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REVIEW

Signals from the gut microbiota to distant organs in physiology and disease

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The ecosystem of the human gut consists of trillions of bacteria forming a bioreactor that is fueled by dietary macronutrients to produce bioactive compounds. These microbiota-derived metabolites signal to distant organs in the body, which enables the gut bacteria to connect to the immune and hormone system, to the brain (the gut–brain axis) and to host metabolism, as well as other functions of the host. This microbe–host communication is essential to maintain vital functions of the healthy host. Recently, however, the gut microbiota has been associated with a number of diseases, ranging from obesity and inflammatory diseases to behavioral and physiological abnormalities associated with neurodevelopmental disorders. In this Review, we will discuss microbiota–host cross-talk and intestinal microbiome signaling to extraintestinal organs. We will review mechanisms of how this communication might contribute to host physiology and discuss how misconfigured signaling might contribute to different diseases.

There are more microbial cells in the gut as human cells in the body[1]. Approximately 1,200 different bacterial species have been identified in at least the same amount of the human gut microbiota, and each individual is host to a distinct set of at least 160 species in the gut[2–6]. The collective microbial genome encodes 500 times more genes than the human genome[3,6,7], and so it is tempting to consider human genes as noise in the storm of microbial signals. Recent data suggest that microbial signals modulate crucial functions of the healthy human body, ranging from host metabolism to brain function. There are also accumulating data suggesting that many human diseases have their origin in distorted gut microbiota composition—or potentially, in microbial metabolites that signal to distant organs (**Table 1**). Here we discuss recent findings of how the gut microbiota signals to peripheral organs distant from the gut, and how this communication affects physiology and disease.

The gut microbiota

In humans, microbial density increases from the proximal to the distal end of the intestine and comprises a biomass of 1.5–2.0 kg, dominated by strictly anaerobic Bacteria[3]. Archaea, Eukarya and viruses are also present, but their relevance for human health has been less studied.

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Although the vast majority of the gut microbial community is composed of only five phyla (Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria and Verrucomicrobia), there is considerable diversity on the species level and their relative abundances. Key members of Firmicutes include the genera *Clostridium, Lactobacillus* and *Ruminococcus*, as well as the butyrate producers *Eubacterium, Fecalibacterium* and *Roseburia*. Members of Bacteroidetes are known to be efficient degraders of dietary fiber and include the genera *Bacteroides, Prevotella* and *Xylanibacter*. *Bifidobacterium* is a major genus within Actinobacteria, and several taxa are used as probiotics. Proteobacteria includes *Escherichia* and *Desulfovibrio*, whereas Verrucomicrobia so far includes only the mucus-degrading genus *Akkermansia.*

The composition of the gut microbiota is influenced by genetic and environmental factors starting early in life (**Box 1**, also recently reviewed elsewhere[8]). It has been postulated that each individual can be grouped into one of three bacterial clusters called enterotypes, which are defined by the relative abundance of the genera *Bacteroides, Prevotella* or *Ruminococcus*[9]. Although the presence of enterotypes has been debated[10] and is probably not as discrete as originally suggested, it is clear that members of the gut microbiota co-occur and rely on each other's metabolic activities[11]. By contrast, competition for similar environmental conditions and nutrients restricts bacterial colonization and leads to niche competition within or between bacterial species[11–13].

Gut microbiota analyses in humans are often based on fecal material, which is easily accessible. Yet microbiota composition varies along the intestinal tract and differs even between the intestinal lumen and mucosa-adherent bacteria that reside in the mucus[4]. Because of oxygen diffusion from the epithelium, even aerobic bacteria have been detected in mouse crypts[14]. Thus, analysis of fecal microbiota composition is a valuable tool and could serve as a biomarker, but it might not accurately reflect the microbes that are in closest contact

Table 1 The intestinal microbiota communicates with peripheral organs in the body and influences processes in health and disease.

Organ	Process influences by gut microbiota	Disease associated with dysbiosis/microbial metabolites	Selected references
Adipose tissue	Adipocyte volume Thermogenesis Browning Inflammation	Obesity/insulin resistance Insulin resistance	20,32,33,62,63,67,80–82
Liver	Bile acid metabolism Lipogenesis Energy expenditure	NAFLD/NASH	41–44,49,50,62,70,87
Pancreas	Insulin secretion	Type 2 diabetes	40
Whole body	Body growth	Undernourishment	34,40,73–75
Cardiovascular system		Stroke Atherosclerosis Thrombosis	91–93,115,117
Brain	Behavior Serotonin metabolism Intestinal gluconeogenesis Blood–brain barrier Appetite regulation	Autism spectrum disorder Stress response Metabolic disease	38,99,101,105,109,111,112
Lung	Gene expression	Allergic asthma	119,120,122

with the host. Moreover, as we will discuss in this Review, many of the physiological effects attributed to the gut microbiota are caused by their metabolites. Because different microbes might produce the same metabolites and those small molecules are less restricted in their spatial diffusion[15], the presence or absence of a single bacterial species is valuable but not sufficient for understanding the detailed interaction between the microbiota and the host.

Gut-microbiota-derived signaling molecules

The intestinal microbiota is in a homeostatic relationship with the gut mucosal immune system, and disruption of this interaction might lead to diseases, which has been reviewed extensively elsewhere[16–18]. Yet, to communicate with distant organs, gut microbial signals first need to be transmitted across the intestinal epithelium. These signals (or molecules) can be either structural components of the bacteria or metabolites produced from the microbiota that can affect distal organs either directly or by signaling through nerves or hormones from the gut.

Immune signals. Microbe-associated molecular patterns (MAMPs) such as lipopolysaccharide (LPS), peptidoglycan, flagellin or other structural components are recognized by pattern-recognition receptors (PRRs), such as Toll-like receptors (TLRs), NOD-like receptors (NLRs) or RIG-1-like receptors (RLRs), on epithelial and immune cells (as reviewed elsewhere[19]), and translocation across the epithelial barrier is generally prevented. However, low amounts of bacterial products such as LPS might reach the lymph and circulation through paracellular diffusion, transcellular transport or co-transport with chylomicrons, which might affect disease development (see Overnutrition)[20–22]. Because the structural composition of LPSs varies between different bacterial species, it will be important to clarify whether the absolute LPS amount in the intestinal lumen determines circulating levels or whether specific LPS structures translocate more efficiently. Remarkably, LPSs derived from different gut microbial species induce TLR4 signaling differently[23] and might also have distinct effects early in life (**Box 1**)[24]. However, the link to human data is currently associative and requires further investigation.

Only a fraction of microbial signaling can be attributed to general recognition of microbial derivatives through PRRs[25], and there are probably more specific microbial signals that regulate host transcription. The transfer of human microbiota into the gut of germ-free mice, however, induced metabolic gene expression but not immune-related

Box 1 Genetic and environmental factors shape the establishment of the gut microbiota.

Monozygotic twins share a larger fraction of their gut microbiota than unrelated individuals, suggesting that the host genotype contributes to microbiota composition[135]. And indeed, the abundance of specific intestinal inhabitants as Christensenellaceae is determined by host genetics, whereas members of the phylum Bacteroidetes seem rather to be modulated by environmental factors[136]. However, since twins commonly share the same social environment in their infancy, it is difficult to disentangle genetic and environmental influence.

Fine-tuning genetic predetermination, early-life events such as mode of delivery, breast- or formula feeding and number of antibiotic courses shape the seeder community in the intestinal bioreactor[137–140]. Moreover, recent data suggest that the embryo in the womb might not be microbiologically sterile[141], so the mother's microbes might have a stronger impact on the maturing intestinal community than previously thought.

During infancy, diet affects the development of a more adult-like microbiota, and through consumption of oxygen, the young community provides a milieu suitable for the anaerobic population of the adult intestine.

Later during life, when a core microbiota has already been established, diet acquisition (for example, hunted, self-prepared, delivered), quantity and quality (for example, content of fat, fiber, carbohydrates) as well as antibiotic perturbations determine the gut microbiota stability in the bioreactor[12,142–145]. Although the overall temporal variability is comparably low, perturbations as dramatically altered diet, an infection or traveling, can alter the community rapidly within a few days[146–148]. On the contrary, an antibiotic-disturbed microbiota may respond much more slowly, and restoration of the initial state can take weeks to months, or not even recover at all[149].

genes in the intestine[12,26–28], which suggests that the metabolic pathways might be more conserved and involve common metabolites that are produced by both human and mouse microbiota.

Gut microbiota such as *Lactobacilli* have also been suggested to catabolize the amino acid tryptophan into the metabolite indole-3-aldehyde, a ligand to the aryl hydrocarbon receptor (AHR)[29]. AHR is expressed by innate lymphoid cells (ILC3s) group 3), and its activation induces the expression of interleukin (IL)-22, a crucial cytokine that regulates intestinal mucosal homeostasis and provides resistance to the fungus *Candida albicans*[29]. Remarkably, impairment of the gut microbiota's ability to generate AHR ligands has recently been associated with inflammatory bowel disease (IBD)[30].

Short-chain fatty acids (SCFAs). Butyrate, propionate and acetate are the major fermentation products that are generated from gut microbial degradation of dietary fiber, and they are estimated to provide humans with 6–10% of the total daily energy requirement[31]. The excess calories from these fermentation products are then stored in the white adipose tissue (WAT) because the gut microbiota can suppress transcription of intestinal angiopoietin-like protein 4, a lipoprotein lipase inhibitor, which results in increased lipid incorporation in adipocytes[32,33]. This pathway is probably more important for hunter–gatherers who have a more fiber-based diet than individuals on a westernized diet with less fiber.

SCFAs are also important signals generated by the gut microbiota (**Fig. 1**). They can bind to the G-protein-coupled receptor (GPCR) GPR41 (also known as FFAR3) and induce expression of the enteroendocrine hormone peptide YY (PYY) in gut epithelial L-cells, which inhibits gut motility and increases energy harvest from the diet in mice[34]. Moreover, the binding of SCFAs to GPR41 or GPR43 (FFAR2) triggers secretion of the hormone glucagon-like peptide (GLP)-1 by intestinal L-cells in mice[35], which has a substantial impact on pancreatic function and insulin release, as well as central effects regulating appetite[36]. Given that GLP-1 has an extremely short half-life, some of the more distant effects of SCFAs might be regulated through the binding of SCFAs to receptors on afferent nerves close to the gut, which then relay these signals to the brain. Furthermore, enteric nerves express GPR41 (ref. 37), which would allow direct signaling from the SCFAs to the nervous system.

The effects of acetate, propionate and butyrate on host physiology are distinct and often vary[38]. Propionate can serve as a precursor for intestinal gluconeogenesis, whereas both propionate and butyrate induce the expression of gluconeogenic enzymes. The resulting glucose molecules can induce periportal afferent neural signaling, which leads to improved metabolism[38]. Similarly, acetate has been suggested to suppress appetite through central mechanisms[39]. By contrast, a recent study, which surprisingly found increased acetate production in rodents fed a high-fat diet as compared to those fed a high-fiber diet, showed that acetate stimulated the vagus nerve of the parasympathetic nervous system, which resulted in increased secretion of the 'hunger hormone' ghrelin and thus increased food intake, promoting obesity[40]. Moreover, vagus nerve stimulation by acetate, but not butyrate, triggered insulin secretion from pancreatic beta cells[40]. However, it remains unclear how important these mechanisms are in humans and whether similar pathways can be induced by other microbial metabolites.

SCFAs have been reported to act directly as hormonal molecules by signaling through the G-coupled-receptor GPR43 in mouse WAT, which suppresses insulin-mediated fat accumulation and stimulates energy expenditure in liver and muscle[41]. However, it is at present unclear whether SCFAs reach concentrations in the blood sufficiently high to exert hormonal functions.

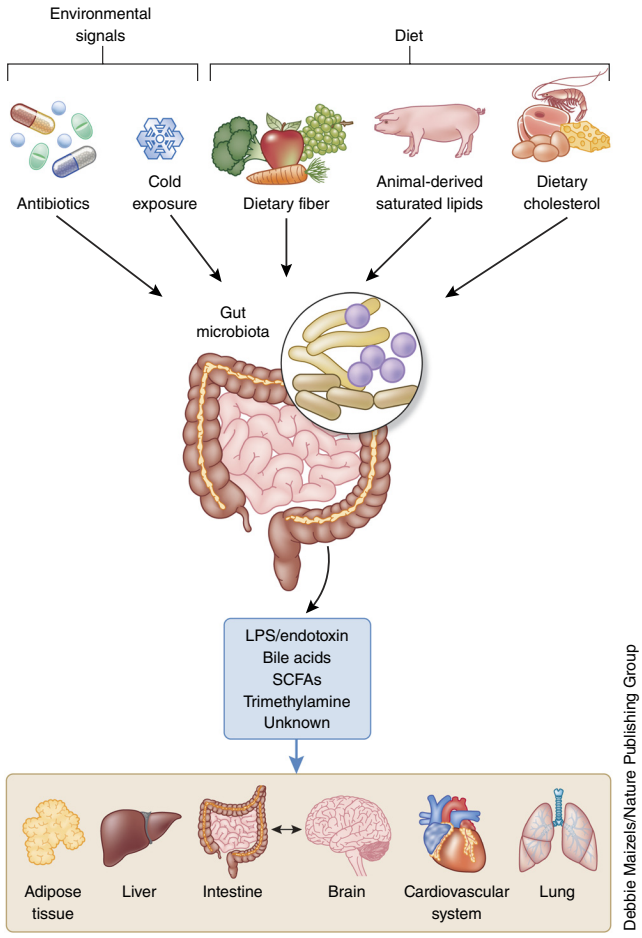

Figure 1 Gut microbiota convert environmental signals and dietary molecules into signaling metabolites to communicate with the host. At the top of the figure are indicated factors that can alter the composition of the gut microbiota. The gut microbiota converts these inputs into metabolites, which can signal to different organs and tissues in the host, as indicated below.

Bile acid metabolism. Bile acids (BAs) are derived from cholesterol in the liver and are further chemically modified by the gut microbiota in the distal small intestine and colon. Recent evidence has identified BAs as important signaling molecules (**Fig. 1**). BAs can activate nuclear receptors, such as farnesoid X receptor (FXR), and G-protein-coupled receptors (GPCRs), such as TGR5, and the microbiota can modify signaling through both receptors via its metabolism of BAs; primary BAs that are secreted into the small intestine can be deconjugated by the ileal gut microbiota, which enables them to escape reabsorption in the small intestine and be subjected to dehydrogenation, dihydroxylation and epimerization by the colonic microbiota[42–44]. By contrast, tauro-β-muricholic acid (TβMCA) is an abundant primary bile acid in mice and was recently identified as an FXR antagonist. Accordingly, microbial metabolism of this bile acid in the ileum relieves FXR inhibition and increases signaling[44]. FXR activation in the intestine induces fibroblast growth factor 15 (FGF15) expression, which suppresses the expression of cholesterol 7α-hydroxylase (CYP7A1) in the liver—the rate-limiting step in bile acid synthesis—and thus leads to

reduced BA levels through a gut–microbiota–liver feedback loop. It will be essential to identify other genes whose expression is induced by the gut microbiota in the small intestine through FXR and elucidate their role in physiology as well as determining whether similar signaling is present in humans.

BAs have also been described to affect the cecal microbiota composition of rats[45]. Although a direct antimicrobial effect of bile acids has been described *in vitro*[46], it is still unclear whether bile acid–mediated microbiota alterations are a direct effect of the bile acids on the bacteria or whether cross-talk with the intestinal mucosa is involved.

Regulation of host metabolism

In addition to regulating bile acid homeostasis, FXR signaling also regulates host metabolism, and it was recently demonstrated in mice that microbial activation of FXR is associated with obesity and steatosis[47–49]. In contrast to FXR, TGR5 predominantly recognizes secondary bile acids, which is suggested to have beneficial metabolic consequences, including increased release of GLP1 from L-cells and increased thermogenesis in brown adipose tissue (BAT)[50,51].

The bile acid–microbiota cross-talk might also be crucially involved in the beneficial effect of bariatric surgery[52,53]. Vertical-sleeve gastrectomy (VSG), a bariatric surgical procedure that reduces body weight and improves glucose regulation in humans[54], is associated with higher circulating bile acids and altered gut microbiota composition in mice[52]. The altered bile acid homeostasis and subsequent signaling might be essential to achieve the beneficial metabolic effects observed after VSG; mice deficient in TGR5 do not exhibit improved glucose metabolism despite weight reduction[55]. However, signaling through FXR also seems to be important because mice deficient in FXR did not exhibit improved glucose metabolism or weight reduction after undergoing VSG[52].

Thus, there are conflicting data on the effects of FXR signaling on host metabolism: in models of obesity, inhibition, of FXR signaling improves metabolism[47–49], but at the same time for example, it seems to be important after VSG to activate FXR signaling for beneficial effects on metabolism[52]. Furthermore, a recent study demonstrated that a supposedly intestine-specific FXR agonist has metabolically beneficial effects[56]. To add to the complexity, stimulation of FXR in different organs might have different metabolic effects, probably owing to the recruitment of specific sets of co-activators and co-repressors[57]. In humans, it has been demonstrated recently that FXR stimulation is beneficial for the treatment of nonalcoholic steatohepatitis (NASH)[58]. Targeting the microbiota to generate a favorable bile acid pool that specifically affects FXR and TGR5 could thus be a therapeutic avenue through which to improve metabolism in humans.

Brown adipose tissue and browning of white adipose tissue. BAT is the major site of thermogenesis in mammals and is characterized by the production of high levels of uncoupling protein-1 (UCP1) by mitochondria[59]. BAT develops during embryogenesis; β-adrenergic stimulation or cold exposure, however, results in 'browning' of precursor cells in WAT depots of mice[60], a process demonstrated to be metabolically beneficial[61]. Two recent studies in mice have demonstrated that a reduction in ambient temperature alters the gut microbiota, and that the altered microbiota might contribute to both the activation of BAT[62] and the browning of WAT[63] (**Fig. 2**). Whereas the increase in BAT activity was associated with increased AMPK phosphorylation in the liver[62], which previously has been linked to the protection of germ-free mice from diet-induced obesity[33], depletion of the microbiota with antibiotics increased the number of beige cells in

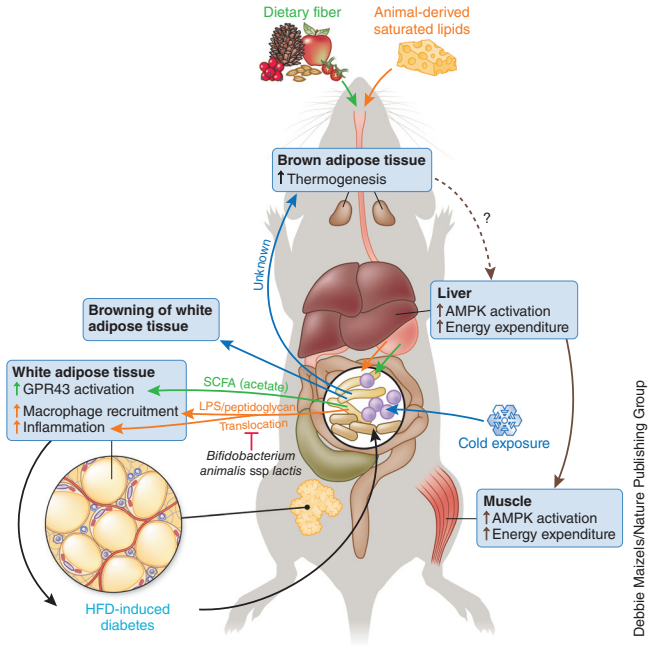

Figure 2 The gut microbiota communicates with host adipose tissue. In mice, animal-derived saturated lipids (orange arrows) promote increased translocation of Gram-negative bacteria and bacterial endotoxin and peptidoglycan into the circulation, resulting in CD14- and NOD1-dependent WAT inflammation, which contributes to the development of type 2 diabetes[20]. The effect can be prevented by application of *Bifidobacterium animalis* ssp. *lactis*[84]. Dietary fiber (green arrows) is fermented by the colonic gut bacteria, generating SCFAs such as acetate, which signal to the host via G-protein coupled receptor 43 (GPR43)[41]. Cold exposure (blue arrows) alters microbial composition, leading to browning of WAT and the activation of BAT[62,63]. The increased thermogenesis affects liver and muscle function through the activation of AMPK, leading to increased energy expenditure.

subcutaneous and visceral WAT, and consequently, stimulated energy expenditure through thermogenesis[64]. This process was dependent on macrophage infiltration and the type 2 cytokines IL-4, IL-13 and IL-5, but the microbial-derived signals leading to immune cell activation and browning still need to be identified. One potential factor could be bile acids because the bile acid profile in cold-exposed mice resembles that of germ-free mice[62].

Thermogenesis in adipocytes is also controlled by the sympathetic nervous system. An increased sympathetic tone to WAT and BAT upon energy shortage or cold exposure activates lipolysis to provide free fatty acids to activate UCP1 (ref. 65). It is currently not known whether gut microbiota and their metabolites can also modulate the sympathetic tone to WAT and BAT during thermogenesis and whether similar microbial activation is at play in humans. However, the microbiota is also altered in larger animals in response to cold environment such as during hibernation[66]. Brown bears have an altered microbiota during winter, which induced less adiposity than the summer microbiota after transfer to germ-free mice[66]. These data suggest that the gut microbiota might be an important factor for energy extraction and a therapeutic target for undernourishment.

Undernourishment. Evidence is accumulating that the gut microbiota is involved in many human diseases (**Fig. 3**). Altered microbial communities as a result of both undernourishment and obesity have been suggested to contribute to the pathogenesis of these

diseases, given that the metabolic phenotype of these disorders can be transferred to germ-free mice, provided the relevant diet, upon fecal transplant[67–71].

As the first dietary encounter, breast milk is a source rich in human milk oligosaccharides (HMOs), which resist degradation in the upper small intestine and nourish the colonic gut bacteria as soluble fiber[72]. Total and sialylated HMOs were reduced in mothers with infants whose growth was severely stunted[73], and supplementation of sialylated bovine milk oligosaccharides promoted growth in gnotobiotic mice and piglets colonized with microbiota from infants with stunted growth[73]. No growth effect was observed in germ-free mice, demonstrating a microbiota-dependent effect. Given these findings, it will be notable to investigate whether sialylated HMOs promote the expansion of distinct microbiota that are beneficial for the host, or whether microbial degradation products such as sialylated monosaccharides or sialic acid are the beneficial metabolite promoting host growth.

Furthermore, undernourished children were found to harbor immature gut microbiota[74], and the presence of a mature community or the addition of *Ruminococcus gnavus* and *Clostridium symbiosum* were sufficient to rescue growth impairment in mice harboring the immature microbiota from undernourished children[70]. Although the underlying molecular signals are unknown, a recent study highlighted that germ-free mice have stunted growth on a diet that was sufficient to promote adequate growth in colonized mice[75]. The stunting was associated with reduced activity of the somatotropic growth hormone (GH)– insulin-like growth factor-1 (IGF1) axis, a major driver of postnatal growth, as revealed by reduced IGF1 serum levels and decreased expression of IGF1 in the liver in germ-free as compared to convetionally raised mice[75]. Mono-colonization of undernourished juvenile GF mice with a selected *Lactobacillus plantarum* strain recovered somatotropic axis signaling and systemic growth. However, mechanisms underlying the increase in IGF signaling remain to be identified, as does the relevance of this pathway in humans whose growth is stunted. But supplementing nutrition therapy with specific probiotic commensals might enhance dietary interventions used to treat undernutrition in the future.

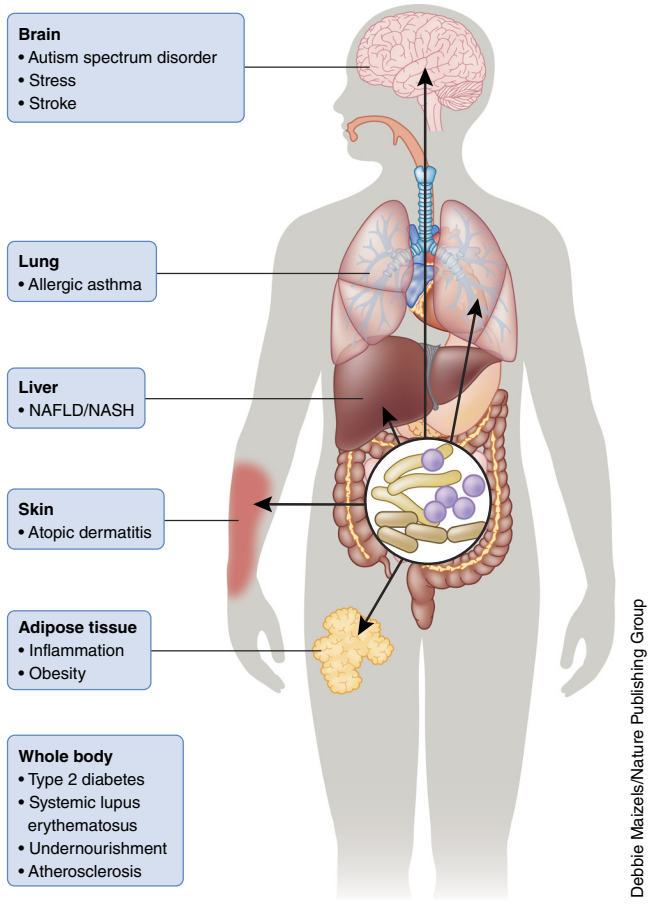

Figure 3 The gut microbiota is associated with various diseases in humans. Alterations in composition, diversity and metabolites derived from the gut microbiota are associated with diseases affecting different organs of the human body. Evidence for a causative role of the gut bacteria is strongest in metabolic disease.

Overnutrition. As discussed above, microbial metabolism of bile acids might regulate host metabolism, but of course, the microbiota can regulate metabolic diseases through other pathways as well. Data obtained from mice strongly suggest that the gut microbiota can contribute to obesity[33,67,68,76,77], and obesity in humans has been associated with reduced microbial diversity as compared to lean controls. However, it is at present unknown whether the reduced microbial diversity contributes to obesity or merely reflects the obesogenic lifestyle and dietary habits, because diets without fiber dramatically reduce microbial diversity and the capacity to metabolize complex carbohydrates[78]. But it is clear that the gut microbiota can affect signaling in many metabolically active tissues[79].

Germ-free mice have reduced adipose inflammation when compared to colonized mice[80,81], which has been associated with impaired glucose metabolism. Interactions between the microbiota and saturated dietary lipids shift the microbiota and increase the abundance of gut microbiota derived pro-inflammatory molecules in the plasma. These activate TLRs on adipocytes that recruit macrophages in a chemokine (C-C motif) ligand 2 (CCL2) dependent fashion[81]. Yet, the nature of the metabolites has not been identified, and besides translocated LPSs from the gut, other bacterial products, saturated lipids or a combination of host and microbial molecules might be responsible for TLR activation and inflammation in the WAT. However, it has been demonstrated in mice that endotoxin is sufficient to induce the proliferation of adipocyte precursors[82], adipose inflammation and impaired glucose metabolism[20] (**Fig. 2**). When fed a HFD, plasma LPS concentration increased as compared to chow-fed mice, and the effect depended on dietary fat content. Diet-induced, LPS-mediated metabolic disease required CD14, the co-receptor for LPS[20]. However, systemic blocking of CD14 to treat metabolic disease might not be a suitable option because this would probably interrupt microbe–host homeostasis at epithelial surfaces and thus increase the risk for bacterial infections. Although it is unlikely that humans would eat the same high-fat diet as the mice above (>70% of calories from fat), it has been shown that dietary lipids increase peripheral endotoxin levels in humans[83].

Gram-negative bacteria can also translocate from the gut and be detected in blood and adipose tissue[84], which is associated with impaired glucose metabolism. However, it is currently unknown whether they are metabolically active in these environments. Treatment with a probiotic *Bifidobacterium animalis* ssp. *lactis* strain reduced the amount of translocating bacteria and substantially improved insulin sensitivity in comparison to untreated mice, which suggests a causative role of the microbiota. Yet, the exact mechanism for bacterial translocation is at present unknown, and it cannot be ruled out that the diabetic state facilitates mucosal-border crossing

owing to impaired immune defense. Indeed, recent data provide evidence that mice fed a HFD, when compared to chow-fed mice, have reduced numbers of small-intestinal Paneth cells, which produce antimicrobial peptides to protect the epithelium from microbial translocation[85], and that a HFD reduces the thickness of the protective colonic mucus layer in mice[86]. However, functional consequences of these findings remain to be studied in mice and humans.

Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. The prevalence of the chronic liver disease nonalcoholic fatty liver disease (NAFLD) is increased in individuals with obesity and proceeds to nonalcoholic steatohepatitis (NASH) in about 20% of people with NAFLD. Recent data in mice suggest that the translocation of gut bacterial molecules to the liver exacerbates this transition[87]. Susceptible mice, in which deficient bacterial recognition through the inflammasome resulted in colitogenic gut microbiota composition[88], had higher bacterial TLR4 and TLR9 agonists in the portal blood when fed a methionine–choline-deficient diet. Activation of the corresponding TLRs in the liver and subsequent tumor necrosis factor (TNF)-α signaling resulted in hepatotoxicity and progression to NASH. Given that no translocation of intact bacteria was observed and that TLR2 agonists were not increased in the circulation following a methionine–choline-deficient diet[87], it will be important to determine whether the detected TLR4 and TLR9 agonists originate from one specific bacterium. In fact, the authors detected enrichment of the bacterial family Porphyromonadaceae in their mouse model, from which the member *Porphyromonas* has been associated with metabolic diseases in humans and mice[89,90]. Future research is required to understand why Porphyromonadaceae can thrive in the colitogenic gut and how alteration in the intestinal microbiota composition can facilitate the uptake of specific microbial products into the circulation. Yet, there is little knowledge on how these findings translate to human (patho)physiology.

Atherosclerosis. Exploiting the diet–microbiota pathway has recently been suggested for the treatment of atherosclerosis. An unhealthy alliance between host and gut microbiota converts choline, found in seafood, cheese and eggs, as well as carnitine, found in red meat, into TMAO, a toxic compound associated with atherosclerosis and cardiovascular disease[91–93] (**Fig. 1**). The primary step is the formation of trimethylamine (TMA) from choline by means of microbial TMA lyases. Consequently, the inhibition of TMA lyases is a promising step toward reducing risk for atherosclerosis. Indeed, 3,3-dimethyl-1-butanol (DMB), a structural analog of choline, was found to inhibit microbial TMA production *in vitro* and in mouse experiments[94]. Furthermore, oral DMB application reduced macrophage-foam-cell formation and aortic-root atherosclerotic plaque development in atherosclerosis-prone *Apoe*$^{-/-}$ mice. Thus, drugging the bugs[95] to treat diseases of the host is a promising concept that needs to be validated in human diseases, especially when complex dietary interactions might complicate the situation. Remarkably, the authors detected the identified inhibitor DHB in various foods and drinks, including balsamic vinegars, cold-pressed extra virgin olive oils and red wines. Whether such a combination reaches a sufficiently high DHB concentration in the human gut and so reduces the risk of atherosclerosis needs to be investigated.

Gut microbial signals in the gut–brain axis

Brain morphology. Investigating the influence of gut microbiota on brain morphology is a challenging task in humans, and thus data are generated mostly in germ-free mice. These have alterations in the structural integrity of the amygdala and hippocampus when compared to colonized mice[96]. Moreover, mice lacking microbiota display increased hippocampal neurogenesis[97] and hypermyelination of the prefrontal cortex[98]. Although mechanistic data are lacking so far, it is evident that the gut microbiota has an effect on the structure of the (mouse) brain. Colonization of germ-free mice with defined bacterial groups or isolated species could reveal whether distinct microbes or a complex community are required to alter the morphology of brain regions. Likewise, it would be intriguing to determine whether the same bacteria are responsible for morphological alterations in different regions of the brain. Yet, given that access to the brain is tightly controlled by the blood–brain barrier (BBB), it is likely that a small and specific set of bacterial metabolites modulates brain morphology.

The gut microbiota is also crucially involved in modulating the BBB. Germ-free mice have a more permeable BBB than do conventionally raised (CONV-R) mice, a phenotype that was reversed after colonization with SCFA-producing *Clostridium tyrobutyricum* or *B. thetaiotaomicron*[99] (**Fig. 4**). However, future research is required to identify the molecular mechanism of how butyrate or other SCFAs exhibit a BBB-modulating effect, especially whether epigenetic or GPCR signaling is involved and whether the results can be translated to the human body.

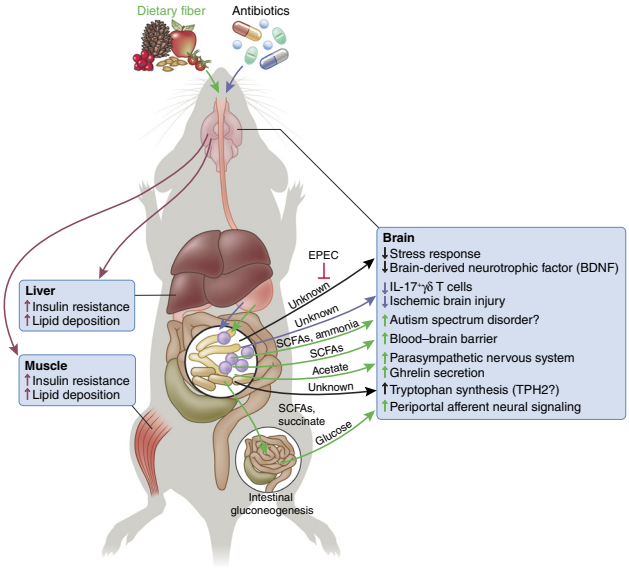

Figure 4 The gut microbiota communicates with the brain through the gut–brain axis. Young germ-free mice (GF, mice that lack microbiota) have increased stress response and increased expression of brain-derived neurotrophic factor (BDNF) in comparison to colonized mice. Colonizing GF mice with enteropathogenic *Escherichia coli* (EPEC) aggravated the stress response[101] by an unknown mechanism (black arrows). Antibiotic treatment alters the gut microbiota composition (violet arrows), which reduces the recruitment of IL-17^{+}γδ T cells to the meninges, thus reducing ischemic brain injury[115]. Dietary fiber (green arrows) is fermented by the colonic gut microbiota into short-chain fatty acids and succinate. Increased levels of the SCFAs acetic, propionic, butyric, isobutyric, valeric and isovaleric acid, as well as ammonia were observed in children with autism spectrum disorder[109]. A causative role of the microbiota, however, remains yet to be determined. SCFAs tighten the BBB[99], while the SCFA acetate activates the parasympathetic nervous system, leading to increased secretion of the hormone ghreline, leading to insulin resistance and lipid deposition in liver and muscle (red arrows)[40]. SCFAs butyrate and propionate as well as succinate activate intestinal gluconeogenesis, which leads to central metabolic improvement via gut–brain neural circuits[38]. The gut microbiota also modulates tryptophan synthesis in the brain[105], probably through a tryptophan hydroxylase 2 (TPH2)-dependent manner.

Although access to the brain is controlled by the BBB, microglia are responsible for immune defense in the brain. Germ-free or antibiotic-treated mice exhibit immature microglia with impaired immune response to bacterial and viral products[100]. Moreover, microglia morphology and distribution were different between colonized and microbiota-deficient mice. Notably, supplementing germ-free mice with a SCFA mix could restore most alterations in microglial function. Similarly, *Ffar2*$^{-/-}$ knockout mice, which lack the SCFA receptor GPR43, displayed alterations in microglia morphology but not in cell densities. Thus, microbiota-derived SCFAs are probably responsible for most, yet not all, effects of gut microbial modulation of microglia function.

Given that *Ffar2* was not expressed in the central nervous system (CNS)[100], GPR43 activation needs to occur in the periphery, but the exact organ or cell type needs to be identified. In addition, direct inhibition of histone deacetylase by butyrate might also occur in the CNS.

Stress response and behavior. Young germ-free mice have an elevated stress response and reduced expression levels of brain-derived neurotrophic factor (BDNF) in the cortex and hippocampus in comparison to specific-pathogen-free (SPF) mice[101]. Colonization with SPF-derived feces or *Bifidobacterium infantis* diminished the stress response when applied at an early developing stage. By contrast, colonization with enteropathogenic *E. coli* (EPEC) aggravated the stress response, revealing that signals from distinct community members of the gut affect brain chemistry and might have differing effects on behavior. As such, one pathway of gut–brain communication involves activation of the vagus nerve as part of the parasympathetic nervous system. In a mouse model of chemically induced colitis, colonization in the gut with *Bifidobacterium longum* reduced anxiety-like behavior via the activation of vagal pathways, independently of brain-derived neurotrophic factor (BDNF) production[102]. Similarly, dietary supplementation of *Lactobacillus rhamnosus* given to mice modulated the expression of γ-aminobutyric acid (GABA) receptors in the brain[103] and thus affected signaling of the major inhibitory neurotransmitter. Consequently, this microbe-dependent communication through the vagus nerve ameliorated stress and anxiety- and depression-related behavior.

Given the diversity of the gut microbiota, it is likely that various other members have the capability to activate vagus nerve signaling. Yet, in both studies described, individual bacterial strains were supplemented in high doses. It will thus be interesting to reveal vagus nerve activation in a stable intestinal community and to determine whether competition exists between vagus-activating and vagus-inhibiting strains.

Besides vagus nerve communication, most routes of communication between the gut and brain are unknown. When transplanting cecal content from a rather exploratory mouse strain into a timid and anxious strain, the behavioral phenotype was transmissible and occurred independently of circulating cytokines, vagus nerve activation or intestinal serotonin and dopamine levels[104]. Studies in germ-free mice indicated that the microbiota can alter concentrations of the tryptophan metabolites serotonin and 5-hydroxyindoleacetic acid in the hippocampus of male, but not female, mice[105]. Thus, the microbiota cannot only regulate tryptophan hydroxylase 1 (TPH1)-dependent serotonin production in the gut, but also TPH2-dependent serotonin synthesis in the brain (**Fig. 4**). Remarkably, the microbiota can even affect behavior in mice through metabolites produced in the gut, such as 4-EPS, which is enriched in mouse models of autism spectrum disorder (ASD)[106].

The gut microbiota might also contribute to ASD. In a study with 99 participants, children with autistic behavior had more frequent ear infections and a history of higher antibiotic courses than typically developing children[107]. Moreover, a higher gut bacterial diversity has been described in ASD children with gastrointestinal symptoms in comparison to controls[108]. Also, three species of the sulfate-reducing Proteobacterium *Desulfovibrio* (*D. piger*, *D. desulfuricans*, *D. intestinalis*) were increased in a small cohort of children with a severe form of autism. Microbial alterations were also reflected in the fermentative profile: ASD children had elevated levels of several SCFAs, including acetic, propionic, butyric, isobutyric, valeric and isovaleric acid, and a higher concentration of ammonia in their feces[109]. However, given that the studies included only limited numbers of participants, the findings need to be confirmed in larger cohorts. Similarly, it is unclear from the human association studies whether alterations in the gut microbiota and their metabolites are indeed causative for the development of ASD[110]. Even though injecting the SCFA propionic acid directly into the ventricular system in the brain of rats resulted in reversible ASD-like behavior[111], it remains to be determined whether elevated levels of fecal SCFAs translate into increased systemic SCFA concentrations in humans.

Social deficits can also be observed in young mice of mothers that were fed a high-fat diet (MHFD mice)[112]. The offspring had altered gut microbiota composition, and co-housing with mice fed a control diet corrected the impaired behavior, as did supplementation with live *Lactobacillus reuteri*. Yet, the application of *Lactobacillus johnsonii* did not rescue the social deficit, indicating that a specific metabolite of *L. reuteri* is required. Of note, fecal transplant from control-fed mice or *L. reuteri* treatment did not correct repetitive behavior or anxiety, which is associated with ASD, in germ-free or MHFD mice[112]. Considering the microbiota-mediated stimulation of the vagus nerve discussed earlier[102,103], it would be notable to determine whether the fecal transplant was lacking potent stimulating bacteria or whether repetitive behavior and anxiety is not modulated by gut bacteria in a healthy (germ-free) mouse. However, social deficits in MHFD mice could be attributed to lower levels of the neuropeptide oxytocin in the hypothalamus and impairment in the mesolimbic dopamine reward system, which were restored after *L. reuteri* supplementation. Identification of the responsible microbial metabolite could lead to improved therapy for behavioral disorders in humans, wherein maternal metabolism similarly affects the behavior of offspring[113,114].

Stroke. Independent of BBB modulation is the involvement of the gut microbiota in ischemic brain injury. Antibiotic-induced dysbiosis of the intestinal community reduced the infarct volume in a mouse model of stroke, an effect that was transmissible through fecal transplant and thus gut-bacteria-dependent[115]. Microbial alteration resulted in augmented dendritic cell (DC)-mediated induction of T regulatory (T_{reg}) cells and inhibition of IL-17^{+}γδ T cell differentiation (**Fig. 4**). As a consequence, fewer IL-17^{+}γδ T cells accumulated at the meninges, which is associated with smaller infarct volume[115]. Still, even though the abundance of distinct bacterial families, including Verrucomicrobiaceae, Prevotellaceae and Clostridiaceae could be used to predict infarct volume, it remains to be investigated how the altered microbial community affects DC function. Given that DCs can sample luminal content[116], it will be of interest to see whether distinct bacterial antigens or their metabolic signature affects acute brain injury. Providing that such a protective mechanism exists in humans, identifying the metabolite or bacterial species will be of even greater interest because altering the bacterial community with antibiotics might have adverse side effects. Yet, the link between microbiota and stroke is more complex, given that brain injury has been found to alter

gut microbiota composition[117]. After stroke, relative abundance of Peptococcaceae increased in the mouse cecum, whereas the proportion of Prevotellacea decreased. This alteration was paralleled by augmented release of noradrenaline and the reduction of mucoprotein-producing cells[117]. Whether this translates into a compromised mucus layer, especially in the colon, would be an important question to follow up. Moreover, transplantation of the altered community into germ-free mice could reveal whether this microbiota composition affects the outcome of experimental stroke.

Gut microbiota and lung disease

Allergic asthma. Delivery by caesarian section, formula feeding, antibiotic treatment early in life and urban living predispose to allergic asthma[118]. Remarkably, all these early-life events alter the gut microbiota composition (**Box 1**), and it is thus likely that signals from our bioreactor are involved in their pathogenesis. Studies in neonatal mice have correspondingly shown that treatment with vancomycin, a non-ribosomal glycopeptide targeting primarily Gram-positive bacteria, resulted in diminished gut microbial diversity and exacerbated asthmatic symptoms[119]. Moreover, vancomycin treatment in neonatal, but not adult, mice shifted the composition of the gut microbiota toward a higher proportion of Lactobacilli and almost entire depletion of Bacteroidetes as compared to untreated mice[119]. A contributing role of the gut microbiota in the pathology of asthma was also demonstrated by a fecal transplant from a child at risk for asthma into germ-free mice. The transplant resulted in severe lung inflammation after challenge with ovalbumin (OVA), an allergen frequently used to induce pulmonary inflammation in mice[120]. Remarkably, supplementing the fecal transplant with the four depleted genera *Lachnospira*, *Veillonella*, *Fecalibacterium* and *Rothia* ameliorated the inflammatory response. These data suggest that there might be critical windows in an individual's life when they are particularly sensitive to disturbances in the microbiota.

Mechanistically, microbial production of SCFAs was reported to protect against allergic airway disease (AAD) in mice, an effect that involved GPR41—but not GPR43—signaling and reduced T_H2 effector cell activation in the lung[121]. Moreover, maternal acetate, generated through microbial fermentation of dietary fiber, regulates gene expression in the mouse fetal lung through inhibition of histone deacetylase 9 (HDAC9), and these epigenetic modifications protected the offspring against AAD[122]. This is potentially also true in humans; preliminary data suggest that increased acetate concentration in mothers was negatively associated with airway diseases in their infants[122]. Consequently, the internal bioreactor translates maternal dietary choices into epigenetic signals that might influence health and disease of the offspring even before birth. Providing that the evidence is substantiated in humans, it will become relevant to examine and potentially adjust maternal microbiota and maternal production of SCFA to prevent the development of allergic asthma in offspring. Crucially, this intervention would be required before birth, which shifts the estimated onset of the disease to an even earlier time point than previously expected.

Conclusions and future directions

Here we presented how the gut microbiota converts dietary and endogenous molecules into metabolites that allow communication with peripheral organs and tissues in the host. Given that an alteration in gut microbiota composition has been linked to different diseases, modulation of gut microbiota composition through dietary intervention represents a promising therapeutic avenue. In a comprehensive study involving more than 900 participants, diet-dependent postprandial blood glucose (PPBG) levels were measured and correlated with individual gut microbiota composition[123]. Because similar diets caused different PPBG responses in different individuals, the authors were able to tailor personalized nutritional recommendations that resulted in improved PPBG levels in a cohort of 26 individuals, which were linked to a consistent alteration in gut microbiota composition. Identifying the relevant microbial metabolites in those individuals could be a step toward the development of personalized medicine.

A rather harsh adaptation of microbial modulation is changing the entire microbial community through fecal microbiota transplant (FMT). For example, FMT is considered to be the best available treatment for recurrent *Clostridium difficile* infection. FMT, despite the small size of the patient group tested so far, has been proven to be beneficial in a small clinical trial for improving metabolic syndrome[124]. Subjects with obesity receiving microbiota from a lean donor ($n = 9$) had improved insulin sensitivity when compared to patients with obesity who received their own microbiota ($n = 9$)[124]. However, only a subset of recipients responded, and thus it will be essential to select the perfect match between donor and recipients. However, FMT will probably remain a research tool for the near future, because it is linked with a substantial health risk. For example, in one case where an individual performed FMT at home, this resulted in bloody diarrhea[125], and in another case, a woman with a *C. difficile* infection was treated with FMT from an overweight stool donor, which caused substantial weight gain in the recipient[126]. In addition, the beneficial effect on insulin resistance was observed for only 6 weeks; this would suggest a need for eight fecal transplants per year if used as treatment, which is not a clinical option.

A major challenge in the field is to translate metagenomic findings into a biologically relevant mechanism. This might be achieved through the isolation of bacterial strains or defined communities, analysis of their response to specific macronutrients in humans and the linking of those findings to disease-specific biomarkers or physiological parameters such as insulin resistance or body weight. The identification of bacterial metabolites modulating physiological processes will advance our mechanistic understanding of how the microbiota affects the host. Signaling pathways can be identified by re-deriving tissue-specific knockout mice as germ-free and administering the bacterial strain or even the metabolite. A first step toward translation would be to perform *in vitro* stimulation of primary human cells with the metabolite in question. Once this is successful, agonists and antagonists for the involved receptors could be developed through classical means.

In addition to the diseases discussed herein, an altered microbiota has also been observed in other diseases, such as atopic dermatitis[127,128], systemic lupus erythematosus[129], inflammatory bowel diseases[130,131], type 1 diabetes[132,133] or multiple sclerosis[134]. Some of them are characterized by rather heterogeneous disease manifestations, and it is likely that the different disease phenotypes are associated with different microbial profiles. It will thus be important to resolve the question of cause or consequence: does an altered gut microbiota contribute to disease, or does it merely reflect a disease status? To this end, prospective, as well as intervention, studies in humans are required.

In conclusion, the gut microbiota actively communicates with the host, similarly to other human organs, and we are only just beginning to decipher their signals and their relevance for human health and disease.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

1. Sender, R., Fuchs, S. & Milo, R. Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. *Cell* **164**, 337–340 (2016).
2. Zoetendal, E.G., Akkermans, A.D. & De Vos, W.M. Temperature gradient gel electrophoresis analysis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. *Appl. Environ. Microbiol.* **64**, 3854–3859 (1998).
3. Qin, J. *et al.* A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464**, 59–65 (2010).
4. Eckburg, P.B. *et al.* Diversity of the human intestinal microbial flora. *Science* **308**, 1635–1638 (2005).
5. Hold, G.L., Pryde, S.E., Russell, V.J., Furrie, E. & Flint, H.J. Assessment of microbial diversity in human colonic samples by 16S rDNA sequence analysis. *FEMS Microbiol. Ecol.* **39**, 33–39 (2002).
6. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature* **486**, 207–214 (2012).
7. Li, J. *et al.* An integrated catalog of reference genes in the human gut microbiome. *Nat. Biotechnol.* **32**, 834–841 (2014).
8. Tamburini, S., Shen, N., Wu, H.C. & Clemente, J.C. The microbiome in early life: implications for health outcomes. *Nat. Med.* **22**, 713–722 (2016).
9. Arumugam, M. *et al.* Enterotypes of the human gut microbiome. *Nature* **473**, 174–180 (2011).
10. Knights, D. *et al.* Rethinking "enterotypes". *Cell Host Microbe.* **16**, 433–437 (2014).
11. Yatsunenko, T. *et al.* Human gut microbiome viewed across age and geography. *Nature* **486**, 222–227 (2012).
12. Kovatcheva-Datchary, P. *et al.* Dietary fiber-induced improvement in glucose metabolism is associated with increased abundance of *Prevotella*. *Cell Metab.* **22**, 971–982 (2015).
13. Kommineni, S. *et al.* Bacteriocin production augments niche competition by enterococci in the mammalian gastrointestinal tract. *Nature* **526**, 719–722 (2015).
14. Pédron, T. *et al.* A crypt-specific core microbiota resides in the mouse colon. *MBio* **3**, e00116–e00112 (2012).
15. Kaiko, G.E. *et al.* The colonic crypt protects stem cells from microbiota-derived metabolites. *Cell* **165**, 1708–1720 (2016).
16. Haag, L.-M. & Siegmund, B. Intestinal microbiota and the innate immune system—a crosstalk in Crohn's disease pathogenesis. *Front. Immunol.* **6**, 489 (2015).
17. Honda, K. & Littman, D.R. The microbiota in adaptive immune homeostasis and disease. *Nature* **535**, 75–84 (2016).
18. Thaiss, C.A., Zmora, N., Levy, M. & Elinav, E. The microbiome and innate immunity. *Nature* **535**, 65–74 (2016).
19. Medzhitov, R. Recognition of microorganisms and activation of the immune response. *Nature* **449**, 819–826 (2007).
20. Cani, P.D. *et al.* Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* **56**, 1761–1772 (2007).
21. Hersoug, L.-G., Møller, P. & Loft, S. Gut microbiota-derived lipopolysaccharide uptake and trafficking to adipose tissue: implications for inflammation and obesity. *Obes. Rev.* **17**, 297–312 (2016).
22. Ghoshal, S., Witta, J., Zhong, J., de Villiers, W. & Eckhardt, E. Chylomicrons promote intestinal absorption of lipopolysaccharides. *J. Lipid Res.* **50**, 90–97 (2009).
23. Bäckhed, F., Normark, S., Schweda, E.K.H., Oscarson, S. & Richter-Dahlfors, A. Structural requirements for TLR4-mediated LPS signalling: a biological role for LPS modifications. *Microbes Infect.* **5**, 1057–1063 (2003).
24. Vatanen, T. *et al.* Variation in microbiome LPS mmunogenicity Contributes to Autoimmunity in Humans. *Cell* **165**, 842–853 (2016).
25. Larsson, E. *et al.* Analysis of gut microbial regulation of host gene expression along the length of the gut and regulation of gut microbial ecology through MyD88. *Gut.* **61**, 1124–1131 (2012).
26. Gaboriau-Routhiau, V. *et al.* The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity* **31**, 677–689 (2009).
27. Chung, H. *et al.* Gut immune maturation depends on colonization with a host-specific microbiota. *Cell* **149**, 1578–1593 (2012).
28. Kashyap, P.C. *et al.* Complex interactions among diet, gastrointestinal transit, and gut microbiota in humanized mice. *Gastroenterology* **144**, 967–977 (2013).
29. Zelante, T. *et al.* Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. *Immunity* **39**, 372–385 (2013).
30. Lamas, B. *et al.* CARD9 impacts colitis by altering gut microbiota metabolism of tryptophan into aryl hydrocarbon receptor ligands. *Nat. Med.* **22**, 598–605 (2016).
31. Bergman, E.N. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiol. Rev.* **70**, 567–590 (1990).
32. Bäckhed, F. *et al.* The gut microbiota as an environmental factor that regulates fat storage. *Proc. Natl. Acad. Sci. USA* **101**, 15718–15723 (2004).
33. Bäckhed, F., Manchester, J.K., Semenkovich, C.F. & Gordon, J.I. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proc. Natl. Acad. Sci. USA* **104**, 979–984 (2007).
34. Samuel, B.S. *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* **105**, 16767–16772 (2008).
35. Tolhurst, G. *et al.* Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. *Diabetes* **61**, 364–371 (2012).
36. Fava, S. Glucagon-like peptide 1 and the cardiovascular system. *Curr. Diabetes Rev.* **10**, 302–310 (2014).
37. Nøhr, M.K. *et al.* GPR41/FFAR3 and GPR43/FFAR2 as cosensors for short-chain fatty acids in enteroendocrine cells vs FFAR3 in enteric neurons and FFAR2 in enteric leukocytes. *Endocrinology* **154**, 3552–3564 (2013).
38. De Vadder, F. *et al.* Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. *Cell* **156**, 84–96 (2014).
39. Frost, G. *et al.* The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat. Commun.* **5**, 3611 (2014).
40. Perry, R.J. *et al.* Acetate mediates a microbiome-brain-β-cell axis to promote metabolic syndrome. *Nature* **534**, 213–217 (2016).
41. Kimura, I. *et al.* The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. *Nat. Commun.* **4**, 1829 (2013).
42. Midtvedt, T. Microbial bile acid transformation. *Am. J. Clin. Nutr.* **27**, 1341–1347 (1974).
43. Swann, J.R. *et al.* Systemic gut microbial modulation of bile acid metabolism in host tissue compartments. *Proc. Natl. Acad. Sci. USA* **108** (Suppl. 1), 4523–4530 (2011).
44. Sayin, S.I. *et al.* Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell Metab.* **17**, 225–235 (2013).
45. Islam, K.B.M.S. *et al.* Bile acid is a host factor that regulates the composition of the cecal microbiota in rats. *Gastroenterology* **141**, 1773–1781 (2011).
46. Kurdi, P., Kawanishi, K., Mizutani, K. & Yokota, A. Mechanism of growth inhibition by free bile acids in lactobacilli and bifidobacteria. *J. Bacteriol.* **188**, 1979–1986 (2006).
47. Li, F. *et al.* Microbiome remodelling leads to inhibition of intestinal farnesoid X receptor signalling and decreased obesity. *Nat. Commun.* **4**, 2384 (2013).
48. Jiang, C. *et al.* Intestine-selective farnesoid X receptor inhibition improves obesity-related metabolic dysfunction. *Nat. Commun.* **6**, 10166 (2015).
49. Parséus, A. *et al.* Microbiota-induced obesity requires farnesoid X receptor. *Gut.* http://dx.doi.org/10.1136/gutjnl-2015-310283 (2016).
50. Thomas, C. *et al.* TGR5-mediated bile acid sensing controls glucose homeostasis. *Cell Metab.* **10**, 167–177 (2009).
51. Broeders, E.P.M. *et al.* The bile acid chenodeoxycholic acid increases human brown adipose tissue Activity. *Cell Metab.* **22**, 418–426 (2015).
52. Ryan, K.K. *et al.* FXR is a molecular target for the effects of vertical sleeve gastrectomy. *Nature* **509**, 183–188 (2014).
53. Tremaroli, V. *et al.* Roux-en-Y gastric bypass and vertical banded gastroplasty induce long-term changes on the human gut microbiome contributing to fat mass regulation. *Cell Metab.* **22**, 228–238 (2015).
54. Schauer, P.R. *et al.* Bariatric surgery versus intensive medical therapy for diabetes—3-year outcomes. *N. Engl. J. Med.* **370**, 2002–2013 (2014).
55. McGavigan, A.K. *et al.* TGR5 contributes to glucoregulatory improvements after vertical sleeve gastrectomy in mice. *Gut.* http://dx.doi.org/10.1136/gutjnl-2015-309871 (2015).
56. Fang, S. *et al.* Intestinal FXR agonism promotes adipose tissue browning and reduces obesity and insulin resistance. *Nat. Med.* **21**, 159–165 (2015).
57. Downes, M. *et al.* A chemical, genetic, and structural analysis of the nuclear bile acid receptor FXR. *Mol. Cell* **11**, 1079–1092 (2003).
58. Neuschwander-Tetri, B.A. *et al.* Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet* **385**, 956–965 (2015).
59. Cypess, A.M. *et al.* Identification and importance of brown adipose tissue in adult humans. *N. Engl. J. Med.* **360**, 1509–1517 (2009).

60. Wang, Q.A., Tao, C., Gupta, R.K. & Scherer, P.E. Tracking adipogenesis during white adipose tissue development, expansion and regeneration. *Nat. Med.* **19**, 1338–1344 (2013).
61. Harms, M. & Seale, P. Brown and beige fat: development, function and therapeutic potential. *Nat. Med.* **19**, 1252–1263 (2013).
62. Ziętak, M. *et al.* Altered microbiota contributes to reduced diet-induced obesity upon cold exposure. *Cell Metab.* **23**, 1216–1223 (2016).
63. Chevalier, C. *et al.* Gut microbiota orchestrates energy homeostasis during Cold. *Cell* **163**, 1360–1374 (2015).
64. Suárez-Zamorano, N. *et al.* Microbiota depletion promotes browning of white adipose tissue and reduces obesity. *Nat. Med.* **21**, 1497–1501 (2015).
65. Collins, S. & Surwit, R.S. The beta-adrenergic receptors and the control of adipose tissue metabolism and thermogenesis. *Recent Prog. Horm. Res.* **56**, 309–328 (2001).
66. Sommer, F. *et al.* The gut microbiota modulates energy metabolism in the hibernating brown bear *Ursus arctos*. *Cell Rep.* **14**, 1655–1661 (2016).
67. Turnbaugh, P.J. *et al.* An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* **444**, 1027–1031 (2006).
68. Turnbaugh, P.J., Bäckhed, F., Fulton, L. & Gordon, J.I. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe.* **3**, 213–223 (2008).
69. Turnbaugh, P.J. *et al.* The effect of diet on the human gut microbiome: A metagenomic analysis in humanized gnotobiotic mice. *Sci. Transl. Med.* **1**, 6ra14 (2009).
70. Blanton, L.V. *et al.* Gut bacteria that prevent growth impairments transmitted by microbiota from malnourished children. *Science* **351**, aad3311 (2016).
71. Smith, M.I. *et al.* Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. *Science* **339**, 548–554 (2013).
72. Engfer, M.B., Stahl, B., Finke, B., Sawatzki, G. & Daniel, H. Human milk oligosaccharides are resistant to enzymatic hydrolysis in the upper gastrointestinal tract. *Am. J. Clin. Nutr.* **71**, 1589–1596 (2000).
73. Charbonneau, M.R. *et al.* Sialylated milk oligosaccharides promote microbiota-dependent growth in models of infant undernutrition. *Cell* **164**, 859–871 (2016).
74. Subramanian, S. *et al.* Persistent gut microbiota immaturity in malnourished Bangladeshi children. *Nature* **510**, 417–421 (2014).
75. Schwarzer, M. *et al. Lactobacillus plantarum* strain maintains growth of infant mice during chronic undernutrition. *Science* **351**, 854–857 (2016).
76. Fleissner, C.K. *et al.* Absence of intestinal microbiota does not protect mice from diet-induced obesity. *Br. J. Nutr.* **104**, 919–929 (2010).
77. Ding, S. *et al.* High-fat diet: bacteria interactions promote intestinal inflammation which precedes and correlates with obesity and insulin resistance in mouse. *PLoS One* **5**, e12191 (2010).
78. Sonnenburg, E.D. *et al.* Diet-induced extinctions in the gut microbiota compound over generations. *Nature* **529**, 212–215 (2016).
79. Tremaroli, V. & Bäckhed, F. Functional interactions between the gut microbiota and host metabolism. *Nature* **489**, 242–249 (2012).
80. Caesar, R. *et al.* Gut-derived lipopolysaccharide augments adipose macrophage accumulation but is not essential for impaired glucose or insulin tolerance in mice. *Gut* **61**, 1701–1707 (2012).
81. Caesar, R., Tremaroli, V., Kovatcheva-Datchary, P., Cani, P.D. & Bäckhed, F. Crosstalk between gut microbiota and dietary lipids aggravates WAT inflammation through TLR signaling. *Cell Metab.* **22**, 658–668 (2015).
82. Luche, E. *et al.* Metabolic endotoxemia directly increases the proliferation of adipocyte precursors at the onset of metabolic diseases through a CD14-dependent mechanism. *Mol. Metab.* **2**, 281–291 (2013).
83. Erridge, C., Attina, T., Spickett, C.M. & Webb, D.J. A high-fat meal induces low-grade endotoxemia: evidence of a novel mechanism of postprandial inflammation. *Am. J. Clin. Nutr.* **86**, 1286–1292 (2007).
84. Amar, J. *et al.* Intestinal mucosal adherence and translocation of commensal bacteria at the early onset of type 2 diabetes: molecular mechanisms and probiotic treatment. *EMBO Mol. Med.* **3**, 559–572 (2011).
85. Beyaz, S. *et al.* High-fat diet enhances stemness and tumorigenicity of intestinal progenitors. *Nature* **531**, 53–58 (2016).
86. Everard, A. *et al.* Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. *Proc. Natl. Acad. Sci. USA* **110**, 9066–9071 (2013).
87. Henao-Mejia, J. *et al.* Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature* **482**, 179–185 (2012).
88. Elinav, E. *et al.* NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. *Cell* **145**, 745–757 (2011).
89. Bajaj, J.S. *et al.* Linkage of gut microbiome with cognition in hepatic encephalopathy. *Am. J. Physiol. Gastrointest. Liver Physiol.* **302**, G168–G175 (2012).
90. Makiura, N. *et al.* Relationship of *Porphyromonas gingivalis* with glycemic level in patients with type 2 diabetes following periodontal treatment. *Oral Microbiol. Immunol.* **23**, 348–351 (2008).
91. Wang, Z. *et al.* Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* **472**, 57–63 (2011).
92. Koeth, R.A. *et al.* Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat. Med.* **19**, 576–585 (2013).
93. Zhu, W. *et al.* Gut microbial metabolite TMAO enhances platelet hyperreactivity and thrombosis risk. *Cell* **165**, 111–124 (2016).
94. Wang, Z. *et al.* Non-lethal inhibition of gut microbial trimethylamine production for the treatment of atherosclerosis. *Cell* **163**, 1585–1595 (2015).
95. Jonsson, A.L. & Bäckhed, F. Drug the bug! *Cell* **163**, 1565–1566 (2015).
96. Luczynski, P. *et al.* Adult microbiota-deficient mice have distinct dendritic morphological changes: differential effects in the amygdala and hippocampus. *Eur. J. Neurosci.* http://dx.doi.org/10.1111/ejn.13291 (2016).
97. Ogbonnaya, E.S. *et al.* Adult hippocampal neurogenesis is regulated by the microbiome. *Biol. Psychiatry* **78**, e7–e9 (2015).
98. Hoban, A.E. *et al.* Regulation of prefrontal cortex myelination by the microbiota. *Transl. Psychiatry* **6**, e774 (2016).
99. Braniste, V. *et al.* The gut microbiota influences blood-brain barrier permeability in mice. *Sci. Transl. Med.* **6**, 263ra158 (2014).
100. Erny, D. *et al.* Host microbiota constantly control maturation and function of microglia in the CNS. *Nat. Neurosci.* **18**, 965–977 (2015).
101. Sudo, N. *et al.* Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *J. Physiol. (Lond.)* **558**, 263–275 (2004).
102. Bercik, P. *et al.* The anxiolytic effect of *Bifidobacterium longum* NCC3001 involves vagal pathways for gut-brain communication. *Neurogastroenterol. Motil.* **23**, 1132–1139 (2011).
103. Bravo, J.A. *et al.* Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc. Natl. Acad. Sci. USA* **108**, 16050–16055 (2011).
104. Bercik, P. *et al.* The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology* **141**, 599–609.e3 (2011).
105. Clarke, G. *et al.* The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Mol. Psychiatry* **18**, 666–673 (2013).
106. Hsiao, E.Y. *et al.* Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell* **155**, 1451–1463 (2013).
107. Niehus, R. & Lord, C. Early medical history of children with autism spectrum disorders. *J. Dev. Behav. Pediatr. JDBP* **27** (Suppl.), S120–S127 (2006).
108. Finegold, S.M. *et al.* Pyrosequencing study of fecal microflora of autistic and control children. *Anaerobe* **16**, 444–453 (2010).
109. Wang, L. *et al.* Elevated fecal short chain fatty acid and ammonia concentrations in children with autism spectrum disorder. *Dig. Dis. Sci.* **57**, 2096–2102 (2012).
110. Mayer, E.A., Padua, D. & Tillisch, K. Altered brain-gut axis in autism: comorbidity or causative mechanisms? *BioEssays* **36**, 933–939 (2014).
111. MacFabe, D.F. *et al.* Neurobiological effects of intraventricular propionic acid in rats: possible role of short chain fatty acids on the pathogenesis and characteristics of autism spectrum disorders. *Behav. Brain Res.* **176**, 149–169 (2007).
112. Buffington, S.A. *et al.* Microbial reconstitution reverses maternal diet-induced social and synaptic deficits in offspring. *Cell* **165**, 1762–1775 (2016).
113. Sullivan, E.L., Nousen, E.K. & Chamlou, K.A. Maternal high fat diet consumption during the perinatal period programs offspring behavior. *Physiol. Behav.* **123**, 236–242 (2014).
114. Connolly, N. *et al.* Maternal metabolic risk factors for autism spectrum disorder-An analysis of electronic medical records and linked birth data. *Autism Res.* **9**, 829–837 (2016).
115. Benakis, C. *et al.* Commensal microbiota affects ischemic stroke outcome by regulating intestinal γδ T cells. *Nat. Med.* **22**, 516–523; advance online publication (2016).
116. Niess, J.H. *et al.* CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science* **307**, 254–258 (2005).
117. Houlden, A. *et al.* Brain injury induces specific changes in the caecal microbiota of mice via altered autonomic activity and mucoprotein production. *Brain Behav. Immun.* http://dx.doi.org/10.1016/j.bbi.2016.04.003 (2016).
118. Arrieta, M.-C., Stiemsma, L.T., Amenyogbe, N., Brown, E.M. & Finlay, B. The intestinal microbiome in early life: health and disease. *Front Immunol.* **5**, 427 (2014).
119. Russell, S.L. *et al.* Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma. *EMBO Rep.* **13**, 440–447 (2012).
120. Arrieta, M.-C. *et al.* Early infancy microbial and metabolic alterations affect risk of childhood asthma. *Sci. Transl. Med.* **7**, 307ra152 (2015).
121. Trompette, A. *et al.* Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat. Med.* **20**, 159–166 (2014).
122. Thorburn, A.N. *et al.* Evidence that asthma is a developmental origin disease influenced by maternal diet and bacterial metabolites. *Nat. Commun.* **6**, 7320 (2015).
123. Zeevi, D. *et al.* Personalized nutrition by prediction of Glycemic Responses. *Cell* **163**, 1079–1094 (2015).
124. Vrieze, A. *et al.* Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* **143**, 913–916.e7 (2012).
125. Hohmann, E.L., Ananthakrishnan, A.N. & Deshpande, V. Case records of the Massachusetts General Hospital. Case 25-2014. A 37-year-old man with ulcerative colitis and bloody diarrhea. *N. Engl. J. Med.* **371**, 668–675 (2014).
126. Alang, N. & Kelly, C.R. Weight gain after fecal microbiota transplantation. *Open Forum Infect. Dis.* **2**, ofv004 (2015).
127. Abrahamsson, T.R. *et al.* Low diversity of the gut microbiota in infants with atopic eczema. *J. Allergy Clin. Immunol.* **129**, 434–440.e2 (2012).

128. Song, H., Yoo, Y., Hwang, J., Na, Y.-C. & Kim, H.S. *Faecalibacterium prausnitzii* subspecies-level dysbiosis in the human gut microbiome underlying atopic dermatitis. *J. Allergy Clin. Immunol.* **137**, 852–860 (2016).
129. Hevia, A. *et al.* Intestinal dysbiosis associated with systemic lupus erythematosus. *MBio* **5**, e01548–14 (2014).
130. Lepage, P. *et al.* Twin study indicates loss of interaction between microbiota and mucosa of patients with ulcerative colitis. *Gastroenterology* **141**, 227–236 (2011).
131. Takahashi, K. *et al.* Reduced abundance of butyrate-producing bacteria species in the fecal microbial community in Crohn's disease. *Digestion* **93**, 59–65 (2016).
132. Kostic, A.D. *et al.* The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. *Cell Host Microbe.* **17**, 260–273 (2015).
133. Alkanani, A.K. *et al.* Alterations in intestinal microbiota correlate with susceptibility to type 1 diabetes. *Diabetes* **64**, 3510–3520 (2015).
134. Chen, J. *et al.* Multiple sclerosis patients have a distinct gut microbiota compared to healthy controls. *Sci. Rep.* **6**, 28484 (2016).
135. Tims, S. *et al.* Microbiota conservation and BMI signatures in adult monozygotic twins. *ISME J.* **7**, 707–717 (2013).
136. Goodrich, J.K. *et al.* Human genetics shape the gut microbiome. *Cell* **159**, 789–799 (2014).
137. Huurre, A. *et al.* Mode of delivery—effects on gut microbiota and humoral immunity. *Neonatology* **93**, 236–240 (2008).
138. Dominguez-Bello, M.G. *et al.* Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc. Natl. Acad. Sci. USA* **107**, 11971–11975 (2010).
139. Bäckhed, F. *et al.* Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host Microbe.* **17**, 690–703 (2015).
140. Azad, M.B. *et al.* Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: a prospective cohort study. *BJOG* **123**, 983–993 (2016).
141. Koleva, P.T., Kim, J.-S., Scott, J.A. & Kozyrskyj, A.L. Microbial programming of health and disease starts during fetal life. *Birth Defects Res. C Embryo Today* **105**, 265–277 (2015).
142. Ley, R.E., Turnbaugh, P.J., Klein, S. & Gordon, J.I. Microbial ecology: human gut microbes associated with obesity. *Nature* **444**, 1022–1023 (2006).
143. Duncan, S.H. *et al.* Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and butyrate-producing bacteria in feces. *Appl. Environ. Microbiol.* **73**, 1073–1078 (2007).
144. Dethlefsen, L., Huse, S., Sogin, M.L. & Relman, D.A. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biol.* **6**, e280 (2008).
145. Cotillard, A. *et al.* Dietary intervention impact on gut microbial gene richness. *Nature* **500**, 585–588 (2013).
146. David, L.A. *et al.* Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **505**, 559–563 (2014).
147. David, L.A. *et al.* Host lifestyle affects human microbiota on daily timescales. *Genome Biol.* **15**, R89 (2014).
148. Hofstra, J.J. *et al.* Changes in microbiota during experimental human Rhinovirus infection. *BMC Infect. Dis.* **15**, 336 (2015).
149. Dethlefsen, L. & Relman, D.A. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proc. Natl. Acad. Sci. USA* **108** (Suppl. 1), 4554–4561 (2011).