and genetic factors play a role in determining energy balance and risk of obesity. The prevalence of excess adiposity and obesity-related factors has paralleled the rise in diseases associated with chronic low-grade inflammation, such as type 2 diabetes, cardiovascular disease, gall bladder disease and several cancers. The human gut microbial community has been identified as another possible factor that may alter host metabolism and adiposity and influence chronic low-grade inflammation [1, 2].

The composition of the gut microbiome (i.e. the collective genomes of the gut microbial community) is hypothesized to be an environmental factor that plays a role in the pathogenesis of obesity and associated diseases. This hypothesis has emerged with the advent of new technologies that have allowed for the generation of important new data related to the composition and characteristics
of the gut microbiome. Recent application of molecular techniques to characterize intestinal bacterial species has allowed for more in-depth identification of gut microbial species and evaluating its relationship with human health and disease [3]. Much of the work has involved the sequencing of the 16S rRNA gene to determine which microbes are present in the gut, or more recently a metagenomics approach, measuring all of the genes in the microbial genomes, to identify the genetic potential of the bacteria present.

The gut microbiota colonize the length of the intestinal tract in varied cell densities, ranging from ~10^4 bacterial cells/ml lumenal contents in the duodenum to 10^{11} cells/ml lumenal contents in the colon and rectum [4]. The gut microbiota consist largely of members of five phyla, the Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, and Verrucomicrobia. The number of different species of bacteria is estimated to range from 300 to 1,000, with the majority of the species diversity distributed between the Firmicutes and Bacteroidetes [5, 6]. There is high interindividual variation in the composition of the communities, mostly at the species level [3], although recent work suggests that certain bacterial community composition patterns are identifiable within human populations [7].

Obesity and Gut Microbial Profiles in Humans and Experimental Animals

Results of several human studies, typically with small sample sizes, suggest that obesity is associated with differences in gut microbial profiles [2, 7–11], as well as reduced bacterial diversity [9], and altered representation of bacterial metabolic pathways [9]. It has been hypothesized that, at the phylum level, an increased ratio of Firmicutes to Bacteroidetes may contribute to the pathophysiology of human obesity [2, 12]; however, this hypothesis has not been supported consistently across studies [13, 14], even in the studies that detect obesity-associated differences in gut microbial community [7, 8, 10].

Recently, in a cross-sectional sample of 115 premenopausal women, we evaluated the association between adiposity and the gut microbial community. We measured percent body fat on these women by dual-energy X-ray absorptiometry and used several approaches to characterize the microbial community in fecal samples. First, using quantitative PCR and terminal restriction length polymorphism (TRFLP), we found that the abundance of Bacteroidetes was positively associated with percent adiposity (r = 0.20; p = 0.02) and that TRFLP peak 473 (indicative of the Bacteroidetes-Prevotella group) was statistically significantly associated with percent adiposity. Multiple regression testing of the relationship between adiposity and gut microbial community, adjusting for energy, carbohydrate, fat and dietary fiber intake, showed that Bacteroidetes explained 15% of the variation in adiposity in these women. In a subset of the women, we also compared the gut microbial community composition by
pyrosequencing the V1–V3 region of the 16S rRNA gene. In total, we analyzed 239,875 sequences. After trimming the sequences (no ambiguous nucleotides, homopolymer ≤8, primer mismatch ≤2, barcode mismatch ≤1, quality score ≥35 in 50 nt window), we aligned the sequences in SILVA (http://www.arb-silva.de) and found that out of 65,492 sequences 13,190 were unique. We found that the bacterial communities in the obese women (35–46% body fat; n = 35) were significantly less diverse than those of the women with average percent adiposity (25–32%; n = 27), a trend similar to that reported by Turnbaugh et al. [9] in a sample of twins. The phylogenetic composition and abundance of the microbial community were also significantly different between lean and obese individuals. Multivariate analysis of the pyrosequencing data, using non-metric multidimensional scaling (NMS), explained 75% variation in our data and NMS axis 2 was positively associated with percent adiposity. In addition, two _Prevotella_ sp in the phylum Bacteroidetes were positively associated with NMS axis 2 and increasing adiposity. Given that the Bacteroidetes are a metabolically diverse group specializing in saccharolytic degradation, this suggests that increased efficiency of energy extraction from the diet may be associated with specific microbial metabolic pathways in a few groups of bacteria in obese individuals.

Studies in experimental animal models also support the hypothesis that gut microbes play an important role in energy regulation and adiposity. Colonization of axenic (i.e. ‘germ-free’) mice with a gut microbiota derived from conventional mice resulted in a 60% increase in body fat mass and development of insulin resistance [15], and the bacterial community composition of the inoculum influenced amount of fat stored [16]. These effects have been seen in the context of a chow diet and a semi-synthetic Western diet [17]; however, in a similar experiment conducted using a high-fat, semi-synthetic diet with the same overall proportions of macronutrients as the Western diet, but composed of different ingredients, axenic mice were not protected from obesity and gained as high or higher amounts of body fat as the conventional mice [18]. Bacterial community composition has also been shown to differ by degree of genetically mediated adiposity; obese ob/ob (i.e. leptin-knockout) mice had 50% lower abundance of Bacteroidetes and proportionally higher amounts of Firmicutes [1].

The results of the association studies in humans and the animal studies raise the question whether compositional change in the gut microbiota precedes the onset of weight gain and whether the microbiota plays a causative role [19]. Unfortunately, no prospective studies have been conducted in humans to examine this. In a retrospective study, Kalliomäki et al. [20] selected overweight and obese (n = 25) and normal-weight (n = 24) Finnish children, 7 years of age, and matching for numerous factors (e.g. gestational age, body mass index, BMI, at birth, etc.), and assessed gut microbial community by fluorescent in situ hybridization (FISH) and qRT-PCR in fecal samples collected at 6 and 12 months. They reported that, in infancy, bifidobacterial numbers were higher in normal-weight children, and _Staphylococcus aureus_ was greater in overweight children. Some
work in mice supports a potentially causative role of the microbes; transfer of
gut microbiota from genetically obese ob/ob mice to lean, axenic mice resulted
in greater body fat gain in these lean animals than in lean axenic mice colonized
with microbiota from conventional lean mice [12].

Further, some, but not all, intervention studies of weight loss in humans also
show changes in gut microbial populations. Studies report that obese individu-
als have fewer [9, 12], increased [8, 11] or no difference [7, 13] in the amount
of Bacteroidetes compared to Firmicutes. Others have shown shifts at the genus
level [10, 21]. Ley et al. [2] showed that weight reduction on either a fat- or
carbohydrate-restricted weight loss diet resulted in a decrease in Firmicutes and
an increase in Bacteroidetes; by 52 weeks, the distribution mimicked that in lean
individuals, and the percentage bodyweight change was linearly associated with
an increase in Bacteroidetes abundance. Similarly, administration of an obe-
sity treatment program to adolescents resulted in significant changes in certain
bacterial groups as monitored by quantitative real-time PCR; the changes were
observed in the group of participants who lost >4.0 kg, whereas no changes were
observed among the group who lost <2.0 kg [21]. Duncan et al. [13] monitored
groups of fecal bacteria using FISH in obese and non-obese individuals under
conditions of weight maintenance and weight loss on two different reduced-
carbohydrate weight loss diets. There was no significant change in Bacteroidetes
in the obese individuals after 4 weeks on the weight loss diets; however, there
was a diet-dependent decrease in a group of butyrate-producing Firmicutes
(Roseburia + E. rectale).

A recent weight loss study in mice examined the effects of weight loss on
the gut microbiota in the context of high- and low-fat diets (60 and 10% of
energy derived from fat, respectively) [22]. The design allowed for comparison
of the effects of weight loss in lean and obese mice, as well as comparison of diet
effects on gut microbial community composition independent of bodyweight.
The investigators showed that the differences in diet composition had the larg-
est effect on differences in the gut microbiota. When evaluating the effect of
weight loss within diet category, in mice fed the high-fat diet, maintenance of
a 20% reduced bodyweight influenced the composition of the gut microbiota.
This effect was not seen in weight-reduced mice fed the low-fat diet. These dif-
ferential responses and the findings of Fleissner et al. [18] speak to the complex
interactions between diet, the gut microbiome, and host physiology (i.e. adi-
posity and associated metabolic parameters), and suggest that human studies
designed to evaluate these relationships in weight reduction studies need to be
stringently controlled to determine the individual impact of diet and fat mass
loss on the gut microbiome.

Several technical factors may contribute to the varied reports on the rela-
tionship between microbial taxa and obesity in both human and mouse studies.
Different methods used to characterize the microbial community may intro-
duce different types and amounts of bias that could influence the strength of the
association and interpretation of the results. DNA extraction [23], PCR primer biases [24, 25], and accuracy of phylogenetic identification [26] are potential sources of variation. For example, oligonucleotide probes, used in FISH to identify shifts in microbiota associated with weight loss [10, 20], are hampered by cell permeability or probe mismatch issues [27]. In addition, on the adiposity side of the equation, relying on BMI rather than procedures that more accurately determine body fat percentage (e.g. dual-energy X-ray absorptiometry) may contribute to misclassification of individuals [28].

The potentially large, but often not considered, contribution of dietary and other lifestyle differences to some of the obesity-related findings of the cross-sectional studies is also a concern. Among those studies that evaluate diet, underreporting of diet, especially associated with studies of obesity, may be a source of bias [29]. Energy intake is, on average, underestimated, especially when using memory recall methods [30]. Several behavioral changes may influence the accuracy of self-report, such as: change in the true intake as a function of recording, lack of awareness of the amount of food consumed, and reluctance to disclose amounts or foods eaten. In addition, some studies have shown that, among dietary items reported, there is evidence for differential underreporting of certain foods [29], creating further bias in self-reported data.

The Gut Microbiome and Obesity: Mechanisms of Action

The obese phenotype is associated with increased microbial fermentation and energy extraction from non-digestible food components; however, until recently it was not clear how relatively small increases in energy extraction could contribute to the large and rapid weight gain observed in the animal studies. Mechanisms by which the gut microbiome influences human metabolism and energy homeostasis and subsequent obesity-associated disease risk include: regulation of energy uptake from diet [13, 31], interaction with signaling molecules involved in host metabolism [32], release of gut hormones [33, 34], and modification of gut permeability [35]. Some of the mechanisms appear to involve effects of products of bacterial metabolism of dietary constituents and others are direct effects of the gut microbes (fig. 1).

Fermentation efficiency and short-chain fatty acid (SCFA) composition can be influenced by the composition of the gut microbiota [16, 36]. Non-digestible carbohydrates (e.g. dietary fibers) are fermented by the gut microbes to produce hydrogen, carbon dioxide, SCFA (e.g. acetate, propionate, butyrate) and other compounds, such as lactate and formate. Estimates vary, depending on the substrates, the gut microbial community composition, and gastrointestinal transit, but fermentation accounts for approximately 10% of human's daily energy intake [37]. Hydrogenotrophic methanogens (i.e. Archaea) and sulfate-reducing bacteria consume the hydrogen produced from the fermentation of polysaccharides
and their rapid uptake of hydrogen helps to drive the process [38]. Thus, hydrogen transfer between hydrogen-producing bacteria and hydrogen-consuming Archaea is hypothesized to increase fermentation efficiency and SCFA production and contribute to increased adiposity – a relationship that has been shown in an experimental animal model [16]. In humans, the results of one study showed that higher numbers of Archaea and the order Methanobacterales were present in obese individuals (n = 3) as compared to normal-weight individuals (n = 3) [8]. However, in an earlier study of 1,293 individuals, obesity was statistically significantly less prevalent in methane producers than non-producers, when producer status was characterized by breath methane excretion [39]. It is likely that unaccounted dietary factors and other host exposures influence the observed relationships.

Fig. 1. The gut microbiome and its metabolites may influence metabolism and energy homeostasis through several mechanisms. SCFA, the products of carbohydrate fermentation, are substrates for lipogenesis, and also are signaling molecules for G protein-coupled receptors GPR41 and GPR43. The presence of the gut microbiota and SCFA modulate release of enteroendocrine hormones [e.g. glucagon-like peptides (GLP-1 and -2) and PYY], affecting hunger and satiety and suppress FIAF. Reduced FIAF in turn decreases hepatic and skeletal muscle fatty acid oxidation and increases lipoprotein lipase (LPL) activity and triacylglycerol storage in adipose. LPS translocation across the gut epithelium leads to higher circulating LPS concentrations and increased low-grade inflammation and macrophage activation in adipose tissue. Adapted from Bäckhed [31] and Delzenne and Cani [43].
In addition to providing energy, gut microbial metabolites, such as SCFA, also play a role as signaling molecules, interacting with receptors in pathways influencing energy uptake [31]. Acetate and propionate and butyrate bind to two G protein-coupled receptors GPR41 and GPR43, also known as free fatty acid receptors FFA3 and FFA2, respectively [40]. GPR43 is expressed in immune cells, adipocytes, and the distal ileum and colon, and Gpr41 is expressed in adipose tissue and several immune function-associated tissues, such as spleen, bone marrow [40]. Work in GPR41-deficient mice suggests that one aspect of the interaction between SCFA and GPR41 may be to increase levels of peptide YY (PYY), an enteroendocrine cell-derived hormone in circulation that reduces gut motility; reduced gut motility may aid in digestion and allow for increased absorption of SCFA – acetate and propionate are substrates for hepatic de novo lipogenesis and gluconeogenesis, respectively [32]. Activation of GPR43 by SCFA inhibits lipolysis and adipocyte differentiation and GPR43-deficient mice are less prone to high-fat diet-induced obesity and have lower macrophage numbers in their adipose tissue [41]. These findings would suggest that high intakes of non-digestible carbohydrate might be detrimental in weight control; however, prebiotic supplementation of animals overexpressing GPR43 and fed a high-fat diet has been shown to reduce adiposity [42].

The impact of gut microbial fermentation products, such as the SCFA, on release of enteroendocrine hormones involved in appetite and bodyweight regulation has been demonstrated in experimental studies in humans and animals. Fermentable carbohydrates from several sources, even though they modulate gut microbial community structure differently, have been shown consistently to decrease food intake and bodyweight and with concomitant increases in production and secretion of two anorexigenic peptides glucagon-like peptide-1 and PYY [reviewed in 43].

Gut bacteria have also been proposed to increase triacylglycerol storage in adipose tissue by suppressing fasting-induced adipocyte factor (FIAF) in the gut epithelium; FIAF suppression in turn stimulates de novo hepatic lipogenesis and promotes lipoprotein lipase-directed incorporation of triacylglycerol into adipocytes [15] and reduces fatty acid oxidation in the liver and in skeletal muscle [17]. In contrast, another study, also in mice, did not support a crucial role of the modulation of intestinal FIAF production in mediating fat storage [18]. Recent in vitro work using coincubations of bacteria and cell lines showed that some bacteria increased and others decreased FIAF expression in intestinal cancer cell lines [44]. Further, incubation with propionate and butyrate, but not acetate, increased FIAF expression and cleavage in colon and hepatic cell lines, suggesting that the SCFA effects on FIAF may not be restricted to the gut epithelium, but that bacterial effects in vivo may be mediated by gut microbial metabolites at other tissue sites.

The gut microbiota also influences low-grade, chronic inflammation [43], a hallmark of obesity-related diseases. Gut microbiome composition has been
associated with altered concentrations of inflammation biomarkers, such as adipokines, C-reactive protein, monocyte chemotactic protein-1, and tumor necrosis factor-α, in circulation and in adipose tissue [45]. One microbiome-mediated pathway that may contribute to low-grade systemic inflammation is the presence of bacterial lipopolysaccharide (LPS; also called endotoxin) in circulation. Low concentrations of LPS in the blood (i.e. subsepticemic) have been linked to obesity and related metabolic disorders [46]. LPS stimulates the innate immune response by binding to toll-like receptors expressed by macrophages in adipocytes and epithelial cells [47]. A higher systemic LPS load accompanies increased translocation of LPS via chylomicron uptake and/or increased intestinal permeability [48]. Thus, diet, particularly high-fat diets, may contribute to the microbiome-inflammation relationship [18, 45]. In contrast, other end-products of microbial metabolism of diet, such as SCFA, have been associated with reduced inflammation through interaction with the TGF-β pathway [49]. Butyrate interacts with TGF-β to block the transfer of NF-κB to the nucleus thereby reducing transcription of inflammatory genes [50] and reducing inflammation.

In summary, the gut microbiome may contribute to altered energy storage and obesity through several mechanisms, including regulation of energy uptake from diet, interaction with signaling molecules involved in host metabolism, modification of gut permeability, and release of gut hormones. Further, the interaction of gut microbes with the host innate immune system influences inflammatory and metabolic processes associated with obesity and important to human health. The complex interplay between the gut microbial community, diet, and host physiology needs to be considered carefully in the design and interpretation of experimental interventions and population-based observational studies.

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References


Discussion

*Dr. Birch:* My question is whether you have information about whether participants were breastfed or formula fed as infants?

*Dr. Lampe:* This was the study of Kalliomäki et al. [1]. Two groups of 7-year-old children, normal weight and overweight or obese, were selected for retrospective evaluation of their gut microbial communities in stool samples collected within the first year of life. The children were part of a follow-up of a cohort of Finnish children who had participated pre- and postnatally in a randomized trial of probiotics and atopic disease.

*Dr. Birch:* Do you know if obesity status at 7 years of age was associated with differences in infant feeding mode?

*Dr. Lampe:* There were no statistically significant differences in infant feeding mode between the normal weight and overweight children. The two groups were matched for several factors, including gestational age, BMI at birth, mode of delivery, duration of breastfeeding, use of antibiotics during infancy, intervention group, and frequencies of atopic diseases and atopic sensitization at 7 years of age. Other studies in breastfed and formula-fed infants show differences in gut microbial populations, which is why matching was important.

*Dr. Haschke:* The Finnish study was a secondary outcome study looking at allergies. All the infants were breastfed until 6 months of age, and there was some intervention in terms of giving mothers probiotics prenatally, so this study has been reported 3 times. It's from Erica Isolauri's group (Department of Pediatrics, University of Turku, Finland), and it could not be reproduced by any other group that there is some difference continuously there, so we have to wait for confirmation. The clinical data have been really weak until now. The hypothesis is attractive, but we still don't have a prospective study showing that certain microbiota could be preventive.

*Dr. Lampe:* Thanks for that clarification. I think you raise an important point, and that is that we really do not have any good prospective studies. We need to encourage cohort studies to collect and store stool samples, so that we can do the robust studies that are needed in order to answer these questions more effectively.

*Dr. Goran:* I always get a little confused with some of these aspects because short-chain fatty acids are being protective in some situations, so resistant starch for example is promoted as a vehicle to induce insulin resistance and it starts to act through short-chain fatty acids. Can you clarify whether there are positive and negative effects of some of these things, especially in the context of your study where you showed that fiber and starch were predictive of the biome? Are they predictive of a beneficial or a harmful profile?

*Dr. Lampe:* All of the individuals in our observational study of diet and gut microbial community were healthy, so we do not have information on differential relationships dependent on metabolic syndrome or other disease phenotypes. Although the dietary data are predictive of the microbiome, they cannot distinguish between physiologically positive and negative effects. Looking at the totality of the predictive value of diet, these diet components help to define different groups of individuals who have different microbiomes. Whether or not one is better from a health standpoint, we don't know in the context of this study. Intervention studies of dietary fiber and gut microbial community show shifts in different groups of bacteria and in directions that typically
have been thought to be beneficial. With regard to resistant starch and diabetes, I think that part of the effect of resistant starch in relation to glycemic load may be its influence on gut transit and absorption of sugars.

Dr. Goran: Were you able to look at in that study or did other studies look at subtypes of fiber, soluble versus insoluble for example, in different types of starches? And then the third factor that comes to mind is fructose, which in large amounts is poorly absorbed and may also contribute to the issue. Have you been able to dissect that?

Dr. Lampe: In this study, when we looked at soluble and insoluble fiber, it was really the insoluble fiber that was explaining most of the relationship between dietary fiber and microbial community. The contribution of soluble fiber was less apparent. Trying to measure resistant starch from a 3-day food record is not very accurate because it's really hard to quantitate it in observational studies. We didn't try to do that.

Dr. Rosenbaum: I don't fully understand, but you only can report relative amounts of different species, so we don't know if as people lose weight they are back to where one number goes up and the other one is coming down. Do you think that it's the balance between the different species or the absolute amount of one or the other, is this some sort of a functional assay that you could do that would determine the absolute amount, how they handle the starch load or something like that?

Dr. Lampe: In order to get at the absolute amount of bacteria, you would need to access the total contents of the lumen. Consequently, much of the focus has been on the relative amounts of different groups of bacteria. I think the ultimate goal is also to move towards measuring mRNA or even looking at protein levels of bacterial enzymes important in the various metabolic steps in the gut. This will give us information on the functionality of the gut community as compared to just what bacteria are there and in what relative amounts.

Dr. Rosenbaum: In the weight loss studies, was there a difference between dynamic weight loss and static weight maintenance, or they didn't look at that?

Dr. Lampe: The study was 52 weeks of a weight reduction intervention; there are no data to indicate that the participants were followed any further than that.

Dr. Rolls: Can you speculate a bit about whether you think this understanding of the microbiome holds some promise for either the treatment or prevention of obesity and how that might work? Could changing intake of a few foods help adults? Do we need to start prenatally or with maternal diets?

Dr. Lampe: It's very early days, but I think there may be some potential for both prevention and treatment. Given what we know so far, early life may be an important time to intervene. Crosstalk between the host and the gut microbial community is critical for development of the innate immune system and the body's ultimate comfort level with what microbes are present in the gut. The microbiota also interact with the adaptive arm of the host's intestinal mucosal immune system, modulating regulatory cells in the gut that are involved in maintaining immune tolerance. Related to this, we know from interventions in adults that it is difficult to get inoculations of probiotic bacteria to colonize and maintain a presence at detectable levels without constantly providing them. I think there will be opportunities for prevention and treatment of obesity, but we have a way to go to understand the basics and long-term consequences before we can effectively modulate the system.

Dr. Drewnowski: Is the gut microbiome established in the first year of life or even before that?
**Dr. Lampe:** As we saw from the one slide of the study of Koenig et al. [2] – and granted that this was just the example from one child – the gut microbiome is pretty well established by weaning. I think the estimate is that by age 2 or 3, the gut microbiome has the functional attributes of the adult microbial community.

**Dr. Lovejoy:** What would happen if you dramatically changed your diet, given the examples of children in Africa versus children in Italy? If somebody went from a largely meat-eating diet to becoming a vegan and staying a vegan, would their microbiome change and stay different or would they maintain the biome that they had since 2 years of age?

**Dr. Lampe:** Keep in mind that in that study, the children were born and raised to their particular environments and diets. Nonetheless, in adults, there would be changes to the microbiome as a result of what the person is eating and therefore what substrates are available to the bacteria. At the same time though, particularly at the genetic level, you are still likely to find those other bacteria that were part of the microbiome prior to the major change in diet. It’s just that there are going to be fewer of them, but they are still likely to be detectable unless there is some major perturbation as a result of antibiotic treatment or disease.

**References**

