Emerging Insights/Controversies Intestinal Microbiome and Weight Determination

The human intestinal microbiome is currently a hotbed for research. It has been suggested that the microbiome plays a role in many aspects of human pathophysiology, including human weight determination. This paper will review the current intestinal microbiome knowledge and its potential role in both obesity and cachexia.

INTRODUCTION

Adult humans are colonized by more microbial cells (“the microbiota”) than human cells. The microbial cells express up to 800 times more genes (herein referred to as “the microbiome”) than human cells. The densest and most complex range of bacteria in the human body inhabit the large intestine. A person’s environment plays a large part in the composition of one’s microbiome, with 22-36% of microbiome variability between persons associated with environmental factors, and only 1.9-9% by genetics.

A person’s microbiome begins to form in the prenatal period, and will continue to mature through the transition into the external world, with mode of delivery (cesarean section vs. vaginal delivery) influencing microbiota composition. For example, deficits in the human microbiome associated with cesarean section deliveries have been implicated in
certain childhood autoimmune diseases, including celiac disease, asthma, and type 1 diabetes. As we introduce dairy products and solid foods to a person’s body, the microbiome continues to evolve. Breastmilk introduces the infant gut to bacteria that can affect communities of bacteria through adulthood. Infants who are breastfed have a higher proportion of Bifidobacterium and Lactobacillus species, compared to formula-fed infants who tend to have a higher proportion of other bacteria. Breastfed infants, in turn, seem to experience a protective effect against autoimmune diseases and even autism spectrum disorder.3

There is research to suggest a significant role of antibiotic use modifying the microbiome during early life. Children exposed to antibiotics have delayed maturation of the microbiome compared to control subjects, however the mechanism behind this is not fully understood. In animal models, peripartum antibiotic exposure in the mother can lead to gut dysbiosis and even colitis in the offspring. Long-term studies have shown that antibiotic exposure can lead to a new steady state different from the original pre-antibiotic intestinal microbiome, and the effects can last as far as 4 years post-exposure.3

Given the significant influence of a person’s environment on their microbiome, beginning even prior to birth, there is enormous potential for influence on a host’s phenotype through interventions and medications that could alter the intestinal microbiome. This in turn leads to potential for medical research into interventions and medications that could alter the intestinal microbiome, thereby altering certain aspects of the human phenotype, in particular, a person’s weight.

Microbiome and Weight Physiology

Influence of Microbiota on Metabolism

One of the most exciting topics surrounding the intestinal microbiome is its potential to affect a person’s weight (Figure 1, Figure 2). The major function of the intestinal microbiome is to aid in the fermentation and energy extraction of indigestible dietary fiber. In addition, the microbiome has been linked to energy homeostasis, immune function, and certain disease states, including weight determination and irritable bowel syndrome.3 Microbiota can influence the calories absorbed in the gut. As an example, microbial enzymes can digest many dietary polysaccharides that are indigestible by human enzymes leading to digestible sources of energy.1

About 90% of the gut microbiota are of the phyla Bacteroidetes or Firmicutes (Figure 3), but there are approximately 1000 species of microbes that populate the human GI tract.4 Interestingly, obese humans have an increased ratio of Firmicutes as compared to Bacteroidetes.1,5 One of the first articles to demonstrate that weight may be modified by the relative abundance of these two dominant bacterial divisions was a study by Gordon et al. published in 2006. This study found that the concentration of Bacteroidetes and Firmicutes affect the metabolic potential in mouse gut microbiota, thereby indicating that the obese microbiome has an increased capacity to harvest energy from the diet.6 Ley et al. further defined the connection between the microbiome and obesity in their work examining leptin deficient mice.7 Since then, increasing attention has been turned to the role of the gut microbiota in obesity, as studies have continued to establish the role of the microbiome in weight determination.

Microbiome and Obesity

Obesity is clearly linked with chronic conditions such as inflammation and insulin resistance, which confer deleterious effects on overall health. Additionally, obesity carries a significant cost, as health care expenditures for obese individuals can be almost double those of non-obese individuals.8 As such, there is considerable interest in exploring whether alterations in the microbiome could be used in the treatment of obesity.

Clear differences have been established between the microbiomes of obese as opposed to lean subjects.9 Early studies looked at differences in the bacterial composition of the microbiomes between obese and non-obese subjects. More recent studies have begun to look at more functional differences, such as variations in energy metabolism and inflammation.10 Multiple studies have shown that the microbiome in obese subjects appears to be able to extract more energy from the diet, compared to non-obese subjects.11
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There is a good deal of published data to suggest the microbiome’s role in supporting obesity in mammals. In one study comparing the distal gut microbiota of obese individuals to their counterparts, the population of Bacteroidetes increased as the obese volunteers lost weight, and the degree of increase in this phylum of bacteria was significantly correlated to weight loss, but not to overall total caloric intake. This poses the question of how energy is harvested by Bacteroidetes, compared to other phyla of bacteria, such as Firmicutes. The results of this study suggest that the obese microbiome has increased capacity to harvest calories (and thus predispose to weight gain) from ingested food.6

Methane-producing bacteria in the gut have been implicated in host weight gain (Figure 1). Given that there are methods to modify the concentration of such bacteria (i.e. by antibiotic administration), as well as to measure methane production (i.e. breath testing), this is another exciting area of research. It is believed that methane-producing bacteria facilitate increased polysaccharide fermentation by neighboring microbes, and also that methane itself slows intestinal transit, both of which may allow increased time for nutrient absorption and thereby predispose to weight gain.4

Multiple studies have demonstrated the role of the microbiome on the development of obesity. Recent research has also shed light on the role of the microbiome may have on the response to dietary modifications aimed at curtailing obesity. Recent data show that individuals with a higher Prevotella-to-Bacteroides ratio had greater reductions in body weight as well as body fat while on a high-fiber diet, as compared to those with lower Prevotella-to-Bacteroides ratios, while on the same, high-fiber diet.12

Microbiome and Genetics
According to one study, the human microbiota is not only influenced by environmental exposures early in life, but it is more similar among related individuals. This study involved transplantation of fecal samples from adult human female twins (one obese twin, one lean twin) to germ-free mice. It was found that, after 15 days, the adipose mass (as determined by quantitative magnetic resonance analysis) of mice receiving the obese twin’s fecal sample was significantly greater than the change in adipose mass of mice who received the lean twin’s fecal sample. This suggests that the “increased adiposity phenotype” was transmissible. Another measure looking at epididymal fat pad weights also showed higher weights in the mice who ingested fecal samples from the obese twin. Fecal analysis also showed that the mice harboring microbiota from the lean twins had a greater capacity to breakdown and ferment polysaccharides compared to their counterparts. Microbial fermentation of non-digestible starches has previously been associated with lower body weight and decreased adiposity. Another interesting aspect of this study showed that co-housing mice who were transplanted the lean twin’s microbiota with the mice who were transplanted the obese twin’s microbiota led to a significantly lower increase in adiposity in the obese mice compared to the control obese mice who were never exposed to mice harboring the lean twin’s microbiota. This is likely related to the fact that mice eat each other’s feces, which further lends to the fact that gut microbiota modulate the obese phenotype.13

Inflammation in the Obese Phenotype
Intestinal microbiota may affect systemic inflammation, thereby modulating weight gain (Figure 1). As a measurable example, there is a higher concentration of Gram negative bacteria in
the obese microbiota leading to increased intestinal permeability and endotoxemia, as characterized by higher concentrations of lipopolysaccharides in the blood. Endotoxemia leads to low-grade inflammation, insulin resistance, and adipocyte hyperplasia. High fat diets have been implicated in increased lipopolysaccharide translocation and therefore systemic inflammation.\(^5\)

Certain changes to a person’s gut microbiota could lead to weight gain by leading to increased energy supply via the fermentation of short chain fatty acids (SCFA) (Figure 1). SCFA oxidation by certain bacteria in the human gut can lead to the formation of extra calories. Certain SCFAs, including acetate, propionate, and butyrate, can indirectly affect gene expression regulation through certain G-protein coupled receptors that are associated with the signaling for increased expression of glucagon-like peptide-1 and Peptide YY. These two proteins are related to hunger and appetite, and may affect intestinal transit thereby leading to increased nutrition absorption (and ultimately increased caloric intake).\(^5\)

Another way in which intestinal microbiota may directly affect host gene expression is via suppression of “Fasting Induced Adipocyte Factor” (FIAF) gene expression (Figure 1). FIAF inhibits circulating lipoprotein lipase, therefore suppression of FIAF may lead to increased lipoprotein lipase activity, which leads to increased triglyceride deposition in adipocytes. Furthermore, one study showed that FIAF knockout mice had higher intestinal fat uptake, and lower fat excretion, compared to mice who expressed FIAF normally.\(^5\)

**Animal Studies in Obesity and the Microbiome**

One study compared the distal gut microbiota of genetically obese mice to their littermates, as well as obese human volunteers to their counterparts. Cecal microbial DNA was analyzed and then microbiota transplantation performed from the obese mice and from the lean mice into germ-free, lean mice. All mice had the same daily caloric intake. Over 14 days, the mice receiving the microbiota from obese mice became obese, and those receiving microbiota from lean mice retained a normal BMI. This suggests that the obese microbiome is somehow transmissible via transplantation of the gut microbiome from one organism to another.\(^6\)
Another experiment looked at the gut microbiota of mice undergoing Roux-en-Y gastric bypass (RYGB) compared to mice undergoing “sham” surgery. While it is known that RYGB patients typically experience rapid weight loss and decreased adiposity following surgical intervention, the exact mechanism that leads to this outcome is not completely understood. One proposed mechanism is that somehow the RYGB restructures the gut anatomy, thereby leading to a restructured gut microbiota. Further, transplantation of the gut microbiota from RYGB mice to non-operated, germ-free mice led to weight loss and decreased adiposity compared to the mice who received microbiota transplantation from sham surgery mice. There was also evidence that the RYGB improved glucose metabolism in both mice and people.\textsuperscript{14}

**Microbiome and Cachexia**

While the gut microbiota has long been implicated in the development of obesity through modulation of systemic inflammation and energy homeostasis, emerging research also suggests an association between dysbiosis and cachexia through similar systemic pathways. Cachexia is a complex, multifactorial syndrome, most commonly seen in patients with cancer, HIV, and advanced stages of many chronic diseases. It is characterized by progressive weight loss due to fat and muscle wasting, fatigue, and asthenia and can have significant effects on lifespan and quality of life in affected individuals.\textsuperscript{15}

**Energy Metabolism and Cachexia**

As previously discussed, the gut microbiota has been found to affect weight determination through suppression of FIAF, resulting in increased fatty acid uptake and deposition as well as decreased fatty acid metabolism. A study by Backhed et al. demonstrated that germ-free mice, which have been found to have elevated levels of FIAF, also exhibited increased expression of peroxisomal proliferator-activated receptor γ coactivator 1α (PGC-1α). PGC-1α is a regulator of cellular energy metabolism and has, through association with FIAF, been found to contribute to fatty acid oxidation and protect against obesity.\textsuperscript{16} Another study showed that PGC-1α has a protective effect on skeletal muscle in preventing atrophy, with overexpression reducing the impact of denervation and fasting on muscle fiber diameter and expression of enzymes that play key roles in the muscle atrophy process via the ubiquitin-proteasome pathway.\textsuperscript{17}

Another proposed pathway linking gut microbiota to muscle wasting is the Toll-like receptors (TLRs)/NF-κB pathway (Figure 2). TLRs are known to recognize various pathogen-associated molecular patterns (PAMPs), e.g. TLR2, -4, -5, -9 recognition of peptidoglycan from Gram-positive bacteria, lipopolysaccharides, flagellin, and virus or bacteria derived nucleic acids, respectively.\textsuperscript{18,19} These TLRs can, in turn, lead to muscle wasting through muscle-specific activation of the NF-κB transcription factor.\textsuperscript{20} This was further demonstrated in a study by Doyle et al. that found evidence supporting TLR4 mediation of muscle atrophy induced by lipopolysaccharide injection.\textsuperscript{21}

**Inflammation and Cachexia**

Gut barrier function and permeability are important factors in determining the extent of extra-intestinal effects of the gut microbiota by influencing systemic bioavailability of components involved in associated pathways. Interestingly, a link between microbiota-related inflammation and cachectic diseases was hypothesized in a 2016 review article by Bindels et al.\textsuperscript{15} Additionally, a

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**Figure 3.** Digitally colorized scanning electron micrograph of a large cluster of Gram-positive, Enterococci sp. bacteria, belonging to the phylum of Firmicutes.\textsuperscript{Janice Haney Carr, CDC Public Health Image Library, public domain}
study by Puppa et al. notes a development of gut barrier dysfunction and endotoxemia, measured by increasing serum lipopolysaccharide levels, with concurrent progression of tumor growth and cachexia.\textsuperscript{22} This introduces the idea that systemic inflammation and cachexia contribute to increasing gut permeability and, with increasing translocation of PAMPs and subsequent downstream activation of associated transcription factors, further contribute to development of muscle atrophy and cachexia. However, more research is needed to better define this association.

Another area of research has been the changes in gut microbiota composition that occur with developing cachexia. Current literature has identified several specific microbial signatures in the cecal microbiome of mice with cancer cachexia, including decreased levels of \textit{Lactobacillus spp.} and increased levels of \textit{Enterobacteriaceae} and \textit{Parabacteroides goldsteinii} \textsuperscript{23}. A 2012 study by Bindels et al. demonstrated decreasing levels of systemic inflammatory cytokines and

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**Table 1. Key Points**

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<th>EARLY LIFE FACTORS</th>
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<td>Peripartum antibiotic exposure</td>
<td>Peripartum antibiotic exposure in the mother can lead to gut dysbiosis and even colitis in the offspring in animal models</td>
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<td>Delivery Method</td>
<td>C-section vs Vaginal Delivery</td>
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<td>Breastfeeding</td>
<td>Breast-fed infants have higher proportion of \textit{Bifidobacterium} and \textit{Lactobacillus spp.}, compared to formula-fed infants who tend to have a higher proportion of other bacteria</td>
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<td>Childhood antibiotic exposure</td>
<td>Children exposed to antibiotics have delayed maturation of the microbiome compared to control subjects</td>
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<th>WEIGHT DIFFERENCES AND DETERMINANTS</th>
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<td>Differences in energy harvest</td>
<td>Gut microbiota can influence calories absorbed in the gut</td>
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<td>Composition differences</td>
<td>Obese humans have increased ratio of \textit{Firmicutes} as compared to \textit{Bacteroidetes}; mice with cancer cachexia have decreased levels of \textit{Lactobacillus spp.} and increased levels of \textit{Enterobacteriaceae} and \textit{Parabacteroides goldsteinii}</td>
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<th>INFLAMMATORY INVOLVEMENT</th>
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<td>Systemic inflammation</td>
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<th>GENE EXPRESSION</th>
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<td>FIAF</td>
<td>Inhibits circulating lipoprotein lipase; suppressed by gut microbiota, leading to increased fat uptake and deposition</td>
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<td>PGC-1a</td>
<td>Regulator of cellular metabolism; protective effects on obesity and muscle atrophy</td>
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<td>TLR/NF-κB pathway</td>
<td>TLRs on muscle cells respond to PAMPs, activating the NF-κB pathway, and leading to muscle wasting</td>
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<th>MICROBIOME AS A THERAPEUTIC TARGET</th>
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<td>Fecal Microbiota Transplant</td>
<td>Potential role for therapeutic alteration of the gut microbiome using FMT for weight loss or improving insulin resistance</td>
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<td>Areas of possible intervention</td>
<td>Fecal Microbiota Transplant, Prebiotics, Probiotics, Synbiotics</td>
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markers of ubiquitin-proteosome and autophagy-lysosomal pathways of muscle atrophy with oral *Lactobacillus* supplementation. These effects were species specific; however, as *L. reuteri* and *L. gasseri* supplementation appeared to decrease systemic inflammation and levels of muscle atrophy markers, *L. acidophilus* supplementation did not.²⁴ Bindels et al. further demonstrate that modulation of the cecal microbiome using synbiotics, in this case a prebiotic composed of inulin-type fructans and a probiotic of live *Lactobacillus reuteri*, resulted in normalization of cecal *Lactobacillus* and *Enterobacteriaceae* levels, reduced cancer cell proliferation and cachexia, and prolonged survival.²³

**Future Directions**

In recent years, there has been soaring interest in both obesity and the intestinal microbiome, individually, however it has become clear that the two are more connected than previously recognized. The gut microbiome represents a complex ecosystem affecting numerous intertwining processes well beyond the intestines. The microbiome composition has been found to exert its effects through nutrient bioavailability, energy homeostasis, systemic inflammation, and gene expression. Clear differences have emerged between the microbiomes of obese and non-obese subjects. These differences present exciting new arenas to consider how we think of, and how we attempt to manage, weight determination.

**Fecal Transplant**

Thus far, most strategies designed to target the microbiome, for either the prevention or treatment of obesity, have primarily looked at prebiotics, probiotics, or fecal microbiota transplant (FMT).¹⁰ While recent attention to therapeutic benefits of FMT is largely derived from its role in the treatment of recurrent, refractory *Clostridium difficile* infection, the therapeutic benefits of FMT date back as far as the 4th century.²⁵ FMT in particular has drawn particular interest, as FMT has been shown to be able to cause changes in the microbiome composition.

Although more research is needed, promising preliminary data has been reported. Hartstra et al., performed a double-blind, randomized control trial, using FMT from lean donors into men with insulin resistance and metabolic syndrome. They found that the group who received the FMT from the lean donors experienced improvement in peripheral insulin sensitivity, as well as increased intestinal microbiota diversity.²⁶

Researchers at the Massachusetts General Hospital are currently performing a randomized, double-blinded, placebo-controlled study examining the impact of FMT on body weight and glycemic control, using oral FMT capsules (ClinicalTrials.gov ID NCT02530385).

The use of FMT in treatment of cachexia, however, is often contraindicated due to the nature of its etiology (i.e. cancer, HIV, severe systemic disease) and concurrent treatments that may lead to additional contraindications, such as immunodeficiency or use of systemic antibiotics (Table 1).

**Pre/pro/syn-biotics**

In theory, the intestinal microbiome composition can also be modified through the use of prebiotics, probiotics, or synbiotics (a combination of the two), to produce the desirable systemic effects. Notably, this has been demonstrated in mice with cancer cachexia that showed reduction in cancer proliferation, muscle wasting, and morbidity as well as prolonged survival following treatment with a synbiotic.⁶ However, data from a systematic review of randomized controlled trials in human subjects has shown no significant alteration of gut microbiome through the use of probiotics despite other potential systemic effects.²⁷

**Antibiotic Resistance**

Antibiotic resistance has become a real challenge as antibiotics are widely prescribed. Aside from their antimicrobial effects on pathogens, antibiotics can also induce significant and durable changes to the microbiome, with far reaching implications, perhaps even on weight determination, as discussed above. Because of this, the importance of antibiotic stewardship becomes even more paramount.

**Cancer Research**

At the forefront of recent developments in cancer treatment, is the role of immunotherapies, therapies as designed to utilize the human immune system.
to attack cancer cells. The pioneering work by Drs. James Allison and Tasuku Honjo on cytotoxic T-lymphocyte antigen-4 (CTLA4) and protein cell death 1 (PD-1) demonstrating that by inhibiting these checkpoints, T cells are more effectively able to kill cancer cells, earned them the 2018 Nobel Prize in Medicine, and their work as served as the foundation for developments of current immunotherapies used to treat a variety of cancers.

Recent studies have shown that alterations in gut microbiome composition can influence the efficacy of immune checkpoint inhibitors. This presents an opportunity to explore the role of the microbiome in not only predicting the success of immune checkpoint inhibitor therapy, but also in learning how alterations in the microbiome may be used to increase the efficacy of these new therapies.28 Similarly, in murine models, researchers found fecal microbiome transplantation could restore sensitivity to anti-PD-L1 treatment and improve the anti-tumor activity in non-responding mice.29 In another human study, researchers found that in patients with melanoma, receiving PD-1 based immunotherapy, significant differences were found with respect to the diversity and composition of the gut microbiome in patients who responders versus non-responders to the PD-1 immunotherapy. Specifically, responders had higher diversity as well as relative abundance of Ruminococcaceae family, compared to non-responders.30 Further research in the field shows promising possibilities in management of cancer patients through modulation of their gut microbiome, providing potential methods to improve quality of life by combatting cancer-associated cachexia as well as possibly increasing efficacy of immunotherapeutic agents.

Challenges
Current understanding of the complex interactions between the gut microbiome, host physiology, and environment remains limited and though research suggests direct links between the microbiome and levels of obesity, systemic inflammation, and insulin resistance, understanding causality between observed outcomes is difficult. Many factors contribute to the lack of clarity within the growing wealth of research with regards to the microbiome and host physiology, including variations in size, design, and quality of human studies, variations in interpersonal response, environment, technological limitations, and perpetuation of research silos without a focus on interdisciplinary cohesion.31 Regardless of attempts to curb the widespread variation in study design, interpersonal variations and environmental influences will remain daunting obstacles.

As previously mentioned, environmental factors contribute to approximately one third of the observed variability in microbiome composition. One notable environmental factor that is often considered is dietary variations. A recent human study by Roager et al. showed that a whole-grain diet, as compared to a refined grain diet, was associated with a reductions in body weight and systemic inflammatory markers CRP and IL-6 without significant changes in gut microbiome or insulin resistance.32 Another recent human study by Wu et al. showed large variations in plasma levels of microbiome-related metabolites, in this case SCFAs and equol, despite only modest differences in microbiome composition in vegans versus omnivores within same environment. This suggests that environmental factors independent of diet may be influencing regional differences in microbiome composition and also that plasma levels of these critical metabolites may vary according to diet with uncertain influence from the actual microbiome composition.33 These studies challenge our understanding of the suspected mechanisms by which changes in the microbiome, environment, and host physiology interact and highlight the need for further investigation to effectively guide development of effective pre/pro/syn-biotics and antibiotic therapies.

SUMMARY
The intestinal microbiome is a complex ecosystem whose sphere of influence extends well beyond the intestine, providing a new lens for how we understand health and disease. For instance, recent research has discussed the “brain-gut-microbiome axis” with probiotics affecting behavior in animal models.34 The intestinal microbiome has been implicated in the development of obesity as well as the pathogenesis of resultant consequences ranging from inflammation to insulin sensitivity. Similarly, it has been found to effect fatty acid metabolism.
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A SPECIAL ARTICLE

References