FOCUS

A healthy microbiota is defined by high diversity and an ability to resist change under physiological stress. In contrast, microbiota associated with disease is defined by lower species diversity, fewer beneficial microbes and/or the presence of pathobionts

Clinical Consequences of Diet-Induced Dysbiosis
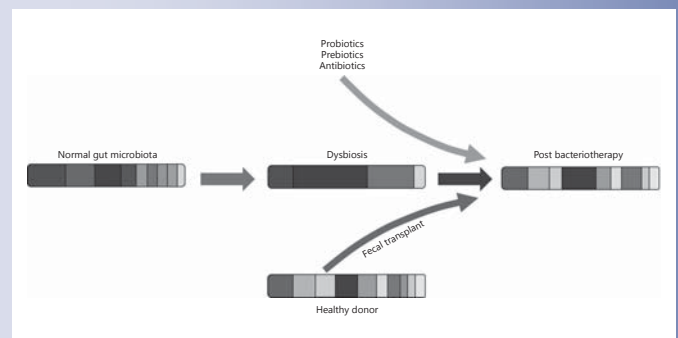by Yee Kwan Chan et al.

Key insights

Various disease states are associated with alterations in the balance between beneficial and harmful bacteria that reside in the intestine. This dysbiosis has far-reaching effects on local and systemic immunity, and underpins the pathogenesis of disorders such as inflammatory bowel disease, colorectal cancer, diabetes, atherosclerosis and nonalcoholic fatty liver disease. Interventions that target the microbial profile of the gut have tremendous potential for addressing these disorders.

Current knowledge

There is a complex tripartite relationship between diet, microbes and the gut epithelium. Beyond the postnatal period, long-term dietary patterns have a strong influence on the composition of gut microbes. For example, regular red meat consumption favors a Bacteroides-rich microflora, whereas Prevotella species tends to dominate in vegetarians. A high-fat diet may induce dysbiosis through the actions of bile, which could affect the growth of some microbes. An examination of the digestive process may offer greater insight into the mechanisms through which diet can influence dysbiosis and disease.

Practical implications

Not surprisingly, diet and gut microbes are two critical factors in the pathogenesis of gastrointestinal diseases. Intestinal dysbiosis has also been linked to systemic conditions such as metabolic and cardiac disorders. Although diet is a tempting intervention, our understanding of how to manipulate diet to promote a healthy microbiota is still in its early days. Bacteriotherapy provides a

Bacteriotherapy is a method which restores the original microbiome or introduces a new healthy one by means of bacterial manipulation. For further details, please refer to the article.

novel approach for restoring healthy homeostasis through the gut microbes. This is achieved through the use of various interventions, including the removal of pathogenic bacteria with antibiotics, supplementation with prebiotics and/or probiotics, and most recently, introduction of a new healthy microbial ecosystem by transplanting fecal bacteria from a healthy donor.

Recommended reading

Adler CJ, Dobney K, Weyrich LS, Kaidonis J, Walker AW, Haak W, Bradshaw CJ, Townsend G, Soltysiak A, Alt KW, et al: Sequencing ancient calcified dental plaque shows changes in oral microbiota with dietary shifts of the Neolithic and Industrial revolutions. Nat Genet 2013;45:450–455.

Clinical Consequences of Diet-Induced Dysbiosis

Yee Kwan Chan   Mehrbod Estaki   Deanna L. Gibson

Department of Biology, University of British Columbia Okanagan, Kelowna, B.C., Canada

Key Messages

- An undesirable alteration of the microbiota resulting in an imbalance between protective and harmful bacteria is termed dysbiosis.
- Dietary patterns alter the intestinal microbiota ecologically and functionally and this results in physiological consequences to the host.
- Dysbiosis has been implicated in many human disease conditions including local gastrointestinal and systemic diseases.
- Restoration and maintenance of a healthy gut microbiota may be an effective, inexpensive and safe remedy to diseases associated with dysbiosis.

quence considering the wide effects that the microbes have on both local and systemic immunity. The goal of this review is to give insight into the importance of gut microbiota in disease development and the possible therapeutic interventions in clinical settings. We introduce the complex tripartite relationship between diet, microbes and the gut epithelium. This is followed by a summary of clinical evidence of diet-induced dysbiosis as a contributing factor in the development of gastrointestinal diseases like inflammatory bowel disease, irritable bowel syndrome and colorectal cancer, as well as systemic diseases like obesity, diabetes, atherosclerosis and nonalcoholic fatty liver disease. Finally, the current dietary and microbial interventions to promote a healthy microbial profile will be reviewed.

© 2013 S. Karger AG, Basel

Abstract

Various disease states are associated with an imbalance of protective and pathogenic bacteria in the gut, termed dysbiosis. Current evidence reveals that dietary factors affect the microbial ecosystem in the gut. Changes to community structure of the intestinal microbiota are not without conse-

Colonization and Diversity of Gut Microbes

Humans have co-evolved with vast amounts of microorganisms that inhabit the body. The average human being harbors 10 times more bacterial cells than their own cell numbers. These microbes colonize the skin, nasal and oral cavity, urogenital and gastrointestinal tract (GIT). Among all sites, the GIT is the most densely populated area with the colon alone harboring over 10^{10}–10^{12} colony-forming units per gram of feces, or 70% of all microbes in the human body [1].

Deanna L. Gibson
Department of Biology, The Irving K. Barber School of Arts and Sciences
University of British Columbia Okanagan, ASC 368, 3333 University Way
Kelowna, BC V1V 1V7 (Canada)
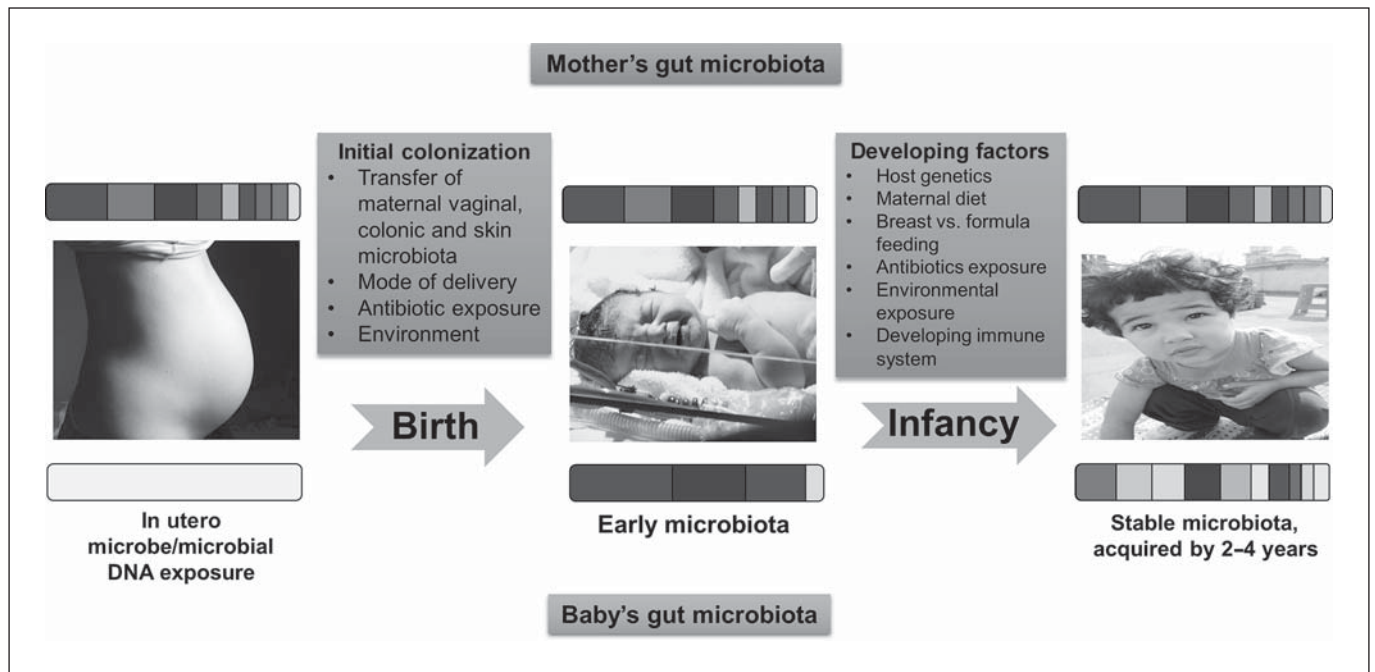E-Mail deanna.gibson@ubc.ca

Fig. 1. Development of gut microbiota in a neonate. The fetal gut in utero is exposed to microbial DNA and potentially maternal microbes. After birth, it is rapidly colonized by bacteria transferring from maternal vaginal, colonic and skin microbiota dependent on the mode of delivery and antibiotic exposure. Environmental factors may also play a role in the acquisition of microbes including: the presence of microbial populations in the place of birth and skin microbiota of persons coming in contact with the baby, like the father's, nurses' or doctors'. Colonization continues to increase the quantity and diversity of bacterial species in the gut and is influenced by various developing factors including: the baby's host genetics, maternal diet, breast or formula feeding, antibiotics and environmental microbial exposure, as well as the developing immune system. Such microbiota becomes more stable and is acquired by the age of 2–4 years.

While it has been thought that a fetus is sterile in utero, there is some evidence that microbial DNA and potentially even microbes are exposed to the fetus and fetal gut through the placenta [discussed by Luoto et al. in this issue]. During birth, microbial colonization of the GIT occurs and develops rapidly thereafter with maternal and environmental microbes. Colonization does not appear to be random but preprogrammed; yet, the mode of infant delivery, antibiotic exposure, nutrition and other extrinsic factors influence microbial ecology (fig. 1). Microbial diversity increases during the first few years of life and then stabilizes by 2–4 years of age resembling that of an adult [2]. Most of these bacteria associate with the intestinal mucosal surface and maintain their specific niches over time as indigenous populations. Newly introduced bacteria either pass through the GIT in the stool or compete with indigenous bacteria to create their niche. While there is evidence that the intestinal microbiota is relatively stable throughout life, extrinsic factors such as stress, alcohol consumption, exercise and dietary choices do change the ecology and function of the microbiota in adults. We do not yet understand how dynamic the ecology of the microbiota is, so microbial changes may only be transient and reversible, but more research is required to understand this plasticity.

Humans carry 500–1,000 bacterial species in the GIT of which the majority belongs in only two phyla: the Firmicutes and Bacteroidetes (>90%). Other phyla present to a lesser extent include: Actinobacteria, Proteobacteria, Fusobacteria, Spirochaetae and Verrucomicrobia. While the dominating phyla are relatively constant between individuals, diversity increases along the taxonomic line with each individual harboring over a hundred unique species. Three distinct clusters of gut microbiota have been identified in humans. These 'enterotypes' are mainly driven by species composition and are not geographical, age or gender specific [3]. An undesirable alteration of the microbiota resulting in an imbalance between protective and harmful bacteria is termed dysbiosis and may cluster as a specific entero-
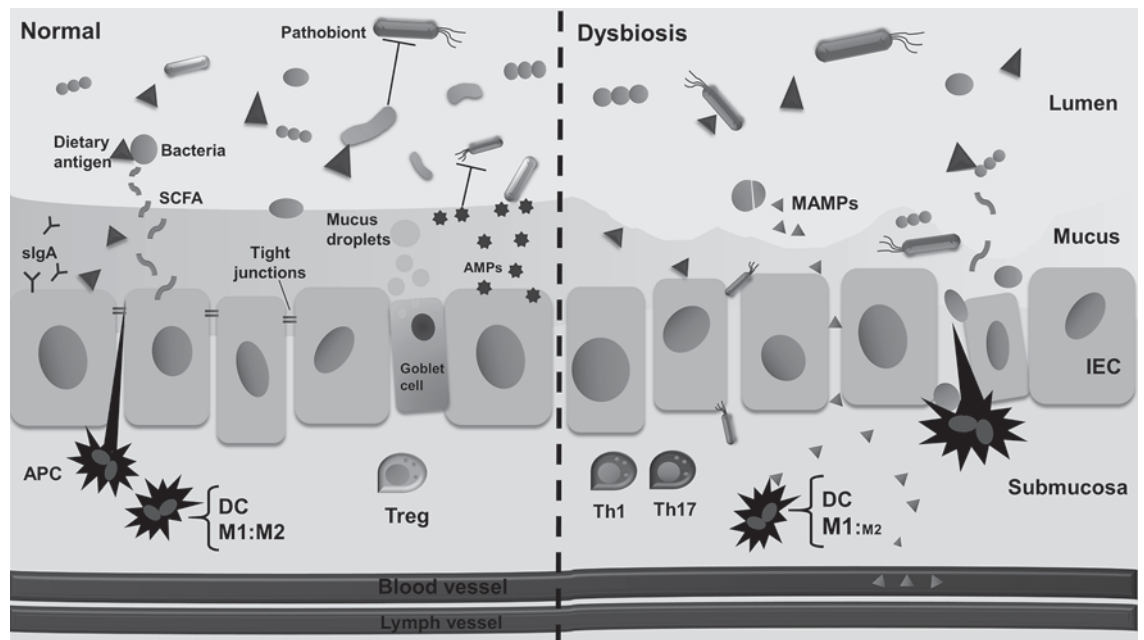
Fig. 2. GIT under homeostatic and dysbiotic conditions. The intestinal microbiota lies strategically at the interface of the internal and external environment of the gut. It plays several important biological roles including: aiding in digestion and absorption of nutrients from partially digested food, production of SCFA – a primary energy source for intestinal epithelial cells (IECs), stimulating immune responses by releasing ligands, and protection against enteropathogens by production of antimicrobial peptides (AMPs). In addition, commensal bacteria also work as a protective barrier against pathobionts through competition for space and food. The highly selective permeable monolayer made up of IECs and adjacent tight junctions acts as the only barrier separating the microbe-rich lumen side from the sterile submucosal area. Damage to this layer or loss of the tight junctions' integrity in a diseased state allows for increased passing of microorganisms and their immune-stimulating molecules such as MAMPs, i.e. lipopolysaccharide, to the submucosa where ultimately they may enter circulation, induce pro-inflammatory signaling and recruit leukocytes. Goblet cells found within the IEC layer replenish the mucus layer covering the epithelium by releasing large glycoprotein polymers such as mucin. The secretion of mucus droplets by the goblet cells is regulated by the microbiota, thus dysbiosis plays a key role in disruption of the mucus layer. Dietary antigens (dark grey triangles) can interact with the microbiota and IECs inducing biological responses in both. After the IEC layer, antigen-presenting cells (APC) act as the next line of cellular defense. APCs, which include dendritic cells (DC), M1 and M2 type macrophages, are part of the innate immune response which protects the host against invading pathobionts. Typically, under dysbiotic conditions, overactivation of the innate immune response leads to higher than normal expressions of activated M1 to M2 type macrophages which increase pro-inflammatory events. Regulatory T cells (Treg) regulate the adaptive immune response by maintaining tolerance to self-antigens and suppressing overactivation of the immune responses. Insufficient Treg expression can lead to increased levels of Th1 and Th17 facilitating chronic inflammatory responses.

type. In support of this, enterotypes have been shown to associate with chronic ailments such as colonic inflammation [3], symptomatic atherosclerosis [4] and nonalcoholic steatohepatitis [5]. Factors such as nutrient load, macro- and micronutrients induce changes to the ecology and functionality of the gut microbiota, and long-term dietary patterns can alter the original enterotype [6]. Identifying dietary factors that promote beneficial microbes and prevent pathobiont intrusion may be an important tactic in the prevention of dysbiosis-associated diseases.

The GIT, Microbes and Diet

The gut microbial ecosystem has tremendous influence on the overall health status of the human host. The microbiota lies at the interface of the internal and external environment in the gut forming a tripartite relationship with the intestinal epithelial cells and dietary antigens (fig. 2). Due to this conspicuous location, the microbiota is able to liaise with both the intestinal mucosal surface and the luminal environment that contain partially digested food. Dietary antigens interact with both the microbes and the intestinal epithelium. Microbes impart physiological changes to the host by interacting with the

intestinal epithelial cells via innate immune receptors [discussed by Walker in this issue]. The intestines contain the largest mass of lymphoid tissue in the body: the gut-associated lymphoid tissue (GALT). The GALT relays signals from the mucosal surface to the rest of the body through various immune cells and immune receptors including innate toll-like receptors (TLRs) and NOD-like receptors (NLRs). The intestinal microbiota plays crucial roles in the GIT development, systemic immunity and colonic homeostasis. Gut microbiota can modulate the function and responsiveness of intestinal immune cells, like T regulatory cells, to bacterial products. This is required to regulate mechanisms that keep both mucosal and systemic immunity in balance, allowing for mucosal surfaces to tolerate harmless bacteria, yet adequately respond to invading pathogens. Production of short-chain fatty acid (SCFA) by gut microbes also plays an important role in regulating homeostasis in the gut. For example, butyrate produced by colonic microbes is not only the main energy source for colonocytes, but also inhibits intestinal cell proliferation which can reduce colitis symptoms [7]. Given the vital relationship between microbes and intestinal health, a normal functioning microbiota is crucial to maintaining a balance of local and systemic immunity. As discussed below, in the absence of a healthy microbiota, immune disorders may arise. Identifying dietary factors that control the intestinal microbial ecology and their role in enteric disease susceptibility could provide insight into the functioning of the microbiota in healthy and diseased individuals. Yet, due to the vast diversity of dietary antigens and gut microbes, we are challenged to define the exact interactions between microbes, dietary antigens and epithelium and their consequences to the host.

Dietary antigens can interact with both the microbiota and the intestinal mucosae, initiating biological reactions in the host. Food contains numerous compounds that shape the chemistry of the gut as well as the whole body. For example, dietary antigens are absorbed through the intestine which results in metabolites in the circulating fluids like blood and lymph [8]. The association of specific metabolites in the body with dominant bacterial taxa in infants suggests that the chemical composition of the diet can define the gut microbial ecology [9]. While dietary factors can directly affect the functionality of intestinal epithelial cells and the underlying

Postnatal nutrition could be used to target specific changes in microbial diversity.

immune cells [10], dietary antigens also alter the intestinal ecosystem by enabling certain microbial populations to proliferate and dampening the dominance of others (reviewed by Brown et al. [11]). The consequences of dysbiosis are not innocent, but detrimental when pathobionts (any disease-causing microorganism) become prominent in the microbial communities. To support this idea, oral microbes sequenced from ancient teeth found in skeletons from various periods of time have become increasingly cariogenic dominant, or rich in microbes that promote dental disease [12]. These microbial changes have occurred during the two greatest dietary shifts in human evolution: the transition from the hunter-gatherer 'Paleolithic' period to the carbohydrate-rich farming 'Neolithic' period (~10,000 years ago) and the initiation of the industrialized period characterized by processed foods (~160 years ago). These findings support the notion that diet induces dysbiosis which alters the health of the host.

Evidence suggests that dietary factors alter intestinal ecology in both rodent models (reviewed by Brown et al. [11]) and in humans, and the changed ecology is associated with clinical consequences (table 1). Neonatal nutrition is critical in the initial development of microbial ecology [13]. For example, formula-fed infants have higher levels of pathobionts like Enterobacteriaceae and less beneficial microbes like *Bifidobacteria* spp. compared to breastfed infants [14]. Interestingly, infants fed cow's milk but not infant formula supplemented with fish oil had increased *Bifidobacteria* spp. [15] suggesting that postnatal nutrition could be used to target specific changes in microbial diversity. Beyond the postnatal period, long-term dietary choices are strongly associated with the gut microbiota composition [6]. In humans, diets that include regular red meat consumption tend to favor a predominantly *Bacteroides*-rich gut ecosystem [16], while *Prevotella* species dominate in vegetarians [17]. European children are deficient in Bacteroidetes and enriched with Enterobacteriaceae compared to rural African children who consume diets rich in fiber [18]. This study may be an important key to understanding the increase in noncommunicable diseases in European children. While it is generally agreed that high-fat diets promote dysbiosis, recent evidence from our laboratory suggests that the specific type of dietary fatty acid as opposed to total calories from fat appears to be important. For example, diets rich in ome-

Table 1. Summary of studies showing that dietary factors change microbial profiles in humans and the associated clinical consequences

Dietary factor implicated	Specific diet	Sample size	Location of microbes analyzed in host	Bacterial population altered	Method of bacterial detection	Associated host effect	Ref. PubMed accession No.
Fat	high fat (shortening) and high sugar	1 man, 15 mice	feces of men	↑ Clostridium innocuum, Catenibacterium mitsuokai, Enterococcus spp. ↓ Bacteroides spp.	multiplex amplicon pyrosequencing	↑ obesity when transplanted into mouse	20368178
	fish oil-supplemented infant formula versus cow's milk	65	feces of 10-month-old infants	consumption of cow's milk and infant formula resulted in different microbial patterns; fish oil supplementation affects the microbial pattern of the cow's milk group only	DGGE	not examined	17460496
Carbo-hydrate	increased carbohydrate-rich foods	34	mouth of ancient skeletons	cariogenic-dominant	454 pyrosequencing	↑ dental disease	23416520
	diets high in resistant starch compared to non-starch polysaccharides and low carbohydrate	14	feces of overweight men	↑ Firmicutes, Eubacterium rectale, Roseburia, Ruminococcus bromii (R-ruminococci)	qPCR	↑ digestibility of starch	20686513
	dietary fiber-rich diets found in rural Africa compared to Western European diets	29	fecal microbiota of children aged 1–6 years	↑ Bacteroidetes ↑ Firmicutes ↑ Prevotella and Xylanibacter ↓ Enterobacteriaceae	454 pyrosequencing	↑ bacterial genes for cellulose and xylan hydrolysis ↑ SCFAs	20679230
	inulin and Brussels sprouts	1 man, 48 rats	feces	↑ Bifidobacterium and Lactobacillus	TTGE	↑ cecal butyrate and acetate when transplanted into rats	15975167
	kiwifruit	10	feces	↑ Bifidobacterium and Bacteroides-Prevotella-Porphyromonas group	qPCR	↑ microbial glycosidases and SCFAs	22576129
	sucrose-free chocolates + maltitol + bulking agents (polydextrose and resistant starch)	40	feces	↑ Bifidobacterium and Lactobacillus	FISH	↑ SCFAs propionate and butyrate	20370946
	bread enriched with arabinoxylan-oligosaccharides	40	feces	↑ Bifidobacteria ↑ Lactobacillus	FISH	↑ butyrate ↓ isovalerate and fatty acids associated with protein fermentation	22657950
Protein	vegetarian	29	feces	↑ in overall bacterial DNA ↓ the amount and changing the diversity of Clostridium cluster IV	DGGE, qPCR	not examined	19641302
	high red-meat diet	24 mice	feces	↑ Bacteroides spp.	qPCR	no functional changes observed when transplanted into mouse	23239972
	gluten-free diet	10	feces	↓ Bifidobacterium and Lactobacillus ↑ Enterobacteriaceae	not mentioned	↓ TNF-α, IFN-γ, IL-8 and IL-10 in peripheral blood mononuclear cells	21327021
Breastfeeding	Breastfeeding compared to formula feeding	not reported	feces of 3-month-old infants	↑ Bacteroidetes ↓ Firmicutes and Verrucomicrobia	454 pyrosequencing	gene networks (inflammation, cell adhesion, barrier function, histamine, etc.) differentially expressed in exfoliated intestinal epithelial cells	22585924
		207	mouth of 3-month-old infants	↑ Lactobacillus spp.	culturing, qPCR	inhibited growth of the cariogenic Streptococcus spp.	22955450
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 the first 3 years of life	↓ Actinobacteria in kwashiorkor twin compared to healthy twin	multiplex shotgun sequencing	severe acute malnutrition caused when kwashiorkor microbiota transplanted into mouse	23363771
	3 cups of coffee daily for 3 weeks	16	feces	↑ Bifidobacterium spp.	DGGE, FISH	↑ metabolic activity of Bifidobacteria spp.	19217682
	dark chocolate	30	urine	not examined	¹H NMR, MS analysis	different energy profiles, hormonal metabolism and gut microbial activity	19810704

DGGE = Denaturing gradient gel electrophoresis; FISH = fluorescence in situ hybridization; ^{1}H NMR = proton nuclear magnetic resonance; MS = mass spectrometry; qPCR = quantitative polymerase chain reaction; TTGE = temporal temperature gradient electrophoresis.

ga-6 polyunsaturated fatty acids (PUFAs) cause blooms of pathobionts, but isocaloric diets supplemented with omega-3 PUFA can reverse such microbial alterations in mice [19, 20].

> *Diets rich in omega-6 PUFAs cause blooms of pathobionts, but isocaloric diets supplemented with omega-3 PUFA can reverse such microbial alterations in mice.*

One of the main functions of the microbiota is to break down food to make it available to the host and as a result, the effect of dysbiosis on metabolism has received considerable attention in current research. 'Humanized' mice, or germ-free mice transplanted with human fecal microbiota, are now being used to test the effects of human gut microbiota on mammalian physiology. Using this model, humanized mice fed a 'Western' diet high in fat and sugar were shown to have increased adiposity as a result of decreased ratios of Bacteroidetes to Firmicutes in the fecal microbiota [21]. Similarly, the gut microbiome was shown to play an important role in the development of kwashiorkor disease, a severe form of malnutrition [22]. In this study, the fecal microbiota of Malawian twins that were discordant for kwashiorkor was transplanted into mice. When fed a Malawian diet, weight loss and metabolic perturbations were more severe in the mice that received microbiota from the twin that had kwashiorkor compared to those that received microbes from the unaffected twin. Another study elegantly links specific nutrition factors to microbial ecology and the complex biological consequences that occur in the intestinal epithelial cells [23]. This study examined infant fecal microbiota with varying human milk oligosaccharide consumption and found that differences in microbiota modulated major gene networks including signal transduction, inflammation, histamine, cell migration and adhesion. GIT motility is another major function that is affected by the intricate interactions amongst diet and microbes. When humanized mice were fed a diet containing fermentable fructooligosaccharides (FOS), gastrointestinal transit time was altered [24].

Dietary factors alter the microbial ecology in the small intestine where food antigens are primarily digested, as well as the cecum and the distal colon where digestion is not a main function of the host but an important

function of the microbes. High-fat feeding induces dysbiosis through the direct antimicrobial activity of bile. Insoluble lipid molecules are broken into small droplets by bile and lipases which become soluble free fatty acids and monoglycerides, which then enter the bloodstream. As shown, bile secreted during high-fat feeding could affect the growth or survival of some microbes [25], although we have found that varying types of fatty acids play more of a role in dysbiosis than high-fat feeding alone [19, 20]. The process of lipid digestion may give more clues as to how microbes could be related to various diseases.

Clinical Evidence for Intestinal Dysbiosis-Associated Diseases

A healthy microbiota is defined by high diversity and an ability to resist change under physiological stress. In contrast, microbiota associated with disease is defined by lower species diversity, fewer beneficial microbes and/or the presence of pathobionts. Given the role of the microbiota in mediating host metabolism and immunity, disruption of the microbiota has been associated with various human diseases of the GIT and systemically throughout the body. Here, we review evidence from recent clinical studies connecting dysbiosis to various diseases, with an emphasis on the involvement of dietary factors.

Intestinal Dysbiosis in Gastrointestinal Diseases

The functional roles of the human GIT include nutrient absorption, waste removal via peristalsis, defense against ingested pathogens and prevention of translocation of food or antigens into the bloodstream. The gut microbiota regulates several of these functions including peristalsis, barrier function and maintaining balanced inflammatory and homeostatic responses. Disruption of the gut microbiota renders the GIT vulnerable to local disease states (fig. 3).

Inflammatory Bowel Diseases

Clinical studies have identified dysbiosis in patients with inflammatory bowel disease (IBD), including both Crohn's disease (CD) and ulcerative colitis (UC). Studies examining twins have shown enriched Actinobacteria and Proteobacteria and reduced Bacteroidetes in the twins with UC as compared to their healthy siblings [26]. An increase in sulfide-generating *Desulfovibrio* subspecies and *Fusobacterium varium* that can invade the epithelium are present in UC patients [27], while anti-in-
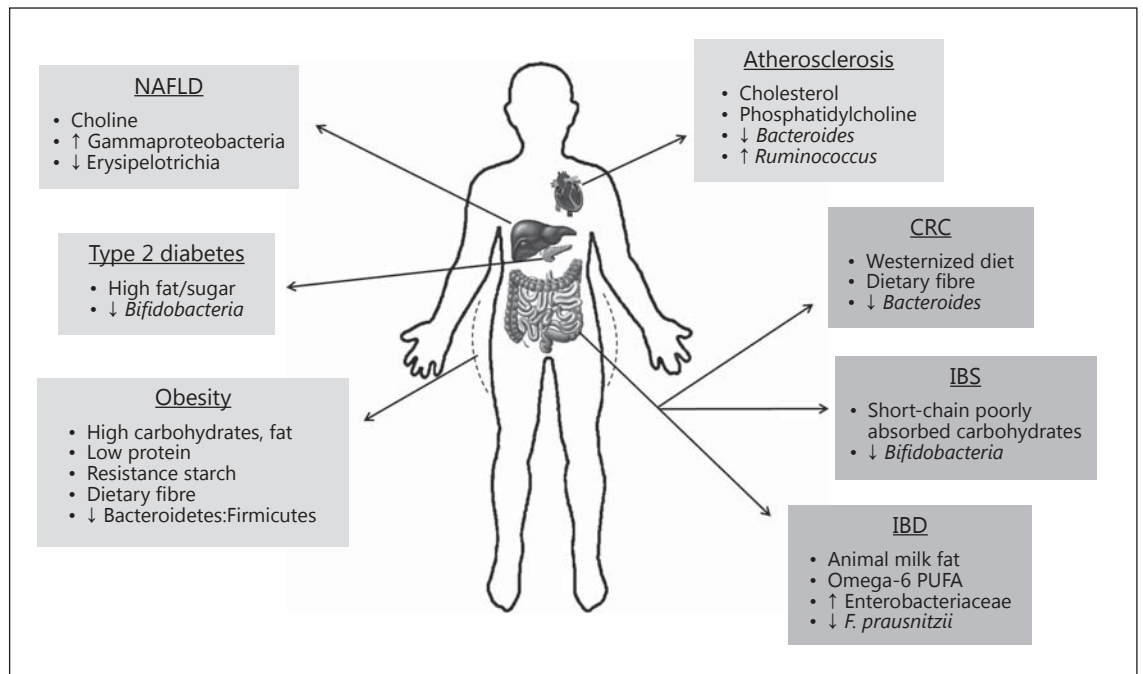
Fig. 3. Diet-induced dysbiosis and diseases. The diagram summarizes the gastrointestinal diseases (in dark grey boxes) and systemic diseases (in light grey boxes), the relevant dietary factors and induced intestinal microbial community changes discussed in this review.

flammatory-associated *Faecalibacterium prausnitzii* is reduced [28]. A typical trait of human IBD patients is reduced gut microbial biodiversity [29, 30]. For example, patients with CD had reduced levels of *Faecalibacterium* and *Roseburia*, increased *Ruminococcus* [30] and Enterobacteriaceae including adherent-invasive *Escherichia coli* [31]. Excessive milk fat [32] and omega-6 PUFA [19] were shown in rodents to exacerbate IBD through dysbiosis, which is supported by a 30% increased risk for UC by excessive consumption of omega-6 PUFA [33].

Colorectal Cancer

The adaptation of African-Americans to Western diets has been shown to increase the incidence of and mortality due to colorectal cancer (CRC) corresponding to altered fecal microbial profiles [34]. CRC patients are shown to have increased levels of certain bacterial species such as *Bacteroides fragilis, Enterococcus, Escherichia/Shigella, Klebsiella, Streptococcus, Peptostreptococcus, Roseburia* and decreased abundance in butyrate-producing Lachnospiraceae [35]. Growing evidence supports an inverse relationship between dietary fiber, fruit and vegetable intake to CRC development risk. Long-term fiber intake can result in a microbiota enterotype that positively

associates with Firmicutes and Proteobacteria and inversely with Bacteroidetes, Actinobacteria [6] and *Bifidobacteria* [36]. This may be through improved intestinal barrier function since beneficial microbes improve barrier integrity and this is associated with decreased complications in patients undergoing colectomy [37]. Dietary fiber intake can also reduce the risk of CRC development by promoting a gut microbiota that is enriched by SCFA production [38].

Irritable Bowel Syndrome

Diet and gut microbiota are two crucial components implicated in the pathogenesis of irritable bowel syndrome (IBS). Poorly absorbed dietary carbohydrates induce prolonged hydrogen production in the intestines of patients with IBS (Rome III criteria), which is important since the amount of methane produced corresponds with disease symptoms [39]. IBS patients have an altered carbohydrate and protein energy metabolism in the gut, accompanied by changes in the diversity of particular gut bacterial genera [40], where enriched Firmicutes and reduced Bacteroidetes are found to be associated with a distinct subset of IBS patients [41]. Studies conducted in diarrhea-dominated IBS patients show reduced fecal

aerobic bacteria, *Bifidobacteria* and *Verrucomicrobium* and an increase in *Lactobacillus, Veillonella, Prevotella* and *Parasporo* [42, 43]. In addition, the increase in *E. coli* and decrease in *Leptum* and *Bifidobacteria* and bacteria involved in bile acid transformation is accompanied by an increase in fecal bile acids, which acts as an endogenous laxative further exacerbating disease symptoms [44].

Intestinal Dysbiosis in Systemic Diseases

In addition to local GIT diseases, intestinal dysbiosis is also associated with systemic diseases such as obesity, diabetes, atherosclerosis and nonalcoholic fatty liver disease (NAFLD) (fig. 3). Indeed, many metabolic diseases are associated with chronic inflammation induced by lipopolysaccharide, a major component of the outer membrane of Gram-negative bacteria. Other causative factors associated with the intestinal microbiota include gut barrier dysfunction, immunomodulation, production of SCFA and other metabolites, as well as changes to metabolic pathways involved in nutrient or energy harvest.

> **Specific bacterial phylotypes are present in atherosclerotic plaques that are common to oral or gut samples from patients with atherosclerosis, where the amount of bacterial DNA correlated with the amount of leukocytes found in the atherosclerotic plaque.**

Obesity

Current evidence reveals that gut microbes are critical in overall energy harvest influencing obesity [45]. Fat- and carbohydrate-restricted diets lead to increased Bacteroidetes and decreased Firmicutes [46]. Other diets with low carbohydrate/high protein content, resistant starch [47] or high dietary fiber [48] also lead to distinct increases in various bacterial populations. Obese children have a microbiota enriched with Enterobacteriaceae [49], reduced *Bacteroides* and Bacteroidetes to Firmicutes ratio that are negatively correlated with body mass index [50]. Obese children also have increased Desulfovibrio and *Akkermansia muciniphila* [49] found important for gut barrier dysfunction [51]. Moreover, obese children also have increased SCFAs and more exhaustive substrate utilization implicating that microbes are capable of increased energy harvest [52]. Similarly, obese adolescents had gut microbes that were more engaged in de novo B12 synthesis and butyrate production [53]. Finnish women with metabolic disorders were found to have increased *Eubac-*

terium rectale-Clostridium coccoides that efficiently harvest energy and nutrients and positively correlated with several metabolic markers [54].

Diabetes

Type 2 diabetes (T2D) is a metabolic disorder defined by insulin resistance, impaired intestinal permeability, endotoxemia and chronic inflammation, all of which are linked to diet-induced dysbiosis [55]. Patients with T2D have been shown to have a fecal microbiota with reduced populations of Firmicutes including microbes from the Clostridia clusters [56]. Recently, a metagenome-wide association study involving 345 Chinese subjects had their fecal microbiota shotgun sequenced and dysbiosis was confirmed in patients with T2D. The results revealed that the patients' fecal microbiota was enriched with more opportunistic pathogens and less microbes involved in butyrate production. This resulted in increased microbial functions involving sulfate reduction and oxidative stress resistance [57]. Another study found that T2D patients of Chinese origin also displayed a microbiota decreased in *Bifidobacteria* spp. [58], a beneficial microbe often shown to be decreased in rodent models of T2D. Although mounting evidence reveals that intestinal microbes are important in T1D pathogenesis, so far, little evidence has linked dietary factors to disease progression.

Atherosclerosis

Recent evidence reveals that gut microbiota participates in atherosclerosis, a chronic inflammatory condition of the arteries with the formation of multiple plaques that restrict blood flow. Various microbial byproducts or so-called microbial-associated molecular patterns (MAMPs) play a pivotal role in atherogenesis [59]. Additionally, the metabolism of dietary phosphatidylcholine and the subsequent generation of cardiovascular disease risk markers are gut microbiota dependent [60]. Specific bacterial phylotypes are present in atherosclerotic plaques that are common to oral or gut samples from patients with atherosclerosis, where the amount of bacterial DNA correlated with 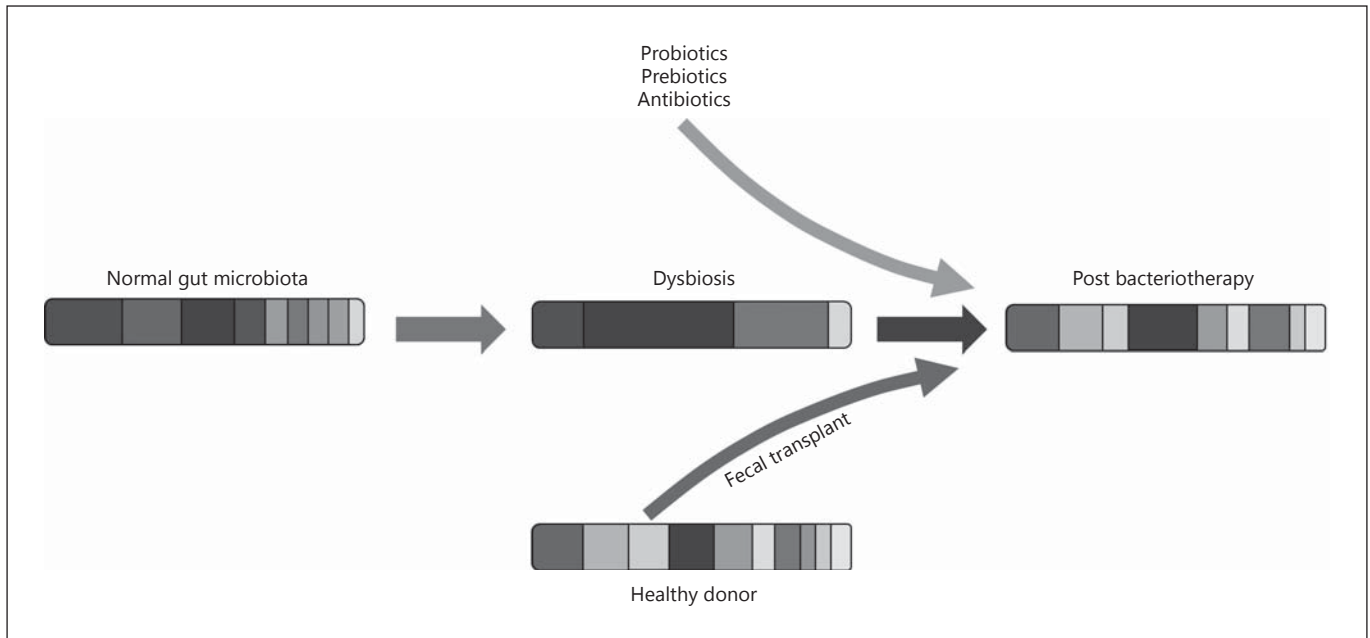the amount of leukocytes found in the atherosclerotic plaque [61]. Shotgun sequencing of fecal samples revealed that the *Bacteroides-* and *Ruminococcus*-domi-

Fig. 4. Bacteriotherapy in the treatment of dysbiosis-associated diseases. Dysbiosis results in an altered microbial composition which is often characterized by significant reduction in species diversity and increased growth of typically nondominating taxa. Bacteriotherapy is a method by which restoration of the original microbiota or a new healthy one is achieved by means of bacterial manipulation. This is achieved by the use of one or a combination of various interventions including: removal of pathogenic bacteria by the use antibiotics, supplementation with prebiotics to promote growth of beneficial bacteria, supplementation with probiotics to compensate inadequate levels of specific bacterial strains and, most recently, introduction of a new microbial ecosystem by transplanting fecal bacteria of a healthy donor.

nated enterotypes were under- and overexpressed, respectively, in atherosclerotic patients. The disease microbiome was enriched in genes encoding peptidoglycan synthesis but depleted in phytoene dehydrogenase required for metabolism of lipid-soluble antioxidants [4]. Although there are limited clinical studies to date, they present a powerful message to potential therapeutic strategies against atherosclerosis targeted to the gut.

Nonalcoholic Fatty Liver Disease

NAFLD is associated with small intestinal bacterial overgrowth (SIBO) and the resulting effects of increased acetaldehyde, trimethylamine, trimethylamine N-oxide and tumor necrosis factor-α [62]. Given that the gut and liver are connected by the portal venous system, it makes the liver more vulnerable to translocation of bacteria, bacterial products, endotoxin or secreted cytokines. An obesogenic microbiota can alternate liver function by stimulating hepatic triglyceride and modulating systemic lipid metabolism that indirectly impact the storage of fatty acids in the liver [63]. In support of this, SIBO corre-

lates with a leaky gut in humans [64] and hepatic steatosis in obese patients [65]. The severity of NAFLD is associated with chronic endotoxin exposure in humans [66]. Choline deficiency and fatty liver development have also been associated with changes in abundance of γ-Proteobacteria and Erysipelotrichi [67]. The diet-induced change of such bacterial abundance further helps predict the risk of fatty liver development.

Bacteriotherapy to Promote a Healthy Microbial Profile

Diet is considered a modifiable intervention; however, our understanding of how to manipulate diet to promote a healthy microbiota is in its infancy, since the effects of many dietary factors are frequently changed, disputed or simply lack evidence. A novel approach to alter our intestinal microbes is by the use of bacteriotherapy (fig. 4). While bacteriotherapy may be an alternative approach to preventing, treating or even curing ailments, there is a lack of clarity as to its efficacy in humans.

Table 2. Summary of clinical studies using probiotics against dysbiosis-induced diseases

Probiotics	Dose, CFU/day	Duration	Sample size	Subject's condition	Gut/fecal microbiota change	Method of bacterial detection	Outcome (generalized)	Ref. PubMed accession No.
B. bifidum MIMBb75	1×10^9	4 weeks	122	IBS	NA	NA	improved IBS symptoms and QOL	21418261
VSL#3	9×10^{11}	8 weeks	24	IBS, diarrhea predominant	no change	microarray hybridization	no major changes	22247743
B. longum L. acidophilus	NA	4 weeks	60	IBS	↑ Bifidobacteria spp. ↑ Lactobacillus spp.	qPCR	improved IBS symptoms	22837798
L. plantarum MF1298	1×10^{10}	6 weeks	28	IBS	no change	direct sequencing, 454 pyrosequencing	aggravated IBS symptoms	22899904
L. casei DG (rectal)	1.6×10^9	8 weeks	26	IBD, mild left-sided UC	↑ Lactobacillus spp. ↓ Enterobacteriaceae	culturing	decreased inflammation	20737210
L. plantarum L. acidophilus B. longum	2.6×10^{14}	6 days pre- and 10 days postoperatively	100	CRC, undergoing colectomy	↑ Bacterial variety	culturing, DGGE	improved gut permeability, improved recovery from surgery	21083585
B. longum W11 FOS Actilight	NA	20 days, 40 days, 60 days	297	chronic constipation with hypocaloric diet	NA	NA	improved constipation	17312986
L. paracasei MGP22043 artichokes	2×10^{10}	15 days	8	constipation	no change	culturing, REP-PCR	altered SCFA conc., fecal metabolic activity, improved constipation	20495470
L. casei Shirota	1.95×10^{10}	3 months	28	metabolic syndrome	NA	NA	increased inflammation	22872030
L. salivarius Ls-33	1×10^{10}	12 weeks	50	obesity	similar change in both groups	qPCR	no change in metabolic biomarkers	22695039
L. rhamnosus GG	1×10^{10}	4 weeks pre- and 6 weeks post-delivery	159	pregnant	NA	NA	restrained excessive weight gain in offspring during their first years of life	20231842
B. longum FOS	NA	24 weeks	66	NASH	NA	NA	reduced inflammation and NASH activity index	21901256
E. faecium M-74, selenium	2×10^9	56 weeks	43	healthy	NA	NA	reduced total cholesterol and low-density lipoprotein	16026136
L. plantarum 299v	5×10^9	6 weeks	36	heavy smokers	NA	NA	reduced cardiovascular risk	12450890
L. plantarum DSM 9843	1×10^{11}	4 weeks	16	incipient arteriosclerosis	↑ Lactobacillus spp. ↑ diversity	culturing, T-RFLP	decreased SCFA conc., no change in blood markers	19608185

CFU = Colony forming units; conc. = concentration; DGGE = denaturing gradient gel electrophoresis; NA = not available; NASH = nonalcoholic steatohepatitis; QOL = quality of life; qPCR = quantitative polymerase chain reaction; REP-PCR = repetitive sequence-based polymerase chain reaction; T-RFLP = terminal restriction fragment length polymorphism.

Probiotics, Prebiotics and Symbiotics

Probiotics are defined as live microorganisms which confer health benefits to the host when taken in adequate quantities. These are discussed by Versalovic in this issue. Prebiotics are nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of beneficial colonic bacteria. A combination of probiotics and prebiotics is termed symbiotics. Probiotics are strain specific and require sufficient dosages and time to exert efficient effects. Various kinds of probiotics have been tested clinically as potential therapeutic agents for both localized and systemic diseases. A recent review of the effects of probiotics on health and disease is shown in table 2. The effects of probiotics in local gastrointestinal diseases are generally positive, although there is usually a lack of evidence that the effects were gut microbiota mediated. While still largely unknown, the effects of probiotics in systemic diseases are more variable.

Antibiotics

It is well documented that antibiotic treatments cause aberrancies in the host microbiota. Though it is generally believed that such changes are normalized within weeks of cessation of antibiotics, recent evidence challenges this notion [68]. For example, significant reduction in diversity of *Bacteroides* persisted up to 2 years following 7 days of Clindamycin administration [68]. In the context of dysbiosis, antibiotics thus can be viewed as a double-edged sword. They are effective in eradicating pathogens but also nonspecifically reduce microbial diversity enabling opportunistic bacteria to colonize the newly hospitable niches in the gut ecosystem. Such is the case of *Clostridium difficile,* an opportunistic pathogen which emerged in the 1970s in patients treated with Clindamycin [69]. Another example of antibiotics' conflicting nature in dysbiosis is their effect on IBD. For instance, the use of ciprofloxacin has been shown clinically to modestly improve symptoms and remission rates of patients with CD [70]; however, antibiotic exposure in childhood has been associated with development of IBD in later years [71]. In a clinical setting, this raises important concern regarding the appropriate use or avoidance of antibiotics. It is important to develop more specific antimicrobial or concurrent therapies to restore or minimize disturbances to the normal microbiota.

Fecal Transplantation

One promising approach for relieving dysbiosis-associated diseases is the re-establishment of normal microbiota via transplantation of a healthy donor's stool into a symptomatic host, called fecal transplantation (FT) (fig. 4). In clinical settings, FT has emerged as a much more effective and safer procedure than standard antibiotics treatment in the immediate and lasting resolution of recurrent *C. difficile*. Currently, this procedure suffers from a lack of standardization; however, its success rate being above 95% [72] and seemingly lack of adverse effects has led experts to investigate its use in treatment of other chronic illnesses such as IBD [73] and metabolic syndrome [74]. As our understanding of the essential role that host microbiota plays in disease and immunity increases, the use of microbiota manipulation therapies becomes more sensible. For example, one possible future venue for FT is the use of a patient's own stored healthy stool to restore their intestinal microbiota following antibiotic treatment or disease onset. Due to its inexpensive nature, FT might be particularly favorable in populations where expensive treatments are not easily accessible.

Conclusions

Interactions between different dietary factors and gut microbes may lead to dysbiosis that exerts distinct immune responses in the host, resulting in higher susceptibility to various gastrointestinal and systemic diseases. Restoration and maintenance of a healthy gut microbiota may be an effective, inexpensive and safe remedy to these diseases.

Disclosure Statement

The authors declare no conflicts of interest. The writing of this article was supported by Nestlé Nutrition Institute.

References

1 Ley RE, Peterson DA, Gordon JI: Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell 2006; 124:837–848.

2 Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R, Angenent LT, Ley RE: Succession of microbial consortia in the developing infant gut microbiome. Proc Natl Acad Sci USA 2011;108(suppl 1):4578–4585.

3 Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, Fernandes GR, Tap J, Bruls T, Batto JM, et al: Enterotypes of the human gut microbiome. Nature 2011;473: 174–180.

4 Karlsson FH, Fak F, Nookaew I, Tremaroli V, Fagerberg B, Petranovic D, Backhed F, Nielsen J: Symptomatic atherosclerosis is associated with an altered gut metagenome. Nat Commun 2012;3:1245.

5 Zhu L, Baker SS, Gill C, Liu W, Alkhouri R, Baker RD, Gill SR: Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. Hepatology 2013;57:601–609.

6 Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, Bewtra M, Knights D, Walters WA, Knight R, et al: Linking long-term dietary patterns with gut microbial enterotypes. Science 2011;334:105–108.

7 Donohoe M: Re: future of board certification in a new era of public accountability. J Am Board Fam Med 2010;23:687; author reply 687–688.

8 Vernocchi P, Vannini L, Gottardi D, Del Chierico F, Serrazanetti DI, Ndagijimana M, Guerzoni ME: Integration of datasets from different analytical techniques to assess the impact of nutrition on human metabolome. Front Cell Infect Microbiol 2012;2:156.

9 Poroyko V, Morowitz M, Bell T, Ulanov A, Wang M, Donovan S, Bao N, Gu S, Hong L, Alverdy JC, et al: Diet creates metabolic niches in the 'immature gut' that shape microbial communities. Nutr Hosp 2011;26:1283–1295.

10 Kuda T, Nakamura S, An C, Takahashi H, Kimura B: Effect of soy and milk protein-related compounds on Listeria monocytogenes infection in human enterocyte Caco-2 cells and A/J mice. Food Chem 2012;134:1719–1723.

11 Brown K, DeCoffe D, Molcan E, Gibson DL: Diet-induced dysbiosis of the intestinal microbiota and the effects on immunity and disease. Nutrients 2012;4:1095–1119.

12 Adler CJ, Dobney K, Weyrich LS, Kaidonis J, Walker AW, Haak W, Bradshaw CJ, Townsend G, Soltysiak A, Alt KW, et al: Sequencing ancient calcified dental plaque shows changes in oral microbiota with dietary shifts of the Neolithic and Industrial revolutions. Nat Genet 2013;45:450–455.

13 Gomez-Llorente C, Plaza-Diaz J, Aguilera M, Munoz-Quezada S, Bermudez-Brito M, Peso-Echarri P, Martinez-Silla R, Vasallo-Morillas MI, Campana-Martin L, Vives-Pinera I, et al: Three main factors define changes in fecal microbiota associated with feeding modality in infants. J Pediatr Gastroenterol Nutr 2013, E-pub ahead of print.

14 Yoshioka H, Iseki K, Fujita K: Development and differences of intestinal flora in the neonatal period in breast-fed and bottle-fed infants. Pediatrics 1983;72:317–321.

15 Nielsen S, Nielsen DS, Lauritzen L, Jakobsen M, Michaelsen KF: Impact of diet on the intestinal microbiota in 10-month-old infants. J Pediatr Gastroenterol Nutr 2007;44:613–618.

16 Ijssennagger N, Derrien M, van Doorn GM, Rijnierse A, van den Bogert B, Müller M, Dekker J, Kleerebezem M, van der Meer R: Dietary heme alters microbiota and mucosa of mouse colon without functional changes in host-microbe cross-talk. PLoS One 2012;7:e49868.

17 Liszt K, Zwielehner J, Handschur M, Hippe B, Thaler R, Haslberger AG: Characterization of Bacteria, Clostridia and Bacteroides in faeces of vegetarians using qPCR and PCR-DGGE fingerprinting. Ann Nutr Metab 2009;54:253–257.

18 De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, Lionetti P: Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci USA 2010;107:14691–14696.

19 Ghosh S, DeCoffe D, Brown K, Rajendiran E, Estaki M, Dai C, Yip A, Gibson DL: Fish oil attenuates omega-6 polyunsaturated fatty acid-induced dysbiosis and infectious colitis but impairs LPS dephosphorylation activity causing sepsis. PLoS One 2013;8:e55468.

20 Ghosh S, Molcan E, Decoffe D, Dai C, Gibson DL: Diets rich in n-6 PUFA induce intestinal microbial dysbiosis in aged mice. Br J Nutr 2013;110:515–523.

21 Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI: The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Sci Transl Med 2009;1:6ra14.

22 Smith MI, Yatsunenko T, Manary MJ, Trehan I, Mkakosya R, Cheng J, Kau AL, Rich SS, Concannon P, Mychaleckyj JC, et al: Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. Science 2013;339:548–554.

23 Donovan SM, Wang M, Li M, Friedberg I, Schwartz SL, Chapkin RS: Host-microbe interactions in the neonatal intestine: role of human milk oligosaccharides. Adv Nutr 2012;3:450S–455S.

24 Kashyap PC, Marcobal A, Ursell LK, Larauche M, Duboc H, Earle KA, Sonnenburg ED, Ferreyra JA, Higginbottom SK, Million M, et al: Complex interactions among diet, gastrointestinal transit, and gut microbiota in humanized mice. Gastroenterology 2013;144:967–977.

25 Islam KB, Fukiya S, Hagio M, Fujii N, Ishizuka S, Ooka T, Ogura Y, Hayashi T, Yokota A: Bile acid is a host factor that regulates the composition of the cecal microbiota in rats. Gastroenterology 2011;141:1773–1781.

26 Lepage P, Hasler R, Spehlmann ME, Rehman A, Zvirbliene A, Begun A, Ott S, Kupcinskas L, Dore J, Raedler A, et al: Twin study indicates loss of interaction between microbiota and mucosa of patients with ulcerative colitis. Gastroenterology 2011;141:227–236.

27 Rowan F, Docherty NG, Murphy M, Murphy B, Calvin Coffey J, O'Connell PR: Desulfovibrio bacterial species are increased in ulcerative colitis. Dis Colon Rectum 2010;53:1530–1536.

28 Varela E, Manichanh C, Gallart M, Torrejon A, Borruel N, Casellas F, Guarner F, Antolin M: Colonisation by Faecalibacterium prausnitzii and maintenance of clinical remission in patients with ulcerative colitis. Aliment Pharmacol Ther 2013;38:151–161.

29 Michail S, Durbin M, Turner D, Griffiths AM, Mack DR, Hyams J, Leleiko N, Kenche H, Stolfi A, Wine E: Alterations in the gut microbiome of children with severe ulcerative colitis. Inflamm Bowel Dis 2012;18:1799–1808.

30 Kang S, Denman SE, Morrison M, Yu Z, Dore J, Leclerc M, McSweeney CS: Dysbiosis of fecal microbiota in Crohn's disease patients as revealed by a custom phylogenetic microarray. Inflamm Bowel Dis 2010;16:2034–2042.

31 Manichanh C, Rigottier-Gois L, Bonnaud E, Gloux K, Pelletier E, Frangeul L, Nalin R, Jarrin C, Chardon P, Marteau P, et al: Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. Gut 2006;55:205–211.

32 Devkota S, Wang Y, Musch MW, Leone V, Fehlner-Peach H, Nadimpalli A, Antonopoulos DA, Jabri B, Chang EB: Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10–/– mice. Nature 2012;487:104–108.

33 Tjonneland A, Overvad K, Bergmann MM, Nagel G, Linseisen J, Hallmans G, Palmqvist R, Sjodin H, Hagglund G, Berglund G, et al: Linoleic acid, a dietary n-6 polyunsaturated fatty acid, and the aetiology of ulcerative colitis: a nested case-control study within a European prospective cohort study. Gut 2009;58:1606–1611.

34 Mai V, McCrary QM, Sinha R, Glei M: Associations between dietary habits and body mass index with gut microbiota composition and fecal water genotoxicity: an observational study in African American and Caucasian American volunteers. Nutr J 2009;8:49.

35 Wang T, Cai G, Qiu Y, Fei N, Zhang M, Pang X, Jia W, Cai S, Zhao L: Structural segregation of gut microbiota between colorectal cancer patients and healthy volunteers. ISME J 2012;6:320–329.

36 Davis LM, Martinez I, Walter J, Goin C, Hutkins RW: Barcoded pyrosequencing reveals that consumption of galactooligosaccharides results in a highly specific bifidogenic response in humans. PLoS One 2011;6:e25200.

37 Liu Z, Qin H, Yang Z, Xia Y, Liu W, Yang J, Jiang Y, Zhang H, Wang Y, Zheng Q: Randomised clinical trial: the effects of perioperative probiotic treatment on barrier function and post-operative infectious complications in colorectal cancer surgery – a double-blind study. Aliment Pharmacol Ther 2011;33:50–63.

38 Chen HM, Yu YN, Wang JL, Lin YW, Kong X, Yang CQ, Yang L, Liu ZJ, Yuan YZ, Liu F, et al: Decreased dietary fiber intake and structural alteration of gut microbiota in patients with advanced colorectal adenoma. Am J Clin Nutr 2013;97:1044–1052.

39 Ong DK, Mitchell SB, Barrett JS, Shepherd SJ, Irving PM, Biesiekierski JR, Smith S, Gibson PR, Muir JG: Manipulation of dietary short chain carbohydrates alters the pattern of gas production and genesis of symptoms in irritable bowel syndrome. J Gastroenterol Hepatol 2010;25:1366–1373.

40 Ponnusamy K, Choi JN, Kim J, Lee SY, Lee CH: Microbial community and metabolomic comparison of irritable bowel syndrome faeces. J Med Microbiol 2011;60:817–827.

41 Jeffery IB, O'Toole PW, Ohman L, Claesson MJ, Deane J, Quigley EM, Simren M: An irritable bowel syndrome subtype defined by species-specific alterations in faecal microbiota. Gut 2012;61:997–1006.

42 Carroll IM, Chang YH, Park J, Sartor RB, Ringel Y: Luminal and mucosal-associated intestinal microbiota in patients with diarrhea-predominant irritable bowel syndrome. Gut Pathog 2010;2:19.

43 Rigsbee L, Agans R, Shankar V, Kenche H, Khamis HJ, Michail S, Paliy O: Quantitative profiling of gut microbiota of children with diarrhea-predominant irritable bowel syndrome. Am J Gastroenterol 2012;107:1740–1751.

44 Duboc H, Rainteau D, Rajca S, Humbert L, Farabos D, Maubert M, Grondin V, Jouet P, Bouhassira D, Seksik P, et al: Increase in fecal primary bile acids and dysbiosis in patients with diarrhea-predominant irritable bowel syndrome. Neurogastroenterol Motil 2012; 24:513–520, e246–e517.

45 Jumpertz R, Le DS, Turnbaugh PJ, Trinidad C, Bogardus C, Gordon JI, Krakoff J: Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. Am J Clin Nutr 2011;94:58–65.

46 Ley RE, Turnbaugh PJ, Klein S, Gordon JI: Microbial ecology: human gut microbes associated with obesity. Nature 2006;444:1022–1023.

47 Walker AW, Ince J, Duncan SH, Webster LM, Holtrop G, Ze X, Brown D, Stares MD, Scott P, Bergerat A, et al: Dominant and diet-responsive groups of bacteria within the human colonic microbiota. ISME J 2011;5:220–230.

48 Shen Q, Zhao L, Tuohy KM: High-level dietary fibre up-regulates colonic fermentation and relative abundance of saccharolytic bacteria within the human faecal microbiota in vitro. Eur J Nutr 2012;51:693–705.

49 Karlsson CL, Onnerfalt J, Xu J, Molin G, Ahrne S, Thorngren-Jerneck K: The microbiota of the gut in preschool children with normal and excessive body weight. Obesity (Silver Spring) 2012;20:2257–2261.

50 Xu P, Li M, Zhang J, Zhang T: Correlation of intestinal microbiota with overweight and obesity in Kazakh school children. BMC Microbiol 2012;12:283.

51 Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, Guiot Y, Derrien M, Muccioli GG, Delzenne NM, et al: Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. Proc Natl Acad Sci USA 2013;110:9066–9071.

52 Payne AN, Chassard C, Zimmermann M, Muller P, Stinca S, Lacroix C: The metabolic activity of gut microbiota in obese children is increased compared with normal-weight children and exhibits more exhaustive substrate utilization. Nutr Diabetes 2011;1:e12.

53 Ferrer M, Ruiz A, Lanza F, Haange SB, Oberbach A, Till H, Bargiela R, Campoy C, Segura MT, Richter M, et al: Microbiota from the distal guts of lean and obese adolescents exhibit partial functional redundancy besides clear differences in community structure. Environ Microbiol 2013;15:211–226.

54 Munukka E, Wiklund P, Pekkala S, Volgyi E, Xu L, Cheng S, Lyytikainen A, Marjomaki V, Alen M, Vaahtovuo J, et al: Women with and without metabolic disorder differ in their gut microbiota composition. Obesity (Silver Spring) 2012;20:1082–1087.

55 Cani PD, Osto M, Geurts L, Everard A: Involvement of gut microbiota in the development of low-grade inflammation and type 2 diabetes associated with obesity. Gut Microbes 2012;3:279–288.

56 Larsen N, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK, Al-Soud WA, Sorensen SJ, Hansen LH, Jakobsen M: Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. PLoS One 2010;5:e9085.

57 Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, Shen D, et al: A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 2012;490:55–60.

58 Le KA, Li Y, Xu X, Yang W, Liu T, Zhao X, Tang YG, Cai D, Go VL, Pandol S, et al: Alterations in fecal Lactobacillus and Bifidobacterium species in type 2 diabetic patients in Southern China population. Front Physiol 2012;3:496.

59 Michelsen KS, Wong MH, Shah PK, Zhang W, Yano J, Doherty TM, Akira S, Rajavashisth TB, Arditi M: Lack of toll-like receptor 4 or myeloid differentiation factor 88 reduces atherosclerosis and alters plaque phenotype in mice deficient in apolipoprotein E. Proc Natl Acad Sci USA 2004;101:10679–10684.

60 Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, Britt EB, Fu X, Chung YM, et al: Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 2011;472:57–63.

61 Koren O, Spor A, Felin J, Fak F, Stombaugh J, Tremaroli V, Behre CJ, Knight R, Fagerberg B, Ley RE, et al: Human oral, gut, and plaque microbiota in patients with atherosclerosis. Proc Natl Acad Sci USA 2011;108(suppl 1):4592–4598.

62 Quigley EM, Stanton C, Murphy EF: The gut microbiota and the liver. Pathophysiological and clinical implications. J Hepatol 2013;58:1020–1027.

63 Backhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JI: The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci USA 2004;101:15718–15723.

64 Miele L, Valenza V, La Torre G, Montalto M, Cammarota G, Ricci R, Masciana R, Forgione A, Gabrieli ML, Perotti G, et al: Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. Hepatology 2009;49:1877–1887.

65 Sabate JM, Jouet P, Harnois F, Mechler C, Msika S, Grossin M, Coffin B: High prevalence of small intestinal bacterial overgrowth in patients with morbid obesity: a contributor to severe hepatic steatosis. Obes Surg 2008;18:371–377.

66 Verdam FJ, Rensen SS, Driessen A, Greve JW, Buurman WA: Novel evidence for chronic exposure to endotoxin in human nonalcoholic steatohepatitis. J Clin Gastroenterol 2011;45:149–152.

67 Spencer MD, Hamp TJ, Reid RW, Fischer LM, Zeisel SH, Fodor AA: Association between composition of the human gastrointestinal microbiome and development of fatty liver with choline deficiency. Gastroenterology 2011;140:976–986.

68 Jernberg C, Lofmark S, Edlund C, Jansson JK: Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. ISME J 2007;1:56–66.

69 Taubes G: The bacteria fight back. Science 2008;321:356–361.

70 Arnold GL, Beaves MR, Pryjdun VO, Mook WJ: Preliminary study of ciprofloxacin in active Crohn's disease. Inflamm Bowel Dis 2002;8:10–15.

71 Kronman MP, Zaoutis TE, Haynes K, Feng R, Coffin SE: Antibiotic exposure and IBD development among children: a population-based cohort study. Pediatrics 2012;130:e794–e803.

72 de Vos WM: Fame and future of faecal transplantations – developing next-generation therapies with synthetic microbiomes. Microb Biotechnol 2013;6:316–325.

73 Anderson JL, Edney RJ, Whelan K: Systematic review: faecal microbiota transplantation in the management of inflammatory bowel disease. Aliment Pharmacol Ther 2012;36:503–516.

74 Vrieze A, Van Nood E, Holleman F, Salojarvi J, Kootte RS, Bartelsman JF, Dallinga-Thie GM, Ackermans MT, Serlie MJ, Oozeer R, et al: Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology 2012;143:913–916, e917.