The gut microbiota in human energy homeostasis and obesity

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Numerous studies of rodents suggest that the gut microbiota populations are sensitive to genetic and environmental influences, and can produce or influence afferent signals that directly or indirectly impinge on energy homeostatic systems affecting both energy balance (weight gain or loss) and energy stores. Fecal transplants from obese and lean human, and from mouse donors to gnotobiotic mice, result in adoption of the donor somatotype by the formerly germ-free rodents. Thus, the microbiota is certainly implicated in the development of obesity, adiposity-related comorbidities, and the response to interventions designed to achieve sustained weight reduction in mice. More studies are needed to determine whether the microbiota plays a similarly potent role in human body-weight regulation and obesity.

Introduction

The human gut microbiota (see Glossary) consists of up to 100 trillion microbes that exist in largely symbiotic relationship with their human hosts, and carry at least 150 times more genes (the microbiome) than are present in the entire human genome [1,2]. There is significant cross-sectional variability in the microbiota between individuals and longitudinal variability within individuals. Both the abundance and the composition of the gut microbial population are influenced by diet, medication, weight, and overall metabolic state (energy balance, etc.) of the host. In turn, and based largely on animal studies, the microbiota is capable of secreting or altering the production of molecules that affect both energy balance (weight gain or loss) and energy stores (fat mass) [3,4]. The microbiota may, in this context, be regarded as a responsive entero-endocrine organ composed of more cells and genes than the host. This manuscript reviews what has been done and what may be done to determine how translatable rodent data are to humans, and what the implications are for probiotic, prebiotic, and possibly targeted antibiotic approaches to the treatment or prevention of human obesity.

Composition of the microbiota

Although phylum-level and even family-level groupings of microbes are very broad, and can conceal variability at finer levels (including at the strain level) that is often important, some general trends emerge. The dominant phyla in the gut are Bacteroidetes (~20–25%), Firmicutes (~60–65%), Proteobacteria (~5–10%), and Actinobacteria (~3%), which together constitute over 97% of the gut microbe population (Table 1). The taxonomic variability in the human gut is much higher than the functional variability, as measured by a variety of methods, suggesting that many different configurations of the microbiota lead to essentially the same functional result [5,6].

Overview of rodent studies of obesity and the microbiota

In rodents, obesity is associated with an increase in the relative size of the Firmicutes versus Bacteroidetes populations in the gut [7], and a decrease in the diversity of the microbiota that is due to both weight and diet composition. The microbiota of diet-induced obese mice (DIO, induced by an ad lib high-fat diet, HFD), which are weight-reduced by a calorie-restricted HFD, is more diverse compared to DIO mice at maximal weight (similar diet, greater weight), and less diverse than control ad libitum fed mice on a Chow diet (different diet, similar weight) [7]. There is much greater variability in human studies. Some studies report a similar increase in the ratio of Firmicutes/Bacteroides, as well as a decrease in the gut biodiversity in obese humans, but there are also numerous studies reporting contradictory findings in obese versus lean humans [8].

Studies of germ-free mice, or previously germ-free mice colonized with a defined microbial community (‘gnotobiotic mice’), have provided substantial evidence that the diversity, as well as the presence and relative proportion, of different microbes in the gut play active roles in energy homeostasis. The overall importance of the microbiome is emphasized by the consequences of its absence and repletion. Germ-free mice are very inefficient at processing food, and gain weight when colonized with almost any gut microbes. Inoculation of germ-free mice with ‘conventional’ microbes results in weight gain to similar levels of fatness to the donor mice. Surprisingly, this weight gain occurs despite an approximately 30% increase in energy expenditure and 30% decline in energy intake, compared to the mice who remain germ-free [9]. As discussed below, weight gain, despite increased energy output and decreased intake, is consistent with a microbiota mediated increase in energy harvest.

The amount of weight gained by gnotobiotic rodents differs substantially depending on which microbes are
inoculated. Administration of microbes from leptin-deficient (Lep\textsuperscript{ob}) mice, that contain higher absolute and relative proportions of Firmicutes, similarly to other obese mice, results in greater weight and fat gain, lower energy expenditure, and increased energy harvest than does administration of microbiota from wild-type mice [10]. Similar donor adiposity-related effects are noted following inoculation of germ-free mice with the microbiota from monozygotic (MZ) and dizygotic (DZ) human twin pairs discordant for obesity. There was a progressively greater increase in both fat mass and fat-free mass in animals receiving microbes from the obese twins, despite no significant differences between groups in daily chow consumption [11].

The consequences of manipulation of the microbiome population in rodents are also dependent on the environment in which they are studied. In the twin study described above [11], gnotobiotic mice developed the microbiota and somatotype of their human donors. Cohousing of lean (Ln\textsuperscript{ch}) and obese (Ob\textsuperscript{ch}) mice results in acquisition by Ob\textsuperscript{ch} mice of the microbiota of lean animals, but no corresponding acquisition of the obese microbiome or somatotype in Ln\textsuperscript{ch}. Ob\textsuperscript{ch} mice fed a low saturated fat, high fruit and vegetable diet demonstrated greater invasion of the Ln\textsuperscript{ch} microbiota. In addition, all mice on this diet with the lean microbiome (Ln/Ln, Ln\textsuperscript{ch}, and Ob\textsuperscript{ch}) gained less fat mass, compared to mice fed a high saturated fat, low fruit, and vegetable diet (Ln/Ln and Ln\textsuperscript{ch}), and to obese animals cohoused with a lean animal (Ob\textsuperscript{ch}) [11]. Similarly, studies of germ-free mice inoculated with microbiota from obesity-prone (OP, higher Firmicutes/Bacteroidetes ratio) versus obesity-resistant (OR) rats, show that there is greater weight gain in the OP-treated group, but only in the setting of a HFD [12].

In mice, there is some influence of host genotype (mouse strain) on the microbiome [13], but the effect of the mouse environment is greater. Ericsson et al. [14] compared the microbiota of six different strains of purchased mice (some strains duplicated) from three different laboratories and from 3.5 weeks of age to 24 weeks of age, during which time they were all housed and fed similarly and at a single site. The found roughly twice as many significant vendor effects within populations of Bacteroidetes, Firmicutes, and Proteobacteria compared to mouse strain effects. There was little change in these populations between 3.5 and 24 weeks of age, suggesting the importance of early gut colonization and strain×vendor interactions. Parks et al. [15] reported significant heritability of weight gain and of gut microbial composition and plasticity (potential for change) in response to a high-fat/high-sugar diet. However, neither the baseline enteroetype nor the degree of plasticity (amount of change in response to diet) were independently predictive of weight gain in mice. In humans, the predominance of the environment in determining the composition of the gut microbiome is also evident [16].

Overview of the human gut microbiome

The pediatric microbiome

In so-called ‘ecological models’, child adiposity is influenced by home and school environments, parental eating behaviors, and food availability, in addition to adiposity of parents [17]. Similarly, the establishment of the gut microbial population in the neonate is a complex process that may involve interactions between the maternal and fetal genes and environment (as exemplified by studies discussed above of the interactions of the microbiome from genetically OP vs OR rats with diet type). These interactions may begin even before birth and progress through multiple stages under the influence of various internal factors, such as the early decline in the abundance of oxygen in the gut that influences the balance of aerobes and anaerobes, and external factors such as diet [18].
The vaginally delivered neonate is initially colonized with the vaginal and distal gut bacteria of the mother, while babies delivered by Caesarean section (C-section) are initially colonized predominantly with the skin bacteria from the mother [19,20]. More specifically, vaginally delivered neonates have relatively and absolutely larger populations of Bacteroides and Bifidobacteria species than those born by C-section [21] that have been reported to persist for months to years [18]. The observations that increased Bacteroidetes populations are present in both obesity and children born by C-section [7] suggest that the infant microbiome may contribute to the subsequent ~40% increased risk of obesity in children [22] and young adults [23,24] delivered by C-section. This is apparent even when corrected for pre- and post-gravid maternal body mass index (BMI) and birth weight for gestational age. Similarly, maternal exposure to antibiotics in the second and third trimesters of pregnancy or early infancy is associated with decreased bacterial diversity of the first stool of the neonate, reduced abundance of lactobacilli and bifidobacteria in the infant gut, and an approximately 80% increased risk of childhood obesity [22,25,26]. The mechanisms for this increase are not clear, but animal studies have suggested that low-dose penicillin administered early in life increases susceptibility to HFD-induced obesity, and that this susceptibility can be transferred to gnotobiotic animals; in other words, it is the microbiome not the antibiotic per se that increases obesity susceptibility [27].

While there are genetic influences on the human microbiome, particularly in the first 3 years of life, these affects are small compared to those attributable to the environment [6,28,29]. The UniFrac distance (unique fraction metric), a measure of the phylogenetic distance between sets of taxa (in this case, summarizing the evolutionary separation between the microbes present in different biological specimens such as stool samples or gut biopsies), is significantly greater in unrelated individuals than between DZ or MZ twins or their mothers. There is substantial variation between fecal communities in different geographic populations (Malawians, Amerindians, and other Americans) at all ages, and the within-community diversity increases in all populations as age increases [28]. Studies comparing industrialized societies such as the USA or Europe with non-industrialized societies, such as the Hadza hunter-gatherers [30] or groups in Papua New Guinea [31], indicate that industrialization

<table>
<thead>
<tr>
<th>Phylum</th>
<th>% Microbiome</th>
<th>Genus or species</th>
<th>Relevant function</th>
<th>Sources</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firmicutes</td>
<td>~60–65%</td>
<td>Clostridium, Eubacterium, Faecalibacterium, Lactobacilli, Roseburia, Ruminococcus</td>
<td>Some species ferment fiber into butyrate; other functions range from symbionts to pathogens</td>
<td>[5,6,9,28,81,82]</td>
<td></td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>~20–25%</td>
<td>Alistipes, Bacteroides, Parabacteroides, Prevotella, Prophyromonas, Prevotella</td>
<td>Polysaccharide degradation, Some species ferment fiber into butyrate</td>
<td>[5,6,8,9,16,81–84]</td>
<td></td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>~5–10%</td>
<td>E. coli</td>
<td>Vitamin biosynthesis</td>
<td>[5,6,9,81,82]</td>
<td></td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>~3%</td>
<td>Bifidobacterium, colinsella</td>
<td>Vitamin biosynthesis</td>
<td>[5,6,9,81,82]</td>
<td></td>
</tr>
<tr>
<td>Minor phyla (&lt;1% of most individuals)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Archaea</td>
<td>&lt;1%</td>
<td>Methanobrevibacter, Methanospirillum</td>
<td>Both convert hydrogen gas to methane (methanogens)</td>
<td>[85,86]</td>
<td></td>
</tr>
<tr>
<td>Deferribacteres</td>
<td>&lt;1%</td>
<td></td>
<td>Degradation</td>
<td>[8,16,83,84]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Iron</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fusobacteria</td>
<td>&lt;1%</td>
<td>Fusobacterium, nucleatum</td>
<td>Proinflammatory</td>
<td>[16,87,88]</td>
<td></td>
</tr>
<tr>
<td>Melainabacteria</td>
<td>&lt;1%</td>
<td></td>
<td>Synthesize vitamins B and K, ferment carbohydrate into ethanol, lactate, and formate</td>
<td>[16,28,89]</td>
<td></td>
</tr>
<tr>
<td>Spirochaetes</td>
<td>&lt;1%</td>
<td>Predominantly Treponema</td>
<td>More common in rural communities and in high-fiber diets</td>
<td>[16,30]</td>
<td></td>
</tr>
<tr>
<td>Verrucomicrobia</td>
<td>&lt;1%</td>
<td>Akkermansia, muciniphila</td>
<td>Degradation mucin, diminish inflammation, and increase gut butyrate and mucus layer thickness</td>
<td>[90]</td>
<td></td>
</tr>
</tbody>
</table>
is associated with lower diversity of fecal bacteria within individuals (α-diversity) and greater diversity between individuals (β-diversity) [30,31]. In smaller studies of 54 [6] and 63 [28] twin pairs of MZ versus DZ twin pairs, the UniFrac distance within human twin pairs was not significantly different based on zygosity, suggesting that the shared environment contributes more than shared genes to the gut microbe composition [6]. However, in a study of 416 twin pairs in the TwinsUK population, there was a significantly higher correlation within MZ twin pairs for particular taxa, in particular the family Christensenellaceae. The abundance of Christensenellaceae species was negatively associated with fatness in humans, and reduces weight gain when transplanted into gnotobiotic mice [29], indicating that physiologically important microbes may still be heritable at narrower taxonomic levels.

**The adult microbiota**

In most [6,10], but not all [8], studies, both the diversity of the microbiota and the fractional proportion of Bacteroidetes species relative to Firmicutes are decreased in obese versus lean individuals. These proportions are extremely sensitive to caloric balance in subjects studied before, during, and after weight loss while ingesting the same liquid formula diet of identical macronutrient composition [32]. Weight loss increased the relative proportion of Bacteroidetes species and microbial diversity, thereby ‘reversing’ some of the microbial characteristics of obese versus lean individuals; this effect is augmented by a low-carbohydrate versus a low-fat weight reduction diet [33]. Similarly, studies of the microbiome have shown that gene richness is diminished in obese versus lean individuals [6] and is increased by dietary weight loss [34].

Studies of humans during weight loss and weight gain indicate that the slope of the line relating degree of overfeeding and underfeeding to changes in the relative abundance of Bacteroidetes and Firmicutes are almost identical – making it unclear whether these changes in the microbiome reflect nutrient balance or nutrient stores [35]. A key question is whether the positive association of the abundance of Firmicutes and negative association of the abundance of Bacteroidetes with changes in body weight represent a direct effect of the degree and duration of negative or positive energy balance, or of changes in energy stores as fat mass. Studies of changes in the microbiome induced by liposuction, in other words reducing fat stores without inducing a negative energy balance, might more directly address this issue.

**Overview of the human gut microbiome and energy homeostasis**

Several mechanisms have been postulated by which the microbiome might affect energy balance. These mechanisms are poorly studied in humans.

It is important to consider these possible mechanisms in the framework of what is known about human energy homeostasis. Briefly, the relative constancy of adult human body weight over time in a stable environment strongly suggests that energy intake and output are coupled in a manner that ‘defends’ a relatively constant level of energy stores over time, such that if energy intake is decreased there is a corresponding decrease in energy expenditure and vice versa [36,37]. Weight gain and weight loss clearly represent an imbalance beyond the coupling capacity of energy intake and output. To induce or sustain changes in body weight, microbiome-related mechanisms must somehow ‘disrupt’ this coupling.

Briefly, the dynamic process of weight loss and the maintenance of a reduced body weight involve similar but not identical declines in energy expenditure (disproportionate to changes in body composition and weight) and increases in energy intake. The decline in energy expenditure is mainly due to increased chemomechanical efficiency of skeletal muscle, while the increases in energy intake reflect increased food reward (hunger) and decreased food restraint (delayed satiation and decreased perception of how much has been eaten) [38–41]. These effects, which do not abate over time [38–41], are largely mediated by the declines in circulating concentrations of the adipocyte-derived hormone leptin, acting directly and via the autonomic nervous (ANS) and neuroendocrine systems. The ANS includes the sympathetic (SNS) and parasympathetic (PNS) nervous systems. SNS tone modulates feeding behavior via effects on various gut peptides and transmission of nutrient-derived signals to the brainstem [42], and directly increases heart rate and secretion of thyroid hormone [43,44], while increased PNS tone slows heart rate and decreased resting energy expenditure [38]. Thyroid hormone increases energy expenditure by increasing heart rate, blood pressure, muscle ATP consumption [largely by stimulating production of muscle ATPase, and favoring expression of the less mechanically efficient myosin heavy chain II (MHCII) isoform] [45,46]. The increase in PNS tone and the declines in SNS tone and circulating concentrations of leptin and bioactive thyroid hormones during and following weight loss effectively conspire to favor the regain of lost weight [47–52].

Body weight regulation in mice is distinct from that of humans in a number of key areas relevant to the energy homeostatic systems described above (Table 2). For example, in addition to differences in natural diet composition (greater carbohydrate and less fat in murine diet), biorhythms relevant to energy intake and expenditure (nocturnal vs diurnal), thermogenesis by brown (BAT) and beige (brite) adipose tissue accounts for over 50% of adaptive thermogenesis in rodents versus <5% in adult humans [53,54]. In mice, both the amount and metabolism of BAT are affected by diet composition and the gut microbiota [55,56]. Given the lower contribution of BAT to human thermogenesis, it is questionable whether such manipulation has potential in the treatment or prevention of human obesity. It is clear that these anatomic, physiological, and behavioral differences must be considered in attempts to extrapolate from mouse to human microbiota.

There are few studies in humans examining the effects of alterations in the gut microbiome on energy expenditure and intake. As discussed below, there are many hypotheses regarding the mechanisms by which the human gut microbiota might affect these phenotypes, but these are largely based on animal studies or indirect measurements of the effects of experimental alterations of gut flora on other
Table 2. Overview of major differences in mouse and human growth and energy homeostasis that may affect extrapolation of rodent studies to humans*

<table>
<thead>
<tr>
<th>System</th>
<th>Mouse</th>
<th>Adult humans</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somatic growth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exogenous Leptin Effects</td>
<td>Decreased energy intake before, during, or after weight loss</td>
<td>Decreased energy intake after weight loss, less during</td>
<td>[54,93,94]</td>
</tr>
<tr>
<td>Thermogenesis</td>
<td>20% of 24 h energy expenditure is devoted to maintaining body temperature and is largely dependent on brown adipose tissue (BAT) thermogenesis</td>
<td>Comparatively little basal thermogenesis and very little brown adipose tissue (BAT) thermogenesis at rest</td>
<td></td>
</tr>
<tr>
<td>Autonomic Nervous System</td>
<td>No α-adrenergoreceptors (most strains)</td>
<td>α- and β-adrenergoreceptors</td>
<td></td>
</tr>
<tr>
<td>Thyroid</td>
<td>T3, T4, TSH with weight gain leading to BAT thermogenesis</td>
<td>T3 during weight gain, little brown adipose tissue (BAT) or beige adipose tissue (brite) thermogenesis</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: *, increased; T3, triiodothyronine; T4, thyroxine; TSH, thyroid stimulating hormone.

molecules known to affect energy output and intake, without direct measures of these variables.

It has been observed that probiotics (defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host [57]) – such as particular species of Lactobacillus – reduce weight and body fat in DIO mice without changing energy intake [58], in contrast to the transfer of intestinal flora from lean mice to gnotobiotic mice that results in weight (fat) gain despite decreased energy intake and increased energy expenditure [9]. This conjunction of phenotypes suggests increased efficiency in extraction of calories from the diet.

In humans, Kocelak et al. [59] examined resting energy expenditure (REE), body composition, and the gut microbial population in 50 obese and 30 lean healthy weight-stable subjects. They reported that the obese subjects had a significantly greater total microbial count without significant differences in the ratio of Bacteroidetes/Firmicutes, both of which had been reported to be lower in obese individuals in other studies [6,10]. Over the entire group of subjects, the size of the population of Firmicutes was positively correlated with fat mass and negatively correlated with REE and the maximal oxygen consumption (VO$_{2\text{max}}$). Total bacterial count was significantly positively correlated with VO$_2$ and negatively correlated with VCO$_2$ (rate of carbon dioxide production in this case during exercise). However, in multiple regression analysis including fat mass, none of these correlations between the microbiota and energy expenditure remained significant [59].

The observation that administration of conventional mouse bacteria to germ-free mice results in decreased energy intake suggests a direct or indirect role of the microbiome in appetite regulation and the efficiency of energy harvest from food [9,60]. Prebiotics, defined as selectively fermented nondigestible food ingredients or substances specifically supporting the growth and/or activity of health-promoting bacteria that colonize the gastrointestinal tract [57], are valuable tools for modulating the human gut microflora [61]. Most relevant, addition of small amounts of dietary inulin-type fructo-oligosaccharides given to humans stimulate the growth of health-promoting Bifidobacterium, Lactobacillus, Roseburia, and Faecalibacterium species [61]. In a double-blind placebo-controlled study of 16 adults, Cani et al. [62] administered an inulin-like prebiotic fiber versus a similar-tasting placebo (dextrin/maltose), and found that prebiotic administration was associated with a significant decrease in hunger and post-prandial glucose excursions, and a significantly greater satiation after a meal, as well as increases in plasma glucagon-like peptide 1 (GLP-1) and peptide YY (PYY), both of which are gut-derived peptides known to promote satiation and glucose homeostasis [63].

Gnotobiotic mice that have been colonized with conventional mouse gut biota [9,10,33] gain weight (increase energy stores) despite decreasing energy intake and increasing energy expenditure. Rather than violating the first law of thermodynamics, this observation raises the possibility that the microbiota affect nutrient absorption (‘energy harvest’). Gut microbiota can facilitate extraction of calories from ingested dietary substances. Turnbaugh et al. [10] reported that the obese (ob/ob) mouse microbiota have an increased capacity to harvest energy from the diet regardless of whether it is present in ob/ob or gnotobiotic mice. More specifically, transplantation of the caecal microbiota of an obese mouse to gnotobiotic mice results in increased energy harvesting, including increased absorption of monosaccharides from the gut. This in turn leads to increased hepatic lipogenesis and stimulation of both hepatic lipoprotein lipase (LPL) and sterol regulatory element-binding proteins (SREBPs). The approximately 10% decrease in kcal lost per gram of stool in gnotobiotic mice colonized with the ob/ob microbiota suggests that associated decreases in energy harvest would be a potential means of reducing energy stores by switching to a leaner microbiome. However, this does not address issues such as ‘if one absorbs less food energy why doesn’t one just eat more or burn less to compensate, as occurs after induction of decreased energy harvest by pharmacological agents such as orlistat?’ [64].

Similar studies examining energy harvest after manipulating the gut microbial population have not, to our knowledge, been done in humans. Jumpertz et al. [35]...
examined the effects of underfeeding (2400 kcal/day) and overfeeding (3400 kcal/day) on the human microbiota of 12 lean and nine obese individuals. They reported that the differences between caloric intake and weight maintenance calories were positively correlated with the relative abundance of Firmicutes species and negatively correlated with the relative abundance of Bacteroidetes species in lean and obese humans. This suggests that the microbiota is responsive to energy balance (degree of overfeeding or underfeeding) as well as actual adiposity. An approximately 20% increase in abundance of Firmicutes and a corresponding decrease in Bacteroidetes abundance (which is what would occurring during weight gain) was associated with a 150 kcal/day (approximately 5% of ingested calories) increase in daily energy harvest [35].

The mechanisms by which the microbiome affects energy harvest are not clear. Nutrient absorption is clearly influenced by the length of exposure to the gut mucosa. Kashyap et al. [65] reported that colonization of germ-free mice with human or rodent bacteria was associated with a decrease in gastrointestinal (GI) transit time, and that shortening transit time or increasing GI transit time resulted respectively in increased abundances of Bacteroidaceae and Porphyromonadaceae. This type of study is clearly replicable in humans.

**Leptin signaling**

Introduction of conventional gut bacteria into gnotobiotic mice increases fatness but does not appear to affect the anticipated increases in the anorexigenic hormones leptin, and hypothalamic pro-opiomelanocortin (POMC) and cocaine–amphetamine-related transcript (CART), and decreased expression of the orexigenic peptides agouti-related peptide (AGRP) and neuropeptide Y (NPY) [66,67]. This suggests that the changes in the adipose–leptin–hypothalamic pathway are induced by increases in adipose tissue mass rather than by a primary centrally mediated effect. However, the colonization of germ-free mice with conventional microbiota significantly blunts both the weight loss and the decline in Agrp and Npy expression following leptin administration [66,67], and prebiotic manipulation of the microbiota to decrease Firmicutes and increase Bacteroidetes phyla in leptin-deficient mice resulted in increased leptin-sensitivity [68], suggesting that the gut microbiota centrally affect leptin signaling. Germ-free mice have significantly increased brainstem expression of proglucagon (Gcg) that encodes the precursor for the incretin and anti-obesity molecule glucagon-like peptide 1 (GLP-1). Reductions are also seen in the anorexigenic brain-derived neurotrophic factor (Bdnf) and leptin resistance-associated suppressor of cytokine signaling 3 (Socs3) expression in both the brainstem and hypothalamus, compared to conventionally raised mice. Microbe-induced suppression of any or all of these molecules might promote weight gain [51,52].

**Short-chain fatty acids**

Short-chain fatty acids (SCFAs), predominantly butyrate, acetate, and propionate, are the end-products of fermentation of dietary fiber by various gut microbes (germ-free mice produce almost no SCFAs [69]) and have distinct actions relevant to energy homeostasis in addition to serving as an energy source for the colonic epithelia (butyrate), liver (propionate), and peripheral tissues (acetate) [70]. The major microbiotic phyla with species affecting SCFA production in the gut are Firmicutes and Bacteroidetes, as well as the minor phyla Melainabacteria and Porphyromonadaceae. This type of study is clearly replicable in humans.

SCFAs bind to two orphan G protein-coupled receptors (GPCRs); GPR41 (also termed free fatty acid receptor 3 or FFAR3) primarily activates G<sub>q</sub> protein and is expressed in the gut, adipocytes, and the peripheral nervous system, and GPR43 (also termed free fatty acid receptor 2 or FFAR2) primarily activates G<sub>q</sub> and G<sub>15</sub> proteins in the gut and adipose tissue. In addition, activation of GPR43 in the intestines by SCFAs has been shown to decrease release of inflammatory cytokines [69], which may also increase hypothalamic sensitivity to leptin [71,72]; GPR41 activation promotes growth and activation of the sympathetic nervous system. The density of Bacteroidetes in the gut microbiota has been positively associated with fecal concentrations of propionate, butyrate, acetate, and SCFAs [73]; in other words, the same bacterial species that are diminished in obese subjects and increased by weight loss are also correlated with changes in SCFAs whose increase would favor weight loss and the maintenance of reduced weight.

SCFAs affect energy homeostasis by discrete mechanisms. GPR41 is activated equally by propionate and butyrate [74,75], whereas GPR43 is more responsive to propionate and acetate than to butyrate [74,75]. Butyrate has been shown to inhibit histone deacetylases (HDACs), thereby inducing histone hypermethylation with subsequent changes in gene transcription affecting genes and pathways involved in fatty acid oxidation, epithelial integrity, and apoptosis [76–78].

Similarly, there are distinct effects of each SCFA on various gut peptides (incretins and hormones) and energy homeostasis. Oral administration of butyrate significantly increases plasma levels of gastric inhibitory peptide (GIP), GLP-1, PYY, insulin, and amylin which would have a net effect of slowing digestion and nutrient intestinal transit, promoting satiety, and increasing plasma insulin. There is a more modest, but still significant, effect of propionate administration on GIP, insulin, and amylin, and no effect of acetate administration on any of these hormones. Acetate is reported to increase leptin release by fat cells; butyric acid and propionate increase G-protein mediated secretion of PYY and GLP-1 in the gut, and rates of lipolysis and lipogenesis in fat cells [74,75].

Butyrate and acetate have been reported to protect against diet-induced obesity without hypophagia, while propionate has been reported to reduce food intake and increase locomotor activity [70]. Collectively, these data would suggest that acetate, because it has little effect on activity or energy intake, exerts its actions primarily via effects on energy harvest and/or basal thermogenesis (as evidenced by increased oxygen consumption in rats given oral acetate [79]), whereas butyrate and propionate have more direct effects on feeding behavior and physical activity.
Concluding remarks and future perspectives
This review focuses on the role of the human gut microbiota in systems regulating energy homeostasis. It should be recognized that the effects of the intestinal microbial population extend to multiple other systems, including glucose and lipid homeostasis, blood pressure, inflammation (Box 1), and other adiposity-related comorbidities [80].

Most systems regulating energy intake are altered following weight loss in a manner that facilitates weight regain. By contrast, weight reduction is associated with a change in the gut microbiota towards that of a non-obese individual. If the obese microbiota facilitates weight gain in humans, then the shift in the microbiota as a result of weight reduction should assist rather than oppose sustained weight reduction.

A review of the mouse literature suggests that the microbiome affects energy intake and expenditure such that specific alterations of the gut microbiota might constitute a potential therapeutic intervention to prevent obesity and/or to promote and sustain weight loss in humans (Box 2). Before such applications are considered in humans, further studies are clearly necessary, using manipulations involving prebiotics, probiotics, and diet composition. In contrast to mechanistic work done in the mouse, studies of the role of human microbiota and energy homeostasis have been predominantly epidemiological in nature and have failed to distinguish whether alterations of the microbiota are a cause or consequence of changes in fat mass.

Some of the same methodologies used in rodent studies are clearly applicable to humans. Manipulation of the microbiota by transplantation, diet, or prebiotics in different metabolic states (obese, formerly-obese, and never-obese) could be used to isolate the possible role(s) of the microbiota in the regulation of energy homeostasis.

In rodent studies discussed above there is substantial variability in the responses of the animals to fecal transplantation based on diet and even the source (vendor) of the rodents. Exactly as there is large interindividual variation in human responses to diet, exercise, pharmacological or other weight-loss interventions, there are likely to be differences between individuals in the salience of the roles of microbiota in energy homeostasis. Data from larger-scale studies could identify specific behavioral, microbiotic, and metabolic phenotypes that might be predictive of response to different types of behavioral, pharmacological, pro-/pre-biotic, or surgical therapies.

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References

Box 1. The relationship between inflammation, gut microbiota, and obesity
It is well known that weight gain is associated with increased circulating concentrations of various inflammatory cytokines such as tumor necrosis factor α and interleukin 6, and that increased inflammation is an independent risk factor for the development of diabetes and other adiposity-related comorbidities. The observation that germ-free mice suffer from immune dysregulation coupled with the known inhibitory effects of SCFAs on lipopolysaccharide (LPS) metabolic endotoxemia suggest that the microbiota in and of itself may independently contribute to inflammation [36]. Increased circulating concentrations of inflammatory cytokines induced by a HFD have been proposed as a mechanism by which a HFD induces obesity and opposes weight loss in the setting of a high-fat versus a chow diet [71,72]. The other major method by which the microbiome has been purported to affect energy balance is via regulated production of LPS as well as via the inflammatory response to it which is inhibited by butyrate [86,87]. Cani et al. [88] reported that mice injected with LPS showed increased weight gain and insulin resistance without any change in energy intake, and suggested that high plasma LPS levels could result from an increased production of endotoxin by the gut microbiota [4,96]. This would be exacerbated by high-fat feeding which promotes a decreased number of Bifidobacteria, which are known to reduce intestinal LPS levels and improve mucosal barrier function in mice [4,99].

Box 2. Outstanding questions
- To what degree can animal studies of the role of the gut microbiota in energy homeostasis be extrapolated to humans?
- Do the alterations in the microbiota that occur as a result of weight reduction in humans play a significant role in the high relapse rate after otherwise successful weight loss?
- Can pre-biotic, pro-biotic, or dietary therapies modifying the gut microbiota be used to assist in weight loss or reduced weight maintenance in humans?


Murphy, R. et al. (2014) The ISACA Phase Three Study Group. Antibiotic treatment during infancy and increased body mass index in: an international cross-sectional study. Int. J. Obes. (Lond.) 38, 1115–1119

Cox, L. et al. (2014) Altering the intestinal microbiota during a critical development window has lasting metabolic consequences. Cell 158, 705–721


Okubo, T. et al. (2013) K/KTas mice administered Lactobacillus plantarum strain no. 14 have lower adiposity and higher insulin sensitivity. Biosci. Microbiota Food Health 32, 93–100

Mestdagh, R. et al. (2012) Gut microbiota modulate the metabolism of brown adipose tissue in mice. J. Proteome Res. 11, 620–630


Scheele, E. et al. (2013) The gut microbiota reduces leptin sensitivity and the expression of the obesity-suppressing neuropeptides proglucagon (GCG) and brain-derived neurotrophic factor (BDNF) in the central nervous system. Endocrinology 154, 3643–3651


Lin, H. et al. (2012) Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid receptor-3 independent mechanisms. PLoS ONE 7, e35240


73 Hester, C. et al. (2015) Fecal microbes, short chain fatty acids, and colorectal cancer across racial/ethnic groups. World J. Gastroenterol. 21, 2759–2769
75 Inoue, D. et al. (2014) Regulation of energy homeostasis by GPR41. Front. Endocrinol. 5, 81
78 Matsuki, T. et al. (2013) Epithelial cell proliferation arrest induced by lactate and acetate from Lactobacillus casei and Bifidobacterium breve. PLoS ONE 8, e64053
89 Soo, R. et al. (2014) An expanded genomic representation of the phylum Cyanobacteria. Genome Biol. Evol. 6, 1031–1035