Colonic flora, probiotics, obesity and diabetes

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Mammals, including humans, have, throughout their evolution, been associated with complex microbial communities, which inhabit the surfaces and alimentary tract of their host and which outnumber the human somatic cells by a factor of 10. The human intestinal microbiota is estimated to be composed of 10^{13} to 10^{14} microorganisms whose collective genome, the microbiome, contains at least 100-fold more genes than the complete human genome (Eckburg et al., 2005; Gill et al., 2006). The gut of a neonate is sterile. However, at birth, the gut is immediately colonized by maternal and environmental bacteria and the complexity of the resulting gut microbiota increases until weaning to solid foods. Although incompletely understood the gut microbiota is implicated in a variety of host functions involving intestinal development and function, including epithelial turnover, immune modulation, gastrointestinal motility, and drug metabolism. The gut microbiota also has important metabolic functions, breaking down dietary toxins and carcinogens, synthesizing micronutrients, fermenting indigestible food substances, assisting in the absorption of certain electrolytes and trace minerals, and affecting the growth and differentiation of enterocytes andocolonocytes through the production of short-chain fatty acids (SCFA). The gut microbiota composition in obese individuals. Further, genetic, diet, immune status, infections, gastrointestinal disorders as well as antibiotics and other drugs interact to determine an individual's unique microbiome. The effect of genotype on the composition of the human microbiota was demonstrated in a study involving monozygotic twins (Zoetendal et al., 2001). According to this report, there were greater similarities between monozygotic twins than between monozygotic twins and their unrelated marital partners. The importance of the initial colonizing microbial community on the eventual microbiota composition of the gut is evident from animal studies. The composition of the mouse gut microbiota is significantly affected by maternal transmission (Ley et al., 2005). Diet is also

Obesity results from alterations in the body's regulation of energy intake, expenditure, and storage. Animal and human data demonstrate that phylogenetic changes occur in the microbiota composition in obese individuals. Furthermore, evidence from animal models suggest that the alterations of the gut microbiota with obesity result in increased energy extraction and lipid deposition, altered release of enterohormones, increased intestinal permeability and metabolic endotoxemia. Treatment with pre- and probiotics may reverse many of the metabolic effects linked with the altered microbiota in obese patients. The gut microbiota is, therefore, a potential nutritional and pharmacological target for the management of obesity and obesity-related disorders.

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a pivotal variable in influencing the composition of the intestinal microbiota. Studies on chemically well-defined diet components have proven a clear correlation between diet and the presence of specific bacterial groups. It has been shown that a diet rich in insulin and related fibers promote an increase in bifidobacteria, whereas the intake of dietary sulfate favors several genera of sulfate-reducing bacteria over methanogenic Archaea. Diet affects not only the microbiota composition, but more significantly the metabolic activities of the microorganisms.

Obesity results from alterations in energy balance, that is, how the body regulates energy intake, expenditure, and storage. Because starvation poses a greater danger to an organism than overabundance, our biological systems are geared to better protect against weight loss than weight gain (i.e., a thrifty genotype). Considerable effort has been made to improve the availability and stability of the food supply, resulting in an abundance of inexpensive, palatable, and energy-dense foods. Consequently, humans adapted for a situation of insufficiency are now confronted with the easy availability of such foods. Recent evidence suggests that the gut microbiota affect nutrient acquisition and energy regulation and that obese and lean people have differences in their gut microbial landscape. These findings raise the possibility that the gut microbiota may have a role in the development of obesity (see Figure 1; DiBaise et al., 2008; Cani and Delzenne, 2009).

In an elegant series of experiments, Backhed et al. (2004) found that young conventionally reared mice have a 40% higher body fat content than germ-free mice (gnotobiotic mice) even though they consumed less food than their germ-free counterparts. The distal gut microbiota from the normal mice were then transplanted into the gnotobiotic mice (a process known as conventionalization), resulting in a 60% increase in body fat within 2 weeks without any increase in food consumption or obvious differences in energy expenditure. The authors proposed that the gut microbiota promotes intestinal monosaccharides absorption, energy extraction from non-digestible food components via SCFA production through fermentation, de novo hepatic lipogenesis and adipocyte fatty acid storage. The gut microbiota influence the expression of host genes expressed in the intestine that control fatty acid absorption, oxidation, and storage. One such target is angiopoietin-related protein 4 (ANGPTL4), a potent lipoprotein lipase inhibitor. ANGPTL4 inhibits the uptake of fatty acids from circulating triglyceride-rich lipoproteins in white adipose and muscle tissues. Backhed et al. (2004) demonstrated that colonizing germ-free mice with gut microbiota leads to a drop in the intestinal expression of ANGPTL4. Several other proteins/systems (such as the endocannabinoid system and the tight junction protein zona occludin-1) are influenced by gut colonization and are changed upon dietary modulation of gut microbiota composition.

**Figure 1** | Proposed mechanism whereby alteration of the bowel flora with a high-fat low-fiber diet alters the microbiota leading to metabolic endotoxemia with increased lipid storage and decreased insulin sensitivity. Adapted with permission from Cani and Delzenne (2009).
which are implicated in the control of inflammation, gut barrier function, gut motility, nutrient oxidation, and storage (Delzenne and Cani, 2011).

Recent data suggests that SCFAs act not only as an energy substrate for the host, but also as a signaling molecule. SCFAs act as ligands for at least two G-protein-coupled receptors, GPR41 and GPR43 (Le Poul et al., 2003). Samuel et al. (2008) have demonstrated that modulation of the intestinal microbiota by using prebiotics in obese mice acts favorably on the intestinal barrier function leading to a decrease of plasma LPS.

Table 1 | Major Bacteria and Archaea phyla and genera found in the human gut microbiota. Reproduced with permission from DiBaise et al. (2008).

<table>
<thead>
<tr>
<th>Phyla</th>
<th>Representative genera</th>
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<tbody>
<tr>
<td>Bacteria</td>
<td>Ruminococcus</td>
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<td>Clostridium</td>
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<td></td>
<td>Peptostreptococcus</td>
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<td>Lactobacillus</td>
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<td>Enterococcus</td>
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<td>Bacteroidetes</td>
<td>Bacteroides</td>
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<td>Proteobacteria</td>
<td>Desulfovibrio</td>
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<td></td>
<td>Escherichia</td>
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<td></td>
<td>Helicobacter</td>
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<td>Verrucomicrobia</td>
<td>Bilobacterium</td>
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<tr>
<td>Actinobacteria</td>
<td>Synergistis</td>
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<tr>
<td>Cyanobacteria</td>
<td>Synergistis</td>
</tr>
<tr>
<td>Archeae</td>
<td>Methanobrevibacter</td>
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To assess the relative abundance of various types of gut bacteria in obese and lean mice, Ley et al. (2005) analyzed bacterial 16S rRNA gene sequences from the cecal microbiota of genetically obese (ob/ob) mice, their lean ob/+ and +/+ siblings, and their ob/+ mothers, all fed the same polysaccharide-rich diet. They found that the ob/ob mice had 50% fewer Bacteroidetes and correspondingly more Firmicutes than their lean littermates, a finding unrelated to differences in food consumption. Cani et al. (2007a, b) demonstrated that induced obesity in rodents markedly reduced the number of Bifidobacterium as well as Bacteroides-related bacteria. Ley et al. (2006) compared the distal gut microbiota of obese and lean human subjects and found that obese people had fewer Bacteroides and more Firmicutes than did lean control subjects. Interestingly, the authors observed that after weight loss (following a fat restricted or a carbohydrate restricted low-calorie diet), the ratio of Bacteroidetes to Firmicutes approached a lean type profile after 52 weeks of diet-induced weight loss (Ley et al., 2006). Collado et al. (2008) observed significant differences in gut microbiota composition according to the body weight during pregnancy. Interestingly, they found significantly higher numbers of Bacteroides group and of S. aureus in the overweight state compared with normal-weight women, and they established a positive correlation between the number of Bacteroides on the one hand, and the weight and BMI (before and over pregnancy), on the other hand. The Bifidobacterium group was present in higher numbers in normal weight than in overweight women and also in women with lower weight gain over pregnancy. In addition, these researchers demonstrated that overweight mothers give birth to neonates that have a decreased number of Bifidobacteria (Collado et al., 2010). The infants’ fecal microbial composition was related to the weight and weight gain of their mothers during pregnancy. This finding may be related to the effect of colonization at birth and/or inheritable obesogenic microbiota.

A recent paper has shown for the first time in humans that differences in the gut microbiota may precede overweight development. Kalliomaki et al. (2008) have shown that Bifidobacterium spp. number was higher in children who exhibited a normal weight during pregnancy. In addition, these researchers demonstrated that overweight mothers give birth to neonates that have a decreased number of Bifidobacterium (Collado et al., 2010). The infants’ fecal microbial composition was related to the weight and weight gain of their mothers during pregnancy. This finding may be related to the effect of colonization at birth and/or inheritable obesogenic microbiota.

Gastric-bypass surgery has been shown to be very effective in reversing insulin resistance (Mingrone et al., 2012; Schauer et al., 2012). This effect has been observed to occur early after surgery and before the patients have had a significant loss of weight. It has been postulated that the surgically altered anatomy and flow of nutrients results in alteration of the microbiome which may explain this finding (Cani and Delzenne, 2011). Li et al. (2011) performed bacterial profiling to determine the effect of Roux-en-Y gastric bypass in a non-obese rat model. At 2 weeks post-surgery they noted substantial shifts of the main gut phyla toward higher concentrations of Proteobacteria (52-fold). Lower concentrations of Firmicutes (4.5-fold) and Bacteroidetes (twofold) in comparison with sham-operated rats were also found.
EFFECT OF PREBIOTICS, PROBIOTICS, AND SYNBIOtICS ON WEIGHT LOSS AND INSULIN RESISTANCE

The best non-surgical strategy for reversing obesity in the popula-
tion may be to promote small but long-term changes in diet and physical activity that take advantage of our biological systems for regulating energy balance and preventing positive energy balance. Although clearly no substitute for proper diet and exercise, manipulation of the gut microbiota may represent a novel approach for treating obesity, one that has few adverse effects.

A probiotic is defined as “a preparation of or a product con-
taining viable, defined microorganisms in sufficient numbers, which alter the microflora of the host and by that exert beneficial health effects in this host” (Schrezenmeir and de Vrese, 2001). The term prebiotic was introduced by Gibson and Roberfroid (1995), who defined prebiotics as “a non-digestible food ingredient that benef-
ifically affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon.” The term synbiotic is used when a product contains both probiotics and prebiotics.

PREBIOTICS

Prebiotics are non-digestible oligosaccharides that act as “fertilizers” of the colonic microbiota, enhancing the growth of beneficial commensal organisms (e.g., Bifidobacterium and Lactobacillus species; Schrezenmeir and de Vrese, 2001). Fructo-
oligosaccharides are prebiotic agents that are fermented by a number of colonic bacteria to modulate the growth of benefi-
cial bacteria (Roberfroid, 2002). The number of Bifidobacteria has been shown to increase in the presence of inulin-type fruc-
tans with prebiotic properties. This increase occurs within a few days, but rapidly disappears upon withdrawal of the prebiotic compounds (after 1 week; Gibson and Roberfroid, 1995). The extent of increase in the number of Bifidobacteria is also depend-
ton their initial number in the gut. Breast-milk contains oligosaccharides with prebiotic properties that contribute to the increase in the number of Bifidobacteria after birth (Bode, 2009; Chichilniski et al., 2011).

Dietary fructans, which are present in various fruits and vegetables and added to food products, are used as an energy substrate by bacteria, including Bifidobacterium spp., that express β-fructofuranosidase, which promotes their development in the gut. A remarkable increase has been observed in the number of Bifidobacterium spp. in mice with diet-induced or geneti-
cally determined obesity that were supplemented with inulin-type fructans (Cani et al., 2007b). Interestingly, the number of Bifi-
dobacteria was inversely correlated with the development of fat mass, glucose intolerance, and LPS level (Cani et al., 2007b).

Inulin-type fructans increased the number of endocrine L cells in the jejunum and in the colon of rodents, and promoted the production and release of the active forms of GLP-1 and GLP-
2 in the portal vein (Cani et al., 2004). GLP-1 participates in prebiotic-driven decreases in appetite, fat mass and hepatic insulin resistance, whereas GLP-2, as mentioned before, contributes to the reduced permeability of the intestinal wall and endoto-
remia that are associated with obesity (Cani et al., 2006, 2009b). Interestingly, a 2-week treatment with inulin-type fructans (16 g per day) in healthy volunteers increased the postprandial release of gut peptides (namely GLP-1 and gastric inhibitory peptide; Cani et al., 2009a).

Cani and colleagues demonstrated that in rats fed a standard or high-fat diet, the addition of oligofructose to the diet reduced energy intake and consumption and protected against weight gain and fat-mass development, effects shown to be mediated by the modulation of endogenous gut peptides involved in appetite and weight regulation (Cani et al., 2004, 2005; Deltze et al., 2005). The authors also reported that oligofructose increased the gut bifidobacterial content of high-fat diet-fed mice and that endoto-
remia significantly and negatively correlated with Bifidobacterium species (Cani et al., 2007b). Parnell and Reimer (2009) random-
ized 48 otherwise healthy adults with a BMI > 25 kg/m² to receive 21 g oligofructose or a placebo (maltodextrin) for 12 weeks. There was a reduction in body weight of 1.05 ± 0.43 kg with oligofructose supplementation, whereas the control group expe-
rienced an increase in body weight of 0.45 ± 0.31 kg over 12 weeks (p = 0.01). A lower area under the curve (AUC) for ghrelin (p = 0.004) and a higher AUC for peptide YY (PYY) with oligofruct-
tose (p = 0.03) coincided with a reduction in self-reported caloric intake (p < 0.05). Glucose decreased in the oligofructose group and increased in the control group between initial and final tests (p = 0.05).

PROBIOTICS AND SYNBIOtICS

Probiotics have generated considerable interest in recent years because studies investigating their use in a variety of clinical conditions have yielded encouraging results (Floch and Montrose, 2005). Probiotics have been demonstrated to be beneficial in recrとうing enterocolitis, in adults and children with infec-
tive diarrhea and for the prevention and treatment of antibiotic associated diarrhea (Floch and Montrose, 2005; Hempel et al., 2012). Probiotics belonging to a number of genera including Lactobacillus, Bifidobacterium, Saccharomyces, Streptococcus, and Enterococcus (Enterococcus faecium SF68) have been used in these studies. The genus Lactobacillus comprises more than 90 species, the most commonly used include L. acidophilus, L. rhamnosus, L. casei, L. bulgaricus, L. plantarum, and L. reuteri. Most of the clinical trials reported to date have used blends of various probi-
otic genera, primarily Lactobacillus spp. in combination with other probiotics. The study of probiotics is complicated by the fact that the efficacy of these agents may be strain specific and the results from one probiotic (or combination) cannot be extrapolated to another. Furthermore, the results of clinical trials may depend on the potency (concentration) and the measures used to ensure the “bio-availability” of the bacteria. While probiotics are regarded as safe, few probiotics have received the FDA GRAS (Generally Recognized as Safe) approval. Probiotics should be used with caution in critically ill and immunocompromised patients. In rare cases, probiotics have been associated with serious adverse effects in these patients including fungemia (Saccharomyces boulardii) and bacterial sepsis (Riquelme et al., 2003; Land et al., 2005). While clinical data supports the use of prebiotics and experimental data supports the use of probiotics, few studies have investigated the role of probiotics in patients with diabetes, insulin resistance, and obesity.
Luoto et al. (2010) randomized 159 women to receive *Lactobacillus rhamnosus* GG or placebo 4 weeks before expected delivery with the intervention extending for 4 months postpartum. Anthropometric measurements of the children were followed for 10 years. The perinatal probiotic intervention appeared to moderate the initial phase of excessive weight gain (up to 48 months), especially among children who later became overweight (Kadooka et al., 2010). Conducted a multicenter, double-blind, randomized, placebo-controlled trial in which subjects (n = 87) with an increased BMI (24.2–30.7 kg/m²) and abdominal visceral fat area (81.2–178.5 cm²) were randomly assigned to receive either fermented milk containing *Lactobacillus gasseri* SBT2055 (active FM, n = 43) or fermented milk without LG2055 (control FM, n = 44) for 12 weeks. Abdominal fat area was determined by computed tomography. In the active FM group, abdominal visceral and subcutaneous fat areas significantly (p < 0.01) decreased from baseline by an average of 4.6 and 3.3% respectively. Body weight and other measures also decreased significantly while, none of these parameters changed significantly in the control group.

**REFERENCES**


