REVIEW

The Role of the Gut Microbiome in the Pathogenesis and Treatment of Obesity

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INTRODUCTION: OBESITY AND THE MICROBIOTA

The human body is colonized by microorganisms that number in the hundreds of trillions (10^{14}), essentially outnumbering the total number of eukaryotic cells (60 trillion) that make up a human.[1] These organisms can be found all over the body and throughout the gastrointestinal (GI) system from the mouth to the rectum, with the highest concentration of organisms localized to the colon (10^{11}-10^{12}). Over time, humans and these microorganisms have found a method to live in symbiosis—in essence helping one another survive. This community of microorganisms forms an ecosystem that exists in and on every human is broadly termed *the microbiome*. There exists a skin microbiome, urogenital microbiome, and a gastrointestinal microbiome (composed of bacteria, archaea, microeukaryotes, fungi, and viruses). This review will focus primarily on the gut microbiome and its relationship to obesity.

CLASSIFICATION

The gut microbiome has been classified into close to 1000 different species.[1] To date, only about 29 to 52 bacterial phyla have been identified. Of these, 10 have been identified to colonize the colon, with 2 phyla predominating (>90%)[2]—the Bacteroidetes (eg, genera Bacteroides and Prevotella) and Firmicutes (eg, genera Clostridium, Ruminococcus, Enterococcus, and Lactobacillus). Other phyla that have been identified include Actinobacteria (eg, genus *Bifidobacterium*, a strict anaerobic bacteria has been implicated in early colonization of newborn babies as early as day 3 and has been thought to play a key role in stabilizing the microbiome during the weaning phase of life[3]); Proteobacteria (eg, genus *Helicobacter* and *Escherichia*); Fusobacteria; Spirochaetae; and Verrucomicrobia.[2]

The number of organisms that constitute the human microbiome outnumber the host's eukaryotic cells by a factor of 10.[4] Additionally, the genome of the microbiome is thought to contain close to 150 times the number of genes in humans.[1] This gives the gut microbiome the symbolic status of an organ consisting of prokaryotic cells working in conjunction with its human host's eukaryotic cells to maintain good health.[5]

FUNCTIONS OF THE GUT MICROBIOME

The microbiome presumably carries out specific functions unable to be performed by the host. In this issue of *Global Advances in Health and Medicine*, Dr Gregory Plotnikoff discusses in detail the various functions of the gut microbiome. Some relevant functions that are pertinent to our discussion of its impact upon the development of obesity include:

1. Synthesis of vitamins and cofactors[6]
2. Digestion and breakdown of complex polysaccharides to short-chain fatty acids (SCFA; eg, propionate, butyrate, acetate, the main nutritional substrate for colonic epithelial cells)[7,8]
3. Regulation of gastrointestinal motility and vascularization of the GI tract.[9-12]
4. Influence fatty acid composition of the retina and lens of the eye[12]
5. Affect bone density[12]
6. Development of adaptive immunity.[13]

EUBIOSIS: THE GUT MICROBIOME IN HEALTH

This issue of *Global Advances in Health and Medicine* is dedicated to the microbiome and its impact upon health and disease. It has been said that "a healthy microbiota is defined by high diversity and an ability to resist change under physiological stress. In contrast, microbiota associated with disease (ie, dysbiosis) is defined by lower species diversity, fewer beneficial microbes, and/or the presence of pathobionts."[2]

The intrauterine environment was previously hypothesized to be sterile; however, recent evidence suggests that this is not the case. Traces of microorganisms, including DNA and cell structures from intestinal bacteria, have been detected in the placenta, amniotic fluid, and fetal membranes.[14-16] It has also been proposed that the colonization of the gut appears to develop according to a prescheduled plan already in place once we are born. Additionally, it is presumably affected by a variety of extrinsic factors such as mode of delivery of the baby, exposure to antimicrobials, and type of nutrition (breast milk, cow milk, formula, and/or other dietary exposures), environment, host genetics, maternal diet, and other extrinsic factors.[2]

Despite the above factors that influence the overall microbiota, further studies with metagenomics suggest that the overall human gut microbiome is predominantly made up of three clusters/subtypes which have been dubbed "enterotypes" and include Bacteroides (enterotype 1), Prevotella (enterotype 2) and Ruminococcus (enterotype 3).[17] These enterotypes were shown to be independent of gender, ethnicity, age, weight, or body mass index (BMI) but do appear to be strongly associated with long-term dietary habits, especially high protein, red meat, and animal fat, which affects enterotype 1-Bacteroides, while consumption of a high-

carbohydrate diet or vegetarian diet affects enterotype 2-Prevotella,[2,5,18] and consumption of a diet high in resistant starch has been identified as affecting enterotype 3-Ruminococcus.[19,20]

DYSBIOSIS: THE GUT MICROBIOME IN DYSFUNCTION

Perturbations to the intestinal ecosystem typically occur on a nearly daily basis given that this is the main portal for a large percentage of the daily intake into our bodies. These perturbations can come about in a variety of ways including nutrition/diet, antibiotics, and exposure to environmental factors, including pathogens and chemicals. It has been hypothesized that short-term insults to the microbiome do not usually result in long lasting effects, as the microbiome has been described as plastic and thus able to adapt to short-term perturbations. On the other hand, prolonged exposure to these insults appear to induce changes in the microbiota, which result in a variety of effects on the host, some deleterious and others not yet understood.

Studies have hypothesized that a dysbiotic (as opposed to a eubiotic) microbiome predisposes the host to a variety of gastrointestinal disorders (Table 1). Imbalances in the gut microbiome have been described for a number of disorders such as irritable bowel syndrome (IBS), functional bowel disorders (FBD),[21,22] inflammatory bowel disease (IBD, eg, ulcerative colitis and Crohn's disease,[23,24] and colorectal cancer,[25,26] as well as global/systemic illnesses such as allergic diseases,[27] non-alcoholic steatohepatitis (NASH),[28, 29] arteriosclerotic diseases,[30,31] and metabolic syndromes, most notably obesity,[32-34] and diabetes,[35,36] which are the subject of this review.

Table 1 Disorders Associated With an Altered Gut Microbiome

Gastrointestinal
Colorectal Cancer
FMF
Gallstones
Gastric cancer and lymphoma
Hepatic encephalopathy
Inflammatory bowel disease
Irritable bowel syndrome
Recurrent *C difficile* infection
Non-gastrointestinal
Anxiety
Arthritis
Asthma
Autism
Autoimmune disorders
Cardiovascular
Chronic fatigue
Chronic kidney disease
Depression
Diabetes
Eczema
Fatty liver
Fibromyalgia
Hypercholesterolemia
Idiopathic thrombocytopenic purpura
Metabolic syndrome
Mood disorders
Multiple sclerosis
Myoclonus dystonia
Obesity
Oxalic kidney stones
Parkinson's disease

DIET AND THE MICROBIOME

The effect of different diets on the gut microbiome has been studied extensively in mouse models. Fewer studies have been done in humans to evaluate the effects of various dietary components on the gut microbiome. The findings in animal and human studies are summarized in Table 2, adapted from Chan et al.[2]

The above studies lend credence to the fact that dietary habits have an immediate and lasting effect on the microbiome, and further studies are required to address what these effects are long term and how we can intervene to lead to better health outcomes.

OBESITY

Several changes have been reported in regards to the microbiome in overweight and obese individuals. These changes have been noted not only in the composition, but also in the diversity of species and metabolic functions of the microbiome.[53] Composition changes previously reported include increase in Firmicutes; decrease in Bacteroidetes, Bacteroides, Bifidobacterium, and Akkermansia muciniphila; and increase/decrease in Desulfovibrionaceae, Ruminococcaceae, and Rikenellaceae, while changes in function include an increase in production of enzymes involved in membrane transport and processing of complex polysaccharides, downregulation of genes involved in transcription processes, synthesis of cofactors, vitamins, and metabolism of nucleotides. These changes have yet to be causally linked in humans. The majority of human studies done have reported some change in the microbiome of subjects with obesity as compared to lean subjects. Select studies are summarized in Table 3[54] and demonstrates the results of a few studies that have evaluated the intestinal microbiome in obese subjects.

RELATIONSHIP OF OBESITY AND THE MICROBIOME

Ley et al were the first to provide a strong link between obesity and the gut microbiome. Their group carried out the study in leptin-deficient mice (homozygous for an aberrant leptin gene ob/ob, which caused weight gain).[65] Using 16S ribosomal RNA (rRNA) gene sequencing, they found that the bacterial components of the ceca of the ob/ob mice were different from lean wild type mice (+/+), or heterozygous (ob/+) mice. They also reported a higher representation of the Firmicutes and fewer Bacteroidetes.

Metagenomic analysis of the same microbial communities revealed an upregulation in genes involved in energy extraction from food in the ob/ob population when compared to their lean counterparts. Turnbaugh

Table 2 Summary of Diet-induced Changes in the Human Gut Microbiome

Diet Class	Specific diet	N	Source of microbes	Bacterial population altered	Method	Host effect	Reference no.
FAT	High fat shortening and high sugar	1 man 15 mice	Feces	Increase clostridium innocuum, Catenibacterium mitsuokai, Enterococcus spp Decrease Bacteroides spp	Multiplex amplicon pyrosequencing	Increased obesity when transplanted into mice	37
	Fish oil-supplemented infant formula vs cows milk	65	Feces	Consumption of cows milk and infant formula resulted in different microbial patterns; fish oil supplementation affects the microbial pattern of cows milk group only	DGGE	Not examined	38
CHO	Increased CHO-rich foods	34	Mouth of skeletons	Cariogenic dominant	454 pyrosequencing	Increased dental disease	39
	Diets high in resistant starch compared to non starch polysaccharides and low CHO	14	Feces	Increase Firmicutes, Eubacterium rectale, Roseburia, Ruminococcus bromii	qPCR	Increased digestibility of starch	40
	Inulin and Brussels sprouts	1 man 48 rats	Feces	Increased Bifidobacterium and Lactobacillus	TTGE	Increased cecal butyrate and acetate when transplanted into rats	41
	Kiwi fruit	10	Feces	Increased Bifidobacterium and Bacteroides-Prevotella-Porphyromonas group	qPCR	Increased microbial glycosidases and SCFAs	42
	Sucrose-free chocolates+maltitol+bulking agents (polydextrose and resistant starch)	40	Feces	Increased Bifidobacterium and Lactobacillus	FISH	Increased SCFAs propionate and butyrate	43
	Bread enriched with arabinoxylan-oligosaccharides	40	Feces	Increased Bifidobacterium and Lactobacillus	FISH	Increased butyrate Decreased isovalerate and fatty acids associated with protein fermentation	44
Protein	Vegetarian	29	Feces	Increase in overall bacterial DNA, Decreased amount and diversity of clostridium cluster IV	DGGE, qPCR	Not examined	45
	High red meat diet	24 mice	Feces	Increased Bacteroides spp	qPCR	No functional changes observed when transplanted into mouse	46
	Gluten-free diet	10	Feces	Increased Enterobacteriaceae Decreased Bifidobacterium and Lactobacillus	Not mentioned	Decrease TNF alpha, IFN-gamma, IL-8, and IL-10 in peripheral blood mononuclear cells	47

PJ et al were able to demonstrate that transplantation of the cecal contents of ob/ob vs ob/+ vs +/+ into germ-free mice (GFM) led to more weight gain in the GFM receiving the obese mice microbiota than recipients of the lean mice microbiota over a 2-week period, despite having equivalent food intake.[32]

Additional studies have been done looking at the microbiome in obese humans; however, the results have been somewhat difficult to translate into clinical practice. Most of the recent studies used varying methods to detect the microbiome in question, and this may in turn account for why the majority of studies are somewhat discordant in their findings.

Depending on the outcome in question, qualitative vs quantitative outcome, this usually drives the diagnostic tool applied such as culture based methods; fluorescence *in situ* hybridization (FISH); quantitative polymerase chain reaction (qPCR); DNA fingerprinting

Table 2 Summary of Diet-induced Changes in the Human Gut Microbiome *(cont)*

Diet Class	Specific diet	N	Source of microbes	Bacterial population altered	Method	Host effect	Reference no.
Breastfeeding compared to formula feeding		Not reported	Feces	Increased Bacteroidetes Decreased Firmicutes and Verrucomicrobia	454 pyrosequencing	Gene networks (inflammation, cell adhesion, barrier function, histamine, etc) differentially expressed in exfoliated intestinal epithelial cells	48
		207	Mouth	Increased lactobacillus spp	Culturing, qPCR	Inhibited growth of the carcinogenic Streptococcus spp	49
Other	Ready to use therapeutic food composed of peanut paste, sugar, vegetable oil, and milk fortified with vitamins and minerals	634	Feces of Malawian twin pairs over first 3 years of life	Decrease Actinobacteria in kwashiorkor twin compared to healthy twin	Multiplex shotgun sequencing	Severe acute malnutrition caused when kwashiorkor microbiota transplanted into mouse	50
	3 cups of coffee daily for 3 weeks	16	Feces	Increased Bifidobacterium spp.	DGGE, FISH	Increased metabolic activity of Bifidobacteria spp.	51
	Dark chocolate	30	Urine	Not examined	H NMR, MS analysis	Different energy profiles, hormonal metabolism and gut microbial activity	52

Abbreviations: CHO, carbohydrate; DGGE, denaturing gradient gel electrophoresis; FISH, fluorescence in situ hybridization; H NMR, proton nuclear magnetic resonance; MS, mass spectrometry; qPCR, quantitative polymerase chain reaction; TTGE, temporal temperature gradient electrophoresis.

Table reproduced with permission from Chan YK, Estaki M, Gibson DL. Clinical consequences of diet-induced dysbiosis. Ann Nutr Metab. 2013;63(suppl 2):28-40.[2]

techniques, such as temperature gradient gel electrophoresis TGGE and denaturing gradient gel electrophoresis DGGE; DNA phylogenetic microarrays, such as MITChip, HITChip; next generation sequencing methods and Shotgun Sanger Sequencing method (shotgun, 454 pyrosequencing, Illumina, and SOLiD).[53] Most studies looking at the same outcomes were performed using different detection techniques, and this may account for their discordant findings. Angelakis et al performed a meta-analysis of the studies of the gut microbiome in obesity with respect to the 2 predominant phyla—Bacteroidetes and Firmicutes—as well as the preponderance of key bacteria including Bifidobacterium and lactobacillus (Figures 1-3).[33,37,56,57,59-62,64-71,72]

PATHOPHYSIOLOGIC MECHANISMS OF THE MICROBIOME AND OBESITY DEVELOPMENT

Data collected from a variety of mice models indicate that an altered microbiome can be found in a variety of metabolic diseases. Additionally, the disordered microbiome does not appear to be only a consequence of this disorder, but plays a key role in driving these metabolic disorders through the following potential mechanisms (Figure 4)[5,54,73-75]:

1. Microbiome and Metabolism

The gut microbiome is necessary for processing dietary polysaccharides that we cannot digest on our own (eg, oligosaccharides, resistant starch, fructose, oligosaccharides, disaccharides, monosaccharide's, and polyols—FODMAPs), and breaking them down to smaller molecules that the body can use.

The function of the microbiota to assist with energy extraction appears to be dependent, in large part, on the composition of the microbiome. Germ-free mice (GFM) have been shown to be extremely resistant to diet induced obesity, and insulin resistance, following introduction of a microbiome there are elevated levels of plasma glucose and short chain fatty acids (SCFAs) thought to induce hepatic lipogenesis, increased absorption of monosaccharides, and increased adiposity.[76,77]

It is hypothesized that the obese microbiome is set up to extract more calories from the daily intake when compared to the microbiome of lean counterparts.[32] Backhed et al[76] were able to demonstrate that germ-free mice consumed more calories to maintain the same weight as non–germ-free mice, suggesting some lower tendency to produce body fat.

There are a few pathways that have been studied

Table 3 Human Gut Microbiome Analyses in Obesity

Author, reference no.	No.	Body weight	Diet	Method	Microbiome findings
Ley et al, 55	14	12 obese; 2 lean	CHO reduced diet vs fat reduced diet	16S rRNA by Sanger; feces	Increase in Bacteroidetes sequences over time, no difference between diets
Turnbaugh et al, 33	154 31MZ 23DZ 46 mums	6 pairs of obese twins 1 pair discordant (lean/overweight)	N/A	16S rRNA by Sanger and 454 pyrose-quencing; metqage-nomics; feces	Obesity microbiome associated with overall reduced diversity, decrease in Bacteroides, with upregulation of energy harvesting genes of the Actinobacteria and Firmicutes
Schwiertz et al, 56	98	33 obese 35 overweight 30 lean	N/A	qPCR for Bacteroidetes, Actinobacteria, Archea; feces	Higher levels of Bacteroidetes in obese and overweight subjects; higher Methanobrevibacter in lean subjects
Collado et al, 57	54	18 overweight 36 lean	Before and during pregnancy	FISH/flow cytometry and qPCR; feces	Higher levels of Bacteroidetes and S aureus in overweight subjects; positive correlation between Bacteroidetes levels and weight gain during pregnancy
Sotos et al, 58	8	8 overweight/obese	Followed as they lost weight	FISH; feces	Found that group with highest weight loss had higher reduction in Enterobacteriaceae and sulfate reducing bacteria. Also noted that Roseburia and Eubacterium were reduced in the group with less weight loss
Duncan et al, 59	47	33 obese 14 lean	Weight loss diet vs weight maintenance diet	FISH; feces	No significant difference in Bacteroidetes levels between groups. Reduced Roseburia and Eubacterium and increased Clostridium spp seen in subjects with reduced intake of dietary CHO
Kalliomaki et al, 60	49	25 obese and overweight 24 lean	N/A	qRT-PCR and FISH/ flow cytometry; feces	Higher levels of Bifidobacteria and lower levels of S aureus in lean subjects at age 7
Santacruz et al, 61	36	36 overweight	10 weeks of Calorie reduced diet and increased physical activity	qPCR; feces	Increased levels of Bacteroidetes, and Lactobacillus spp with increased weight loss, while Bacteroides fragilis increase was correlated to CHO intake
Nadal et al, 62	39	39 overweight/obese subjects	10 weeks of calorie restricted diet and increased physical activity	qPCR; feces	Increased Bacteroidetes and Provotella with increased weight loss. Decrease in *Clostridium histolytica*, *C coccoides*, and *E rectale* with weight gain
Sabate et al, 63	177	137 obese 40 lean	Gastric bypass for obese participants	Glucose-hydrogen breath test for H_2 and liver biopsy	Small intestinal bacterial overgrowth is more common in obese vs lean subjects
Zhang et al, 64	9	3 post gastric bypass 3 obese 3 lean	N/A	Sanger and 454 sequencin, qPCR; feces	Firmicutes more abundant in lean subjects, lowest after gastric bypass, Gama proteobac-teria and Verrucomicrobia enriched after gastric bypass; higher archea in obese sub-jects, communities of obese and post gastric bypass subject more similar than lean subjects

Abbreviations: CHO, carbohydrate; DZ, Dizygotic; FISH, fluorescent in-situ hybridization; MZ, monozygotic.

Table reproduced with permission from Ley RE. Obesity and the human microbiome. Curr Opin Gastroenterol. 2010,26(1):5-11.[54]

and show that the gut microbiome may have a significant role to play in regulation of these pathways most of them have been in murine models and will need validation in large prospective human studies. One such pathway was studied by Backhed et al,[76] who were able to demonstrate that the gut microbiota is responsible for regulation of Angiopoietin-like Protein 4 (Angptl4), another alias is Fasting-Induced Adipose Factor (Fiaf), its an important regulator of fatty acid oxidation in muscle and adipose tissue[78] Angptl4 appears to be a circulating antagonist of lipoprotein lipase (LPL), found in the liver, adipose tissue, and the intestinal tract[79]—in differentiated gut epithelial cells,[77] suppression of Angptl4 leads to increased LPL activity and in the adipocyte will lead to increased uptake of free FFA and triglyceride deposition in adipose tissues. Germ-free mice lacking Angptl4 have been shown not to be immune to diet induced obesity.[76] Human studies looking at the ANGPTL4 gene have shown that functional variants of the gene appear more prevalent in people with low triglyceride levels,[80] and also plasma levels correlated with fasting levels of fatty acid and adipose tissue lipolysis.[81]

Another plausible pathway, which has been studied, is the Adenosine Monophosphate AMP-activated protein kinase (AMPK) – which is an enzyme that is thought to act via modulation of cell energy state.[82] As

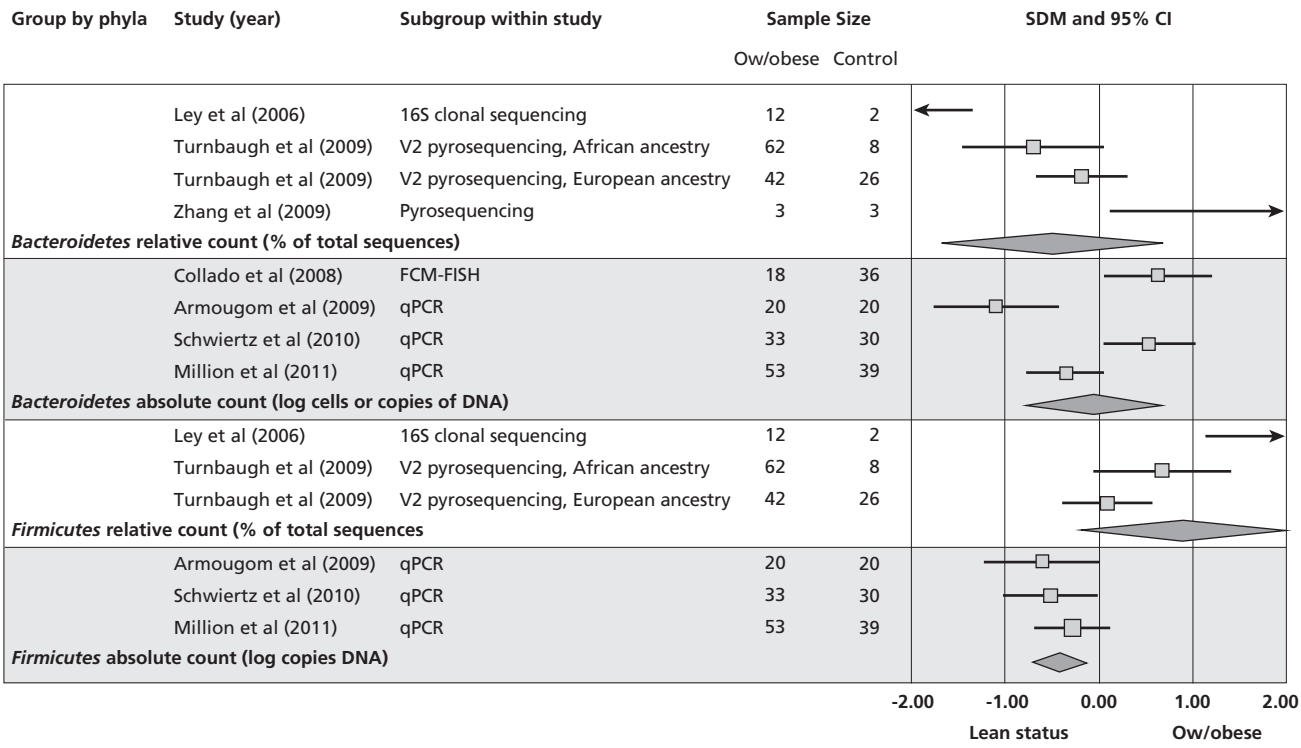

Figure 1 Meta-analysis of the obesity-associated gut microbiota alterations at the phylum level.

Meta-analysis was performed with the comprehensive meta-analysis software version 2 (Biostat, Englewood, New Jersey). Each line represents a comparison between an obese group (right) and a control group (left). The first reported alteration[65] was a decrease in the relative proportion of *Bacteroidetes* (percentage decrease) represented by a deviation of the square (standardized difference in the means) to the left. The size of the square represents the relative weight of each comparison (random model). The length of the horizontal line represents the 95% CI and the diamond represents the summarized effect. The presence of a square to the right and left of the midline means studies with conflicting results corresponding to a substantial heterogeneity (I2 >50%). Here, the only reproducible and significant alteration at the phylum level is the decrease in the absolute number of sequences of *Firmicutes* in obese subjects. Relative count of *Bacteroidetes* (n=4; SDM=–0.51; 95% CI=–1.7-0.67; *P*=.40 [I^2=81%]); absolute count of *Bacteroidetes* (n=4; SDM=–0.07; 95% CI=–0.78-0.65; *P*=.86 [I^2=85]); relative count of *Firmicutes* (n=3; SDM=0.88; 95% CI=–0.21-1.97; *P*=.11 [I^2=79%]); absolute count of *Firmicutes* (n=3; SDM=–0.43; 95% CI=–0.72 to –0.15; *P*=.003 [I^2=0%]).

Abbreviations: FCM, Flow cytometry; Ow, Overweight; qPCR, Quantitative PCR; SDM, standardized difference in the means.

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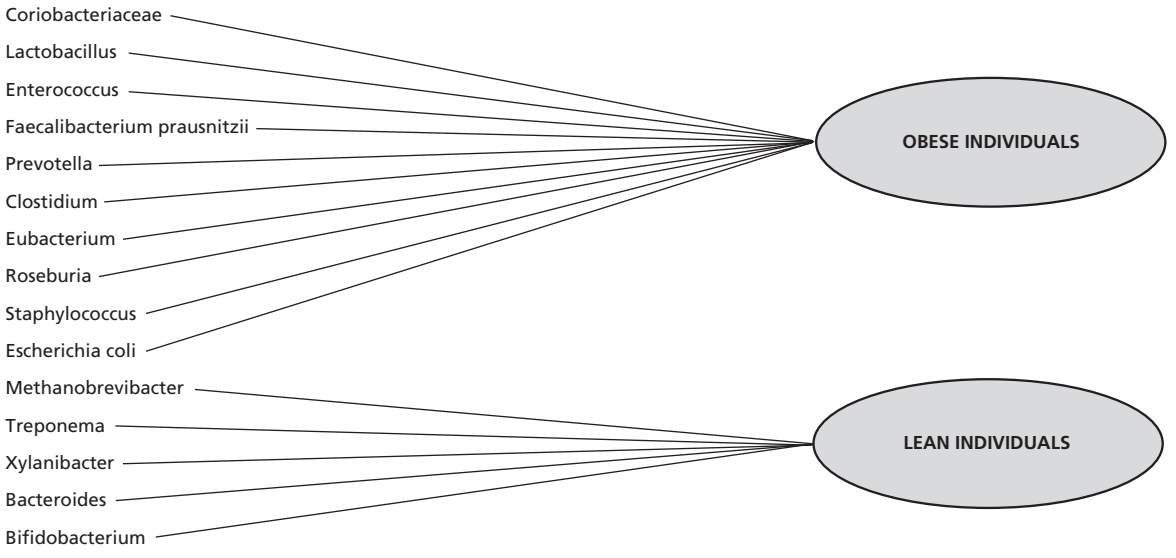

Figure 2 Population of bacteria found to increase in obese and lean individuals.

Summary of evidence for consistent changes in the gut microbiome of obese human subjects versus lean individuals.
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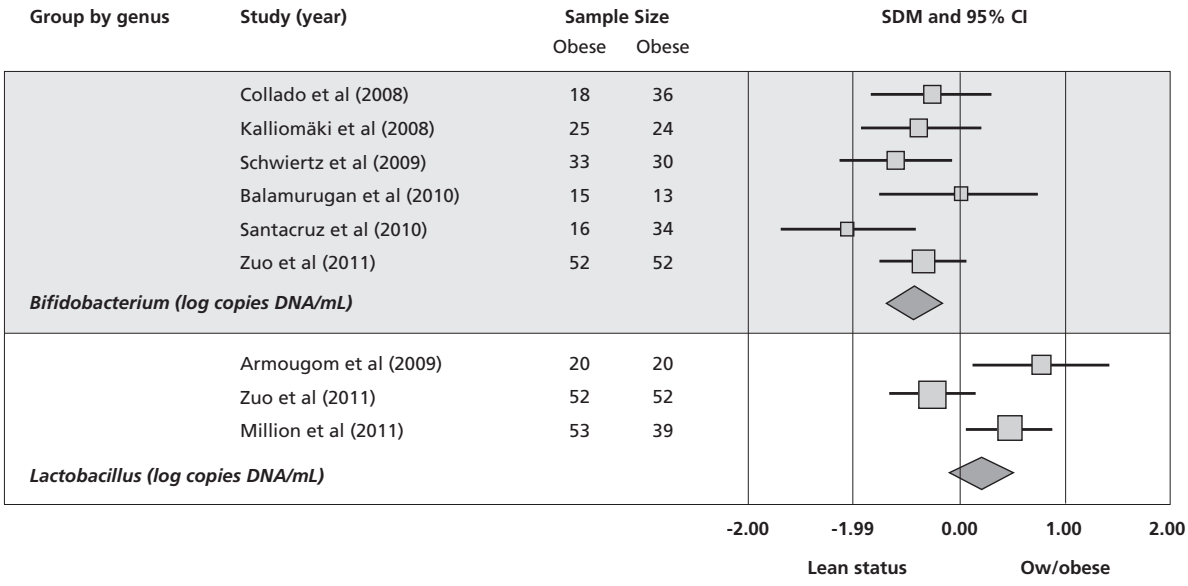

Figure 3 Meta-analysis of the obesity-associated gut microbiota alterations at the genus level for Bifidobacteria and Lactobacilli comparing the absolute number of sequences generated by genus-specific quantitative PCR.

For *Bifidobacteria*, a consistent difference was found by our meta-analysis between 159 obese subjects and 189 controls from six published studies showing that the digestive microbiota of the obese group was significantly depleted in *Bifidobacteria*. Low heterogeneity (I^2=17%) shows that this result is very robust. Additional tests have shown that there was no small studies bias (Egger's regression intercept test, *P*=.92; no change after Duval and Tweedie's trim and fill). For *Lactobacilli*, no consistent and significant summary effect was found comparing 127 obese subjects and 110 controls from three studies. *Bifidobacterium* spp (n=6; SDM=–0.45; 95% CI=–0.69 to –0.20; *P*<.001 [I^2=17%]); *Lactobacillus* spp (n=3; SDM=0.29; 95% CI=–0.31-0.90; *P*=.34 [I^2=80%]).

Abbreviations: Ow: overweight; SDM, atandardized difference in the means.

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mentioned before GFM are known to be resistant to diet induced obesity and this is due to an associated increased activity of phosphorylated AMPK levels,[76] which leads to oxidation of fatty acid in peripheral tissues and decreased glycogen in the liver.

An additional hypothesized pathway is via the use of some of the products of dietary breakdown of complex plant polysaccharides—(carried out by the microbiome as humans are not equipped to carry out these functions)—down to monosaccharides, and SCFAs—eg, butyrate, propionate, and acetate. The last two SCFAs have been shown to be ligands for a couple of G-protein-coupled receptors (GPCRs) Gpr41, and Gpr43.[83,84] A study done with mice models deficient in Gpr41 showed that it might help regulate the host energy balance through pathways that are the purview of the microbiome and their metabolic potential.[85] The metabolic pathways purported to be involved in the pathogenesis of obesity are illustrated in Figure 4. by Brown RK et al and published in the April 2012 issue of the journal *Nutrition in Clinical Practice*.[72]

2. Changes of the Microbiome in Obesity

Some studies have demonstrated a change in the normal distribution of the gut microbiota in obesity. Further, it seems that the obese microbiota is more attuned to energy extraction from the diet of the host. It has been difficult; however, replicating the microbiota changes found in obesity in humans, with multiple studies demonstrating varying results.

In mouse models, ingestion of a high-fat diet was noted to affect the composition of the microbiome in the following ways:

a. Populations of Lactobacilli/Enteroccocci and Bacteroides remained grossly unchanged; however, there was significant reduction in the population of *Clostridium coccoides* group and the *Bifidobacterium* spp.[86]
b. Decrease in populations of *Eubacterium rectale*/*C coccoides* group and also *Bifidobacterium* spp—when mice were fed a high-fat diet for 14 weeks.[87]
c. Populations of Ruminococcaceae and Rikenellaceae were notably increased in mice fed a high-fat diet.[88]
d. Populations of Akkermansia muciniphila were reduced at least 100- to 1000-fold in obese mice that were genetically induced or fed a high-fat diet. This spp has been associated with mucin degradation and has been found to colonize the mucin layer.[89] In other studies, it has also been shown to negatively correlate to body weight,[90] and type I[91] and type II[92] of diabetes that is present.
e. Decrease in the population of bifidobacteria in ceca of mice fed a high-fat diet, with associated higher rate of endotoxemia.[87]

3. Microbiome and Inflammation

The gut microbiota has been implicated in inducing low-grade chronic inflammation in the gut directly or increasing systemic loads of microbial ligands,

this effect has been most notable in subjects that consume a high-fat diet.[93,94]

A high-fat diet has been shown to increase the serum levels of lipopolysaccharide (LPS).[95] LPS is a component of the cell wall of gram-negative bacteria (eg, Bacteroidetes), which leads to the production of pro-inflammatory cytokines in different tissues of the body. Another study demonstrated that microbiome changes associated with obesity led to increased metabolic endotoxemia, inflammation, and other associated disorders through a GLP-2 mediated gut permeability mechanism.[87] Cani et al found that subcutaneous infusion of LPS can lead to weight gain and insulin resistance in mice without changes in dietary intake.[86]

Other investigators reported that mice who were deficient in the toll-like receptor (TLR) 5 (a transmembrane protein expressed in the intestinal mucosa, which functions to recognize bacterial flagellin, and is also involved in the innate immune system that helps defend against infection), had subsequent development of similar features of metabolic syndrome including hyperphagia, obesity, insulin resistance, and hepatic disease. TLR5 deficiency was also noted to affect the microbiome and transfer of the microbiome of the TLR5 deficient mice to healthy mice resulted in development of similar features of metabolic syndrome.[96]

Another mouse model demonstrated that a high fat diet induced obesity also led to changes in the microbiome and activation of TLR4 (acts by recognition of LPS) leading to subsequent gastrointestinal inflammation associated with the obese phenotype.[94] One murine study showed that mice lacking this TLR4 were as resistant to diet induced obesity and insulin resistance as germ free mice are.[97] Another study found that mice transplanted with an endotoxin-producing Enterobacter cloacae B29 strain that was isolated from an obese human led to development of obesity and disorders of glucose metabolism if the germ free mice were fed with a high fat diet, but not a normal diet.[98]

Other investigators described a link of low-grade inflammation due to endotoxemia to obesity through the gut microbiome via upregulation of the endocannabinoid (eCB) system tone.[99] Indeed, the eCB system appears to control gut permeability and adipose tissue physiology through an LPS-eCB system regulated cycle, and also plays a part in obesity and adiposity (Figure 5).[100]

THERAPEUTIC TARGETS

The question that begs to be answered is whether the intestinal microbiota is a plausible target for obesity treatment. The authors believe that the microbiome offers a logical approach that may be targeted to potentially ameliorate a variety of metabolic and gastrointestinal diseases.

In that regard, certain obligatory steps will be necessary to target the microbiota prior to resulting in reasonable, long-term results; these steps include

1. standardization of detection techniques for most microbiome studies;
2. better collaboration among researchers who are studying the microbiome;
3. larger, prospective studies on humans evaluating the effects of a variety of diets on the microbiota using similar detection techniques; and
4. identifying specific species within the microbiome that can be easily targeted and manipulated without resulting in harmful effects to the host.

Therapies directed at the microbiome in an attempt to correct some of the underlying problems associated with obesity, including metabolic syndrome (hyperphagia, insulin resistance, liver disease, chronic low grade inflammation), may likely be beneficial in the long run to reduce the burden of obesity on the healthcare system. Obesity and its sequelae were estimated to cost the healthcare sector $147 billion in 2008. Additionally, it was also estimated to cost an obese person $1429 per year more as compared to a normal weight individual for medical treatment.[102]

Some plausible targets for future therapies could look at manipulation of the pathways that have been associated with the microbiome and development of possible obesity and metabolic syndrome, including

1. Angptl4 pathway,
2. TLR4 and LPS pathway,
3. TLR5 and Bacterial flagellin pathway,
4. GPR41/Gpr43 and SCFAs pathways,
5. AMPK and oxidation of fatty acids, and
6. eCB and LPS pathway.

PREBIOTICS

Prebiotics are usually comprised of "nondigestible" elements that can serve as fuel for the microbiome and usually consist of oligosaccharides: fructooligosaccharides (FOS) and galactooligosaccharides (GOS). Some studies have shown that they can have beneficial health effects on the host by leading to changes in both activity and function of beneficial organisms in the microbiome,[103] and also by reducing adiposity.[104]

Inulin is a naturally derived prebiotic (from plants, and FOS compounds) and is one of the most studied prebiotics, it has been shown to specifically stimulate the growth of Bifidobacteria, decrease weight gain, improvement in glucose metabolism,[98-100] and also reduction of metabolic endotoxemia.[87,108] It has been found to increase *A muciniphila*, shown to negatively correlate with body weight,[89] and it also has an influence on production of gut hormones like glucagon like peptide-1 (GLP-1), peptide yy (PYY), and ghrelin, both in mice[108-111] and humans.[112-114]

A summary of a few prebiotic studies done in both mouse and humans are summarized here:

1. Prebiotic fiber supplemented in obese mice showed a reduction in the ratio of Firmicutes to Bacteroidetes in the microbiome and also reduction in hepatic *de novo* lipogenesis and a such help ameliorate the effects of nonalcoholic fatty liver disease (NAFLD).[109]

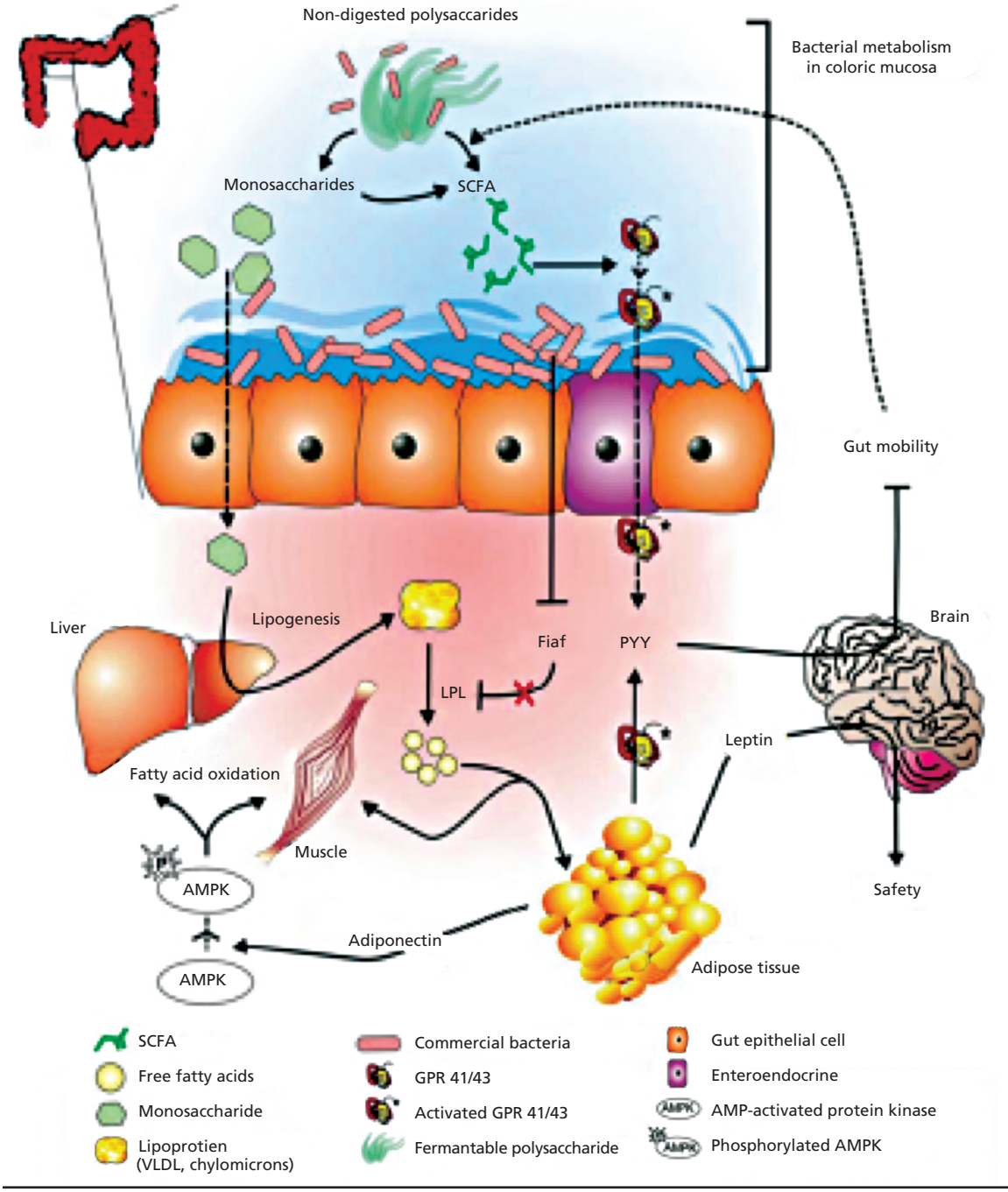

Figure 4 The gut microbiome has a regulatory function on host energy metabolism.

By breaking down nondigestible polysaccharides, gut microorganisms produce monosaccharides and short-chain fatty acids (SCFAs). SCFAs bind to GPR 41/43 receptors and stimulate peptide YY (PYY) production, which inhibits gut motility and allows gut microbes to digest more polysaccharides. Gut microbes also regulate energy metabolism by reducing the expression of fasting-induced adipocyte factor (Fiaf) from gut epithelial cells. Suppressed Fiaf release results in the degradation of lipoproteins and deposition of free fatty acids in adipose tissues. The adiposity in liver and skeletal muscles is also regulated by microorganisms through the changes of phosphorylated adenosine monophosphate-activated protein kinase (AMPK) levels.

Abbreviations: LPL, lipoprotein lipase; VLDL, very low density lipoprotein.

Reproduced with permission from Brown RK, Zehra-Esra I, Dae-Wook K, DiBaise JK. The Effects of gut microbes on nutrient absorption and energy regulation. Nutr Clin Pract. 2012;27:201-214.[72]

2. Supplementation with fungal chitin glucan and noted that there was a significant increase in bacteria related to Clostridium cluster XIVa, including Roseburia spp, accompanied by a decrease in fat production and weight gain.[115]
3. Supplementation with galactooligosaccharides (GOS) in healthy subjects for 12 weeks led to an increase in the Bifidobacterium spp and a decrease in Bacteroides.[116]
4. Ninety-seven adolescents were supplemented with a prebiotic, and noted that subjects that received the prebiotic for 1 year had a smaller increase in their body mass index and fat mass index when compared to controls.[117]

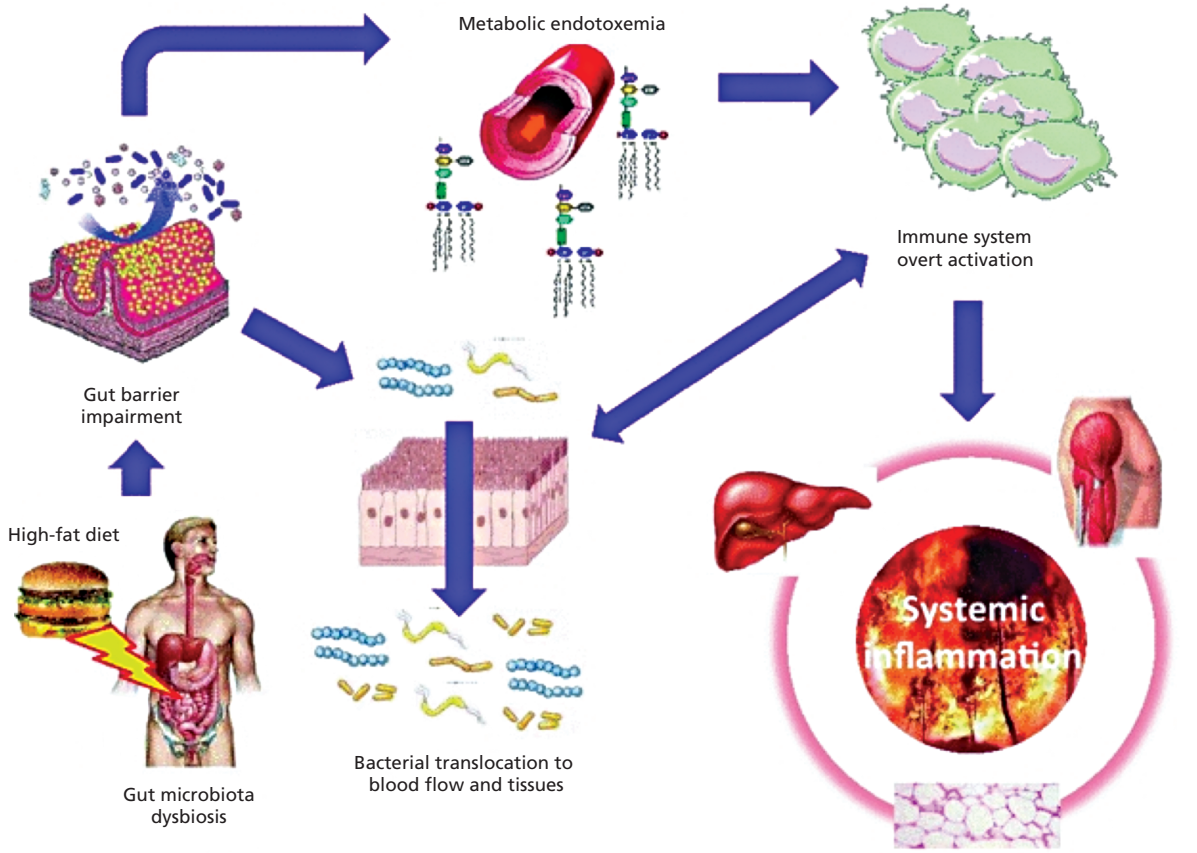

Figure 5 Gut microbiota dysbiosis and the role of inflammation in the metabolic impairments of obesity.

The origin of metabolic diseases is multifactorial but the impact of deleterious feeding habits is certainly the major factor responsible. This directly modifies intestinal ecology and we first showed that upon an increased intestinal permeability it led to an increased circulating concentration of LPS from Gram-negative bacteria of intestinal origin86,101 called metabolic endotoxemia. The inflammatory factors LPS and other bacterial fragments can translocate toward target tissues such as the blood, the liver, and the adipose depots or the arterial wall to interfere with cells from the immune system to generate the chronic low-grade inflammation required for the development of metabolic and cardiovascular diseases.

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5. Mice fed a high-fat diet and arabinoxylans derived from wheat were found to selectively restore the *Bacteroides/Prevotella* spp, *Roseburia* spp, and increase the number of *Bifidobacterium* spp, especially *Bifidobacterium animalis lactis*.[118]
6. Seven patients with NASH were given FOS vs placebo for 8 weeks and the prebiotic subjects were found to have reductions in LFTs and insulin levels that were significant.[119]
7. Inulin supplementation in diet of obese women was shown to increase Bifidobacterium spp and F praunsnitzii and decrease Bacteroides intestinalis, B vulgates, and Propionibacterium; there was only a slight non-significant decrease in fat mass associated with these changes in the microbiome.[120]

PROBIOTICS

Probiotics are living, commensal microbes that have a purported beneficial effect on the host. They are essentially thought to act by modulating the gut microbiome equilibrium, preventing translocation of bacteria, epithelial invasion, inhibiting adherence to mucosal surfaces of harmful bacteria, and stimulate the immunity of the host. The efficacy of probiotics remain highly debatable and studies to date have had varied results especially with regards to anti-obesity effects. A Cochrane review on use of probiotics in NAFLD, suggested the need for more large scale randomized controlled trials in NAFLD patients, but noted that some of the randomized trials did find it useful in this patient population.[121]

Some applications of probiotics with good evidence include prevention of antibiotic associated diarrhea,[122] treatment of necrotizing enterocolitis in premature infants,[123] enhancing urogenital health in females,[124] and preventing infection and allergies as related to pulmonary function.[125,126]

Delzenne et al reviewed the effects of prebiotics and probiotics on obesity and diabetes and is summarized in Table 4.[128]

SYMBIOTICS

Symbiotics are a combination of prebiotics and probiotics, based on the hypothesis that this will be a synergistic relationship and possibly lead to better outcomes when either is administered alone. In the table from Chan et al, mentioned previously in the probiotic section, there were a total of three studies

Table 4 Effects of Probiotics or Carbohydrates With Prebiotic Properties in Patients With Overweight or Diabetes Mellitus

Microbiota	Study design	No.	Duration	Treatment	Results
Probiotics					
Lactobacillus acidophilus NCFM[129]	Randomized, double-blind intervention	45 individuals with glucose intolerance and/or diabetes mellitus	4 weeks	Probiotic (1010 CFU/day) versus SiO2/lactose (placebo)	Systemic inflammation upon LPS challenge in both groups Probiotics prevented loss of insulin sensitivity observed in the placebo group
Lactobacillus gasseri SBT2055[130]	Randomized, multicenter, double-blind, placebo-controlled intervention	87 individuals with a BMI of 24.2–37.0 kg/m^2 and visceral adiposity	12 weeks	Fermented milk with probiotics (1011 CFU/day) or without probiotics (placebo)	Reduced body weight, BMI, waist and hip circumference, visceral and subcutaneous fat mass in the probiotic versus the placebo group
Prebiotics (nondigestible carbohydrates)					
Arabinoxylan[a131]	Randomized cross-over intervention	15 individuals with type 2 diabetes mellitus	5 weeks	Bread and muffins with 14% arabinoxylan (0% for placebo)	Reduced fasting glycemia, ↓ post-OGTT glycemia and insulinemia No difference in blood lipid level, fat mass and blood pressure
Arabinoxylan [132,133]	Single-blind, controlled, cross-over intervention	11 individuals with impaired glucose tolerance	6 weeks	15 g arabinoxylan supplied daily via bread and powder or isocaloric bread rolls without arabinoxylan (placebo)	Reduced fasting and post-LMCT glycemia and triglyceridemia Reduced total post-LMCT ghrelin No difference in leptin, adiponectin, insulin, resistin and FFA levels
Inulin-type fructans[b134]	Randomized, double-blind, placebo-controlled intervention	48 individuals with overweight or obesity	12 weeks	21 g per day oligofructose or maltodextrin (placebo)	Reduced body weight, caloric intake, GIP No difference in fasting glucose, insulin, ghrelin, GLP-1, PYY and leptin levels After MTT: reduced glycemia, insulin, AUC for ghrelin, AUC for PYY, AUC for leptin, but no difference in GIP level or AUC for GLP-1
Inulin-type fructans[135]	Randomized, double-blind, cross-over intervention	10 individuals with type 2 diabetes mellitus	4 weeks	20 g short-chain fructans or 20 g sucrose (placebo)	No difference in caloric intake, body weight, levels of glucose, insulin, HDL, LDL and total cholesterol, triglyceride, apolipoprotein A1 and B, lipoprotein(a), FFA, hepatic glucose production, insulin-stimulated glucose metabolism
Inulin-type fructans[136]	Randomized, double-blind, cross-over, placebo-controlled intervention	7 overweight patients with nonalcoholic steatohepatitis	8 weeks	16 g per day oligofructose or maltodextrine (placebo)	Reduced aspartate aminotransferase and fasting insulin levels No difference in levels of triglycerides, fasting glucose and cholesterol

[a] Arabinoxylans are complex carbohydrates found in the endosperm and the aleurone layer and in pericarp tissues of cereals. Their fermentation is associated with proliferation of *Bifidobacteria* and *Lactobacilli*. Arabinoxylans represent a new class of prebiotics that have a prebiotic index comparable to that of well-established prebiotics.[103]

[b] Inulin-type fructans are well-established prebiotics that can selectively stimulate the growth of *Bifidobacteria* and, in some cases, *Lactobacilli*, which markedly changes the composition of the gut microbiota. Most of the potential health benefits associated with their prebiotic effects were discovered and demonstrated using the same food ingredients and/or supplements.[126]

Abbreviations: AUC, area under curve; CFU, colony-forming unit; GIP, gastric inhibitory polypeptide; GLP1, glucagon-like peptide 1; LMCT, liquid meal challenge test; LPS, lipopolysaccharide; MTT, meal tolerance test; FFA, free fatty acids; OGTT, oral glucose tolerance test; PYY, peptide YY.
Reproduced with permission from Nathalie M. Delzenne, Audrey M. Neyrinck, Fredrik Bäckhed, Patrice D. Cani Targeting gut microbiota in obesity: effects of prebiotics and probiotics. Nature Reviews Endocrinology 7, 639-646 (November 2011).[127]

looking at symbiotics, which all had favorable outcomes.[2] One other study looking at symbiotics in a pediatric population[137] did report a significant increase in microbial counts when compared to participants that were given a placebo, although this was not compared to just a probiotic alone.

To date, there has not been any significant benefit reported over administering only probiotics or prebiotics as compared to symbiotics. This area merits further investigation.

ANTIBIOTICS

Antibiotics have been used for a variety of applications since they were first discovered to cure bacterial illnesses. One use of antibiotics has been to help livestock gain weight. Cho and colleagues demonstrated and the level of antibiotics present in meat and poultry is sufficient to cause weight gain in animal models of diet-induced obesity. It has been hypothesized that antibiotic use early in life may lead to disruptions in the microbiome, perhaps predisposing the host to the

development of obesity.[138] Antibiotic use has also been implicated in the pathophysiological development of IBS[139,140] and Crohn's disease. It has further been reported that antibiotic use during episodes of acute bacterial gastroenteritis may lead to long-term gastrointestinal sequelae[141]

It has also been hypothesized that eradication of *H pylori* in the industrialized nations might have some relationship to the obesity epidemic, through alterations in the serum/gastric ghrelin and gastric leptin levels.[142] Francois et al demonstrated that eradication of *H pylori* led to significant increases in circulating meal-associated leptin and ghrelin levels and BMI, providing some direct evidence that colonization with *H pylori* does in fact play a role in regulation of these gut hormones with consequent effects on the morphology of the body.[143] Large population-based dataset analyses; however, have not shown any association between *H pylori* and obesity to date. Thus, further prospective studies are required.

Despite this, there may be a role for the short-term use of intraluminal/poorly absorbed antibiotics or antimicrobial herbals in the management of obesity on the horizon.

SUMMARY AND CONCLUSIONS

In summary, the intestinal microbiota contributes to key vital functions for the host, including immunity and nutritional status. Prior studies have linked an altered microbiota to a number of metabolic, gastrointestinal, and systemic illnesses. In obesity, changes in the intestinal microbiota composition and function have also clearly been implicated in the control of inflammation, fat storage, and abnormal glucose response. Byproducts of the gut microbiota, such as short-chain fatty acids, also appear to modulate adiposity by producing hormones that regulate appetite, intestinal permeability and inflammation.

Prior studies have reported varying data regarding alterations in the composition of the gut microbiota in obesity. It is clear from the current literature; however, that specific changes in the microbiota composition do occur in overweight or obese individuals presumably leading to inflammation, and altered glucose and lipid homeostasis, thus predisposing to adiposity. Manipulation of the microbiota through diet and/or antibiotics may therefore promote weight loss by altering intestinal function and metabolism. Probiotics and prebiotics may also be of relevance of specific bacteria in obesity. Prebiotics have additionally been postulated to improve weight loss in obese subjects by modulation of gut peptides involved in the control of appetite and intestinal barrier function. There is a tremendous scope of research ongoing in this field, with exciting discoveries being made as new deep sequencing techniques emerge. We are hopeful that this research will subsequently translate into clinical practice for the treatment not only of obesity, but for a variety of metabolic disorders. This will likely only be achieved with increased collaboration among researchers and institutions, standardization of detection techniques, and large-scale, prospective trials.

REFERENCES

1. Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature 2010; 464:59-65.
2. Chan YK, Estaki M, Gibson DL. Clinical consequences of diet-induced dysbiosis. Ann Nutr Metab. 2013;63(suppl 2):28-40.
3. Gioia D, Aloisio I, Mazzola G, et al. Bifidobacteria: their impact on gut microbiota composition and their applications as probiotics in infants. Appl Microbiol Biotechnol. 2014;98:563-77.
4. Sekirov I, Russell SL, Antunes LC, et al. Gut microbiota in health and disease. Physiol Rev. 2010;90:859-904.
5. Fukuda S, Ohno H. Gut microbiome and metabolic diseases. Semin Immunopathol. 2013 Nov 6. http://www.ncbi.nlm.nih.gov/pubmed/24196453.
6. Resta SC. Effects of probiotics and commensals on intestinal epithelial physiology: implications for nutrient handling. J Physiol. 2009;587:4169-74.
7. Donohoe DR, Garge N, Zhang X, et al. The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. Cell Metab. 2011;13: 517-26.
8. Hamer HM, Jonkers D, Venema K, et al. Review article: the role of butyrate on colonic function. Alimentary Pharmacology & Therapeutics, 2008;27:104-19.
9. Anitha M, Vijay-Kumar M, Sitaraman SV, et al. Gut microbial products regulate murine gastrointestinal motility via Toll-like receptor 4 signaling. Gastroenterology. 2012;143:1004.
10. Husebye E, Hellstrom PM, Midtvedt T. Intestinal microflora stimulates myoelectric activity of rat small intestine by promoting cyclic initiation and aboral propagation of migrating myoelectric complex. Dig Dis Sci. 1994;39:946-56.
11. Husebye E, Hellstrom PM, Sundler F, et al. Influence of microbial species on small intestinal myoelectric activity and transit in germ-free rats. Am J Physiol Gastrointest Liver Physiol. 2001;280:G368-80.
12. Oresic M, Seppanen-Laakso T, Yetukuri L, et al. Gut microbiota affects lens and retinal lipid composition. Experimental Eye Research. 2009;89;604-7.
13. Lee YK, Mazmanian SK. Has the microbiota played a critical role in the evolution of the adaptive immune system? Science. 2010;330:1768-73.
14. Satokari R, Gr.nroos T, Laitinen K, et al. Bifidobacterium and lactobacillus DNA in the human placenta. Lett Appl Microbiol. 2009;48:8-12.
15. Rautava S, Collado MC, Salminen S, et al. Probiotics modulate host-microbe interaction in the placenta and fetal gut: a randomized, double-blind, placebo-controlled trial. Neonatology. 2012;102:178-84.
16. Jimenez E, Fernandez L, Marin ML, et al. Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. Curr Microbiol. 2005;51:270-4.
17. Arumugam M, Raes J, Pelletier E, et al. Enterotypes of the human gut microbiome. Nature. 2011;473:174-80.
18. Kurokawa K, Itoh T, Kuwahara T, et al. Comparative metagenomics revealed commonly enriched gene sets in human Gut microbiomes; DNA RESEARCH. 2007;14:169-81.
19. Walker AW, Ince J, Duncan SH, et al. Dominant and diet-responsive groups of bacteria within the human colonic microbiota. ISME J. 2011 Feb;5(2):220-30. Epub 2010 Aug 5.
20. Ze X1, Duncan SH, Louis P, et al. Ruminococcus bromii is a keystone species for the degradation of resistant starch in the human colon. ISME J. 2012 Aug;6(8):1535-43. Epub 2012 Feb 16.
21. Parkes GC, Brostoff J, Whelan K, et al. Gastrointestinal microbiota in irritable bowel syndrome: their role in its pathogenesis and treatment. Am J Gastroenterol. 2008;103:1557-67.
22. Spiller R, Garsed K. Postinfectious irritable bowel syndrome. Gastroenterology. 2009;136:1979-88.
23. Nell S, Suerbaum S, Josenhans C. The impact of the microbiota on the pathogenesis of IBD: lessons from mouse infection models. Nat Rev Microbiol. 2010; 8:564-77.
24. Sokol H, Pigneur B, Watterlot L, et al. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci USA. 2008;105:16731-6.
25. Arthur JC, Perez-Chanona E, Muhlbauer M, et al. Intestinal inflammation targets cancer-inducing activity of the microbiota. Science. 2012;338:120-3.
26. Scanlan PD, Shanahan F, Clune Y, et al. Culture independent analysis of the gut microbiota in colorectal cancer and polyposis. Environ Microbiol. 2008;10:789-98.
27. McLoughlin RM, Mills KH. Influence of gastrointestinal commensal bacteria on the immune responses that mediate allergy and asthma. J Allergy Clin Immunol. 2011;127:1097-107.
28. Abu-Shanab A, Quigley EM. The role of the gut microbiota in nonalcoholic fatty liver disease. Nat Rev Gastroenterol Hepatol. 2010;7:691-701.
29. Henao-Mejia J, Elinav E, Jin C, et al. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. Nature. 2012;482:179-85.
30. Wang Z, Klipfell E, Bennett BJ, et al. Gut flora metabolism of phosphatidylcho-

line promotes cardiovascular disease. Nature. 2011;472:57-63.
31. Koeth RA, Wang Z, Levison BS, et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat Med. 2013;19:576-85.
32. Turnbaugh PJ, Ley RE, Mahowald MA, et al. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006;444:1027-31.
33. Turnbaugh PJ, Hamady M, Yatsunenko T, et al. A core gut microbiome in obese and lean twins. Nature. 2009;457:480-4.
34. Cani PD, Bibiloni R, Knauf C, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat dietinduced obesity and diabetes in mice. Diabetes. 2008;57:1470-81.
35. Wen L, Ley RE, Volchkov PY, et al. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature. 2008;455:1109-13.
36. Musso G, Gambino R, CassaderM. Interactions between gut microbiota and host metabolism predisposing to obesity and diabetes. Annu Rev Med. 2011; 62:361-80.
37. Turnbaugh PJ, Ridaura VK, Faith JJ, et al. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Sci Transl Med. 2009 Nov 11;1(6):614.
38. Nielsen S, Nielsen DS, Lauritzen L, et al. Impact of diet on the intestinal microbiota in 10-month-old infants. J Pediatr Gastroenterol Nutr. 2007 May;44(5):613-8.
39. Adler CJ, Dobney K, Weyrich LS, et al. Sequencing ancient calcified dental plaque shows changes in oral microbiota with dietary shifts of the Neolithic and Industrial revolutions. Nat Genet. 2013 Apr;45(4):450-5, 455e1. Epub 2013 Feb 17.
40. Walker AW, Ince J, Duncan SH et al. Dominant and diet-responsive groups of bacteria within the human colonic microbiota. ISME J. 2011 Feb;5(2):220-30. Epub 2010 Aug 5.
41. Humblot C, Bruneau A, Sutren M, et al. Brussels sprouts, inulin and fermented milk alter the faecal microbiota of human microbiota-associated rats as shown by PCR-temporal temperature gradient gel electrophoresis using universal, Lactobacillus and Bifidobacterium 16S rRNA gene primers. Br J Nutr. 2005 May;93(5):677-84.
42. Parkar SG, Rosendale D, Paturi G, et al. In vitro utilization of gold and green kiwifruit oligosaccharides by human gut microbial populations. Plant Foods Hum Nutr. 2012 Sep;67(3):200-7.
43. Beards E1, Tuohy K, Gibson G. A human volunteer study to assess the impact of confectionery sweeteners on the gut microbiota composition. Br J Nutr. 2010 Sep;104(5):701-8. Epub 2010 Apr 7.
44. Walton GE, Lu C, Trogh I, Arnaut F, et al. A randomised, double-blind, placebo controlled cross-over study to determine the gastrointestinal effects of consumption of arabinoxylan-oligosaccharides enriched bread in healthy volunteers. Nutr J. 2012 Jun 1;11:36.
45. Liszt K, Zwielehner J, Handschur M, et al. Characterization of bacteria, clostridia and Bacteroides in faeces of vegetarians using qPCR and PCR-DGGE fingerprinting. Ann Nutr Metab. 2009;54(4):253-7. Epub 2009 Jul 27.
46. IJssennagger N, Derrien M, van Doorn GM, et al. Dietary heme alters microbiota and mucosa of mouse colon without functional changes in host-microbe cross-talk. PLoS One. 2012;7(12):e49868. Epub 2012 Dec 11.
47. Sanz Y. Effects of a gluten-free diet on gut microbiota and immune function in healthy adult humans. Gut Microbes. 2010 May-Jun;1(3):135-7. Epub 2010 Mar 16.
48. Donovan SM, Wang M, Li M, et al. Host-microbe interactions in the neonatal intestine: role of human milk oligosaccharides. Adv Nutr. 2012 May 1;3(3):450S-5S.
49. Holgerson PL, Vestman NR, Claesson R, et al. Oral microbial profile discriminates breast-fed from formula-fed infants. J Pediatr Gastroenterol Nutr. 2013 Feb;56(2):127-36.
50. Smith MI, Yatsunenko T, Manary MJ, et al. Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. Science. 2013 Feb 1;339(6119):548-54. Epub 2013 Jan 30.
51. Jaquet M, Rochat I, Moulin J, et al. Impact of coffee consumption on the gut microbiota: a human volunteer study. Int J Food Microbiol. 2009 Mar 31;130 (2):117-21. Epub 2009 Jan 23.
52. Martin FP, Rezzi S, Peré-Trepat E, et al. Metabolic effects of dark chocolate consumption on energy, gut microbiota, and stress-related metabolism in free-living subjects. J Proteome Res. 2009 Dec;8(12):5568-79.
53. Cani PD. Gutmicrobiota and obesity: lessons from the microbiome. Briefings In Functional Genomics. 2013;12(4):381-7 (Advance Access publication date 24 April).
54. Ley RE. Obesity and the human microbiome. Current Opinion in Gastroenterology. 2010;26:5-11.
55. Ley RE, Backhed F, Turnbaugh P, et al. Obesity alters gut microbial ecology. Proc Natl Acad Sci USA. 2005;102:11070-5.
56. Schwiertz A, Taras D, Schafer K, et al. Microbiota and SCFA in lean and overweight healthy subjects. Obesity. 2009. doi: 10.1038/oby.2009.167. [Epub ahead of print].
57. Collado MC, Isolauri E, Laitinen K, et al. Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. Am J Clin Nutr. 2008;88:894-9.
58. Sotos M, Nadal I, Marti A, et al. Gut microbes and obesity in adolescents. Proc Nutrition Soc. 2008;67:E20.
59. Duncan SH, Lobley GE, Holtrop G, et al. Human colonic microbiota associated with diet, obesity and weight loss. Int J Obesity. 2008;32:1720-4. J Pediatr (Rio J).2013.
60. Kalliomaki M, Collado C, Salminen S, et al. Early differences in fecal microbiota composition in children may predict overweight. Am J Clin Nutr. 2008;87:534-8.
61. Santacruz A, Marcos A, Warnberg J, et al. Interplay between weight loss and gut microbiota composition in overweight adolescents. Obesity; 2009. doi: 10.1038/oby.2009.112 [Epub ahead of print].
62. Nadal I, Santacruz A, Marcos A, et al. Shifts in clostridia, bacteroides and immunoglobulin-coating fecal bacteria associated with weight loss in obese adolescents. Int J Obesity. 2009; 33:758-67.
63. Sabate J, Jouet P, Harnois F, et al. High prevalence of small intestinal bacterial overgrowth in patients with morbid obesity: a contributor to severe hepatic steatosis. Obesity Surg. 2008;18:371-7.
64. Zhang H, DiBaise J, Zuccolo A, et al. Human gut microbiota in obesity and after gastric bypass. Proc Natl Acad Sci USA. 2009;106:2365-70.
65. Ley RE, Turnbaugh PJ, Klein S, et al. Microbial ecology: human gut microbes associated with obesity. Nature. 2006;444:1022-3.
66. Angelakis E, Armougom F, Million M, et al. The relationship between gut microbiota and weight gain in humans. Future Microbiol. 2012 Jan;7(1):91-109.
67. Armougom F, Henry M, Vialettes B, et al. Monitoring bacterial community of human gut microbiota reveals an increase in Lactobacillus in obese patients and Methanogens in anorexic patients. PLoS ONE4(9),e7125(2009).
68. Million M, Maraninchi M, Henry M, et al. Obesity-associated Gut microbiota is enriched in Lactobacillus reuteri and depleted in Bifidobacterium animalis and Methanobrevibacter smithii. Int J Obesity. 2011 [Epub ahead of print].
69. Zuo HJ, Xie ZM, Zhang WW et al. Gut bacteria alteration in obese people and its relationship with gene polymorphism. World J Gastroenterol. 2011:17(8),1076-81.
70. Balamurugan R, George G, Kabeerdoss J, et al. Quantitative differences in intestinal Faecalibacterium prausnitzii in obese Indian children. Br J Nutr. 2010;103(3):335-8 (2010).
71. Larsen N, Vogensen FK, van den Berg FW, et al. Gut microbiota in human adults with Type 2 diabetes differs from non-diabetic adults. PLoS ONE5(2),e9085 (2010).
72. Brown RK, Zehra-Esra I, Dae-Wook K et al. Effects of gut microbes on nutrient absorption and energy regulation. Nutr Clin Pract. 2012 April; 27(2): 201-14. Published online 2012 February 24.
73. Tilg, H. Obesity, Metabolic Syndrome, and Microbiota Multiple Interactions. J Clin Gastroenterol. 2010 September;44(1).
74. Duseja A, Chawla YK. Obesity and NAFLD The Role of Bacteria and Microbiota Clin Liver Dis. 2014;18:59-71.
75. Tsai YT, Cheng PC, Pan TM. Anti-obesity effects of gut microbiota are associated with lactic acid bacteria. Appl Microbiol Biotechnol. 2014;98:1-10.
76. Backhed F, Manchester J, Semenkovich C, et al. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proc Natl Acad Sci USA. 2007; 104:979-84.
77. Backhed F, Ding H, Wang T, et al. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci USA. 2004;101:15718-23.
78. Mandard S, Zandbergen F, van Straten E, et al. The fasting-induced adipose factor/angiopoietin-like protein 4 is physically associated with lipoproteins and governs plasma lipid levels and adiposity. J Biol Chem. 2006;281:934-44.
79. Yoon JC, Chickering TW, Rosen ED, et al. Peroxisome proliferator-activated receptor gamma target gene encoding a novel angiopoietin-related protein associated with adipose differentiation. Mol Cell Biol. 2000;20:5343-9.
80. Romeo S, Pennacchio L, Fu Y, et al. Population-based resequencing of ANGPTL4 uncovers variations that reduce triglycerides and increase HDL. Nat Genet. 2007;39:513-6.
81. Staiger H, Haas C, Machann J, et al. Muscle-derived angiopoietin-like protein 4 is induced by fatty acids via PPAR {delta} and is of metabolic relevance in humans. Diabetes. 2008;53:579-89.
82. Kahn BB, Alquier T, Carling D, et al. AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. Cell Metab. 2005;1:15-25.
83. Brown AJ, Goldsworthy SM, Barnes AA, et al. The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. J Biol Chem. 2003;278:11312-9.
84. Le Poul E, Loison C, Struyf S, et al. Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. J Biol Chem. 2003; 278:25481-9.
85. Samuel BS, Shaito A, Motoike T, et al. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. Proc Natl Acad Sci USA. 2008;105:16767-72.
86. Cani PD, Amar J, Iglesias MA, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes. 2007;56:1761-72.

87. Cani PD, Neyrinck AM, Fava F, et al. Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. Diabetologia. 2007;50:2374-83.
88. Kim KA, Gu W, Lee IA, et al. High fat diet induced gut microbiota exacerbates inflammation and obesity in mice via the TLR4 signaling pathway. PLoS One 2012; 7:e47713.
89. Belzer C, de Vos WM. Microbes inside—from diversity to function: the case of Akkermansia. ISME J 2012;6:1449-58.
90. Santacruz A, Collado MC, Garcia-Valdes L, et al. Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. Br J Nutr. 2010;104:83-92.
91. Hansen CH, Krych L, Nielsen DS, et al. Early life treatment with vancomycin propagates Akkermansia muciniphila and reduces diabetes incidence in the NOD mouse. Diabetologia. 2012;55:2285-94.
92. Qin J, Li Y, Cai Z, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature. 2012;490:55-60.
93. Cani PD, Possemiers S, Van deWiele T, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut. 2009;58:1091-103.
94. de La Serre CB, Ellis CL, Lee J, et al. Propensity to high-fat diet-induced obesity in rats is associated with changes in the gut microbiota and gut inflammation. Am J Physiol Gastrointest Liver Physiol. 2010; 299:G440-8.
95. Aggarwal J, Swami G, Kumar M. Probiotics and their effects on metabolic diseases: an update. J Clin Diagn Res. 2013;7:173-7.
96. Vijay-Kumar M, Aitken JD, Carvalho FA, et al. Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. Science. 2010;328:228-31.
97. Tsukumo D, Carvalho-Filho M, Carvalheira J, et al. Loss-of-function mutation in Toll-like receptor 4 prevents diet-induced obesity and insulin resistance. Diabetes. 2007;56:1886-98.
98. Fei N, Zhao L. An opportunistic pathogen isolated from the gut of an obese human causes obesity in germfree mice. ISME J. 2013;7:880-4.
99. Muccioli GG, Naslain D, Backhed F, et al. The endocannabinoid system links gut microbiota to adipogenesis. Mol Syst Biol. 2010;6:392.
100. Burcelin R, Sermino M, Chabo C, et al. Gut microbiota and diabetes: from pathogenesis to therapeutic perspective. Acta Diabetol. 2011 Dec;48(4):257-73.
101. Amar J, Burcelin R, Ruidavets J, et al. Energy intake is associated with endotoxemia in apparently healthy men. Am J Clin Nutr. 2008;87:1219-23.
102. Finkelstein EA, Trogdon JG, Joel W, et al. Annual medical spending attributable to obesity: payer- and service-specific Eestimates. Health Aff. 2009;28(5): w822-31. (Published online July 27, 2009; 10.1377/hlthaff.28.5.822).
103. Maslowski KM, Vieira AT, Ng A, et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature. 2009;461:1282-6.
104. Marchesi JR, Holmes E, Khan F, et al. Rapid and noninvasive metabonomic characterization of inflammatory bowel disease. J Proteome Res. 2007;6:546-51.
105. Neyrinck AM, Delzenne NM. Potential interest of gut microbial changes induced by non-digestible carbohydrates of wheat in the management of obesity and related disorders. Curr Opin Clin Nutr Metab Care. 2010;13:722-8.
106. Cani PD, Daubioul CA, Reusens B, et al. Involvement of endogenous glucagon-like peptide-1(7–36) amide on glycaemia-lowering effect of oligofructose in streptozotocin-treated rats. J Endocrinol. 2005;185:457-65.
107. Cani PD, Knauf C, Iglesias MA, et al. Improvement of glucose tolerance and hepatic insulin sensitivity by oligofructose requires a functional glucagonlike peptide 1 receptor. Diabetes. 2006;55:1484-90.
108. Delmee E, Cani PD, Gual G, et al. Relation between colonic proglucagon expression and metabolic response to oligofructose in high fat diet-fed mice. Life Sci. 2006;79:1007-13.
109. Mazmanian SK, Kasper DL. The love-hate relationship between bacterial polysaccharides and the host immune system. Nat Rev Immunol. 2006;6:849-58.
110. Parnell JA, Reimer RA. Prebiotic fibres dose-dependently increase satiety hormones and alter Bacteroidetes and Firmicutes in lean and obese JCR:LA-cp rats. Br J Nutr. 2012;107:601-13.
111. Cani PD, Neyrinck AM, Maton N, et al. Oligofructose promotes satiety in rats fed a high-fat diet: involvement of glucagon-like Peptide-1. Obes Res. 2005;13: 1000-7.
112. Cani PD, Lecourt E, Dewulf EM, et al. Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal. Am J Clin Nutr. 2009;90:1236-43.
113. Parnell JA, Reimer RA. Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. Am J Clin Nutr. 2009;89:1751-9.
114. Verhoef SP, MeyerD, Westerterp KR. Effects of oligofructose on appetite profile, glucagon-like peptide 1 and peptide YY3-36 concentrations and energy intake. Br J Nutr. 2011;106:1757-62.
115. Neyrinck AM, Possemiers S, Verstraete W, et al. Dietary modulation of clostridial cluster XIVa gut bacteria (Roseburia spp.) by chitin-glucan fiber improves host metabolic alterations induced by high-fat diet in mice. J Nutr Biochem. 2012; 23:51-9.
116. Davis LM, Martinez I, Walter J, et al. Barcoded pyrosequencing reveals that consumption of galactooligosaccharides results in a highly specific bifidogenic response in humans. PLoS One. 2011;6:25200.
117. Abrams SA, Griffin IJ, Hawthorne KM, et al. Effect of prebiotic supplementation and calcium intake on body mass index. J Pediatr. 2007;151:293-8.
118. Neyrinck AM, Possemiers S, Druart C, et al. Prebiotic effects of wheat arabinoxylan related to the increase in bifidobacteria, Roseburia and Bacteroides/Prevotella in diet-induced obese mice. PLoS One. 2011;6:20944.
119. Daubioul CA, Horsmans Y, Lambert P, et al. Effects of oligofructose on glucose and lipid metabolism in patients with nonalcoholic steatohepatitis: results of a pilot study. Eur J Clin Nutr. 2005;59:723-6.
120. Dewulf EM, Cani PD, Claus SP, et al. Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. Gut. 2013;62:1112-21.
121. Lirussi F, Mastropasqua E, Orando S, et al. Probiotics for non-alcoholic fatty liver disease and/or steatohepatitis. Cochrane Database Syst Rev. 2007;(1):CD005165.
122. Goldenberg, JZ, Ma SS, Saxton JD. et al. Probiotics for the prevention of Clostridium difficile-associated diarrhea in adults and children. Cochrane Database Syst. 2013;Rev. 5: CD006095.
123. Bernardo WM, Aires FT, Carneiro RM. et al. Effectiveness of probiotics in the prophylaxis of necrotizing enterocolitis in preterm neonates: a systematic review and meta-analysis. J Pediatr. 2013;(Rio. J.)89:18-24.
124. Macklaim JM, Cohen CR, Donders G. et al. Exploringa road map to counter misconceptions about the cervicovaginal microbiome and disease. Reprod. Sci. 2012;19: 1154-62.
125. Hao Q, Lu Z, Dong BR, et al. Probiotics for preventing acute upper respiratory tract infections. Cochrane Database Syst. Rev. 2011;CD006895.
126. Isolauri E, Rautava S, Salminen S. Probiotics in the development and treatment of allergic disease. Gastroenterol. Clin North Am. 2012;41:747-62.
127. Erridge C, Attina T, Spickett CM, et al. A high-fat meal induces low-grade endotoxemia: evidence of a novel mechanism of postprandial inflammation. Am J Clin. Nutr. 2007;86:1286-92.
128. Delzenne NM, Neyrinck AM, Bäckhed F, et al. Targeting gut microbiota in obesity: effects of prebiotics and probiotics. Nat Rev Endocrinol. 2011 Aug 9;7(11):639-46.
129. Andreasen, A. S. et al. Effects of Lactobacillus acidophilus NCFM on insulin sensitivity and the systemic inflammatory response in human subjects. Br J Nutr. 2010;104:1831-8.
130. Kadooka Y, Sato M, Imaizumi K, et al. Regulation of abdominal adiposity by probiotics (Lactobacillus gasseri SBT2055) in adults with obese tendencies in a randomized controlled trial. Eur J Clin Nutr. 2010;64:636-43.
131. Lu ZX, Walker KZ, Muir JG, O›Dea K. Arabinoxylan fibre improves metabolic control in people with type II diabetes. Eur J Clin Nutr. 2004;58:621-8 (2004).
132. Garcia AL, Steiniger J, Reich SC, et al. Arabinoxylan fibre consumption improved glucose metabolism, but did not affect serum adipokines in subjects with impaired glucose tolerance. Horm Metab Res. 2006;38:761-6.
133. Garcia AL, Otto B, Reich SC, et al. Arabinoxylan consumption decreases postprandial serum glucose, serum insulin and plasma total ghrelin response in subjects with impaired glucose tolerance. Eur J Clin Nutr. 2007;6:334-41.
134. Parnell JA, Reimer RA. Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. Am J Clin Nutr. 2009;89:1751-9.
135. Luo J1, Van Yperselle M, Rizkalla SW, Rossi F, Bornet FR, Slama G. Chronic consumption of short-chain fructooligosaccharides does not affect basal hepatic glucose production or insulin resistance in type 2 diabetics. J Nutr. 2000;130:1572-7.
136. Daubioul CA, Horsmans Y, Lambert P, Danse E Delzenne NM. Effects of oligofructose on glucose and lipid metabolism in patients with nonalcoholic steatohepatitis: results of a pilot study. Eur J Clin Nutr. 2005;59:723-6.
137. Kelishadi R, Farajian S, Safavi M, et al. A randomized triple masked controlled trial on the effects of synbiotics on inflammation markers in overweight children. J Pediatr (Rio J). 2013.
138. Riley LW, Raphael E, Faerstein E. Obesity in the United States - dysbiosis from exposure to low-dose antibiotics? Front Public Health. 2013 Dec 19;1:69.
139. Maxwell PR, Rink E, Kumar D, et al. Antibiotics increase functional abdominal symptoms. Am J Gastroenterol. 2002;97:104-8.
140. Mendall MA, Kumar D. Antibiotic use, childhood affluence and irritable bowel syndrome (IBS). Eur J Gastroenterol Hepatol. 1998;10:59-62.
141. Barbara G, Stanghellini V, Berti-Ceroni C, et al. Role of antibiotic therapy on long-term germ excretion in faeces and digestive symptoms after Salmonella infection. Aliment Pharmacol Ther. 2000;14:1127-31.
142. Atherton JC, Blaser MJ. Coadaptation of Helicobacter pylori and humans: ancient history, modern implications. J Clin Invest. 2009 September 1;119(9):2475-87.
143. Francois F, Roper J, Joseph N, et al. The effect of H. pylori eradication on meal-associated changes in plasma ghrelin and leptin. BMC Gastroenterol. 2011;11:37. Published online 2011 April 14.