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REVIEW

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Metabolic networks of the human gut microbiota

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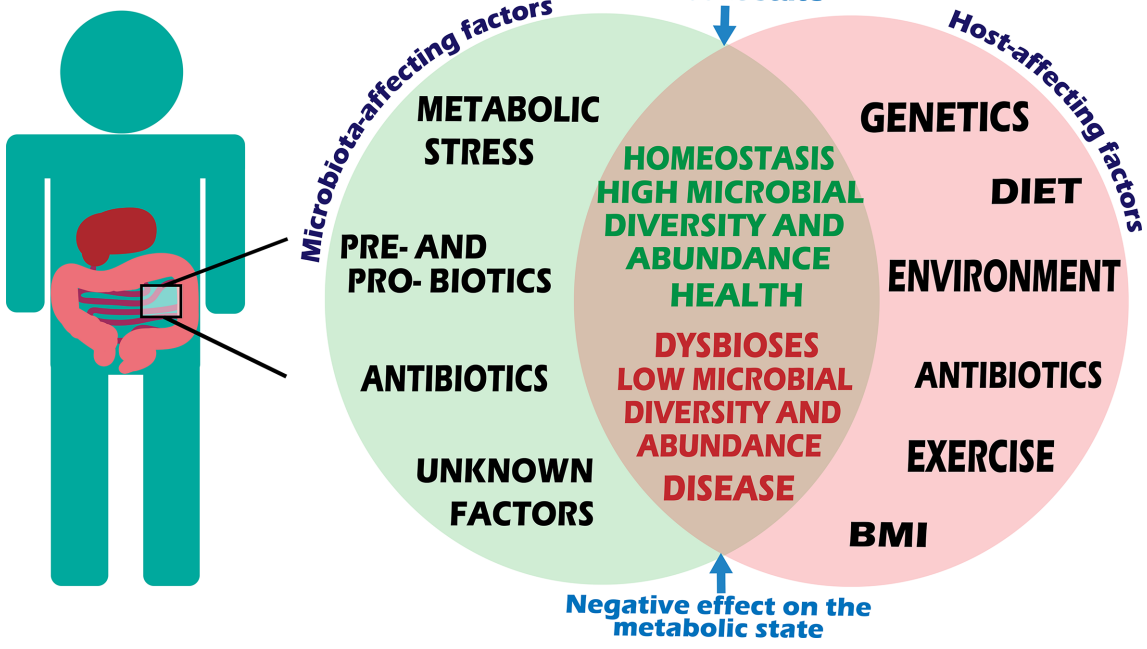

Graphical abstract

Numerous interplaying factors affect the host and/or the microbiota housed within the gastrointestinal tract, yielding different metabolic states.

Abstract

The human gut microbiota controls factors that relate to human metabolism with a reach far greater than originally expected. Microbial communities and human (or animal) hosts entertain reciprocal exchanges between various inputs that are largely controlled by the host via its genetic make-up, nutrition and lifestyle. The composition of these microbial communities is fundamental to supply metabolic capabilities beyond those encoded in the host genome, and contributes to hormone and cellular signalling that support the dynamic adaptation to changes in food availability, environment and organismal development. Poor functional exchange between the microbial communities and their human host is associated with dysbiosis, metabolic dysfunction and disease. This review examines the biology of the dynamic relationship between the reciprocal metabolic state of the microbiota–host entity in balance with its environment (i.e. in healthy states), the enzymatic and metabolic changes associated with its imbalance in three well-studied diseases states such as obesity, diabetes and atherosclerosis, and the effects of bariatric surgery and exercise.

FOREWORD

This review is the result of a pedagogical project carried out during a third-year microbiology undergraduate course at Concordia University in Montréal. The purpose of this activity was to examine the web of metabolic interactions between the intestinal microbiota and the human host from a biological perspective, to learn relevant course topics actively. The endeavour taught the students how to research the primary scientific literature and identify relevant information to write a collaborative review as well as experience first hand the dynamics of a collaborative scientific undertaking. Thus, the final choice of cited sources was influenced by the pedagogical scope of this project and we apologize to the colleagues whose important contributions could not be cited.

INTESTINAL MICROBIOTA, GENETICS AND ENVIRONMENT

The digestive tract constitutes the largest surface area in the human body, with a size of 30–40 m^2 in adults [1]. Such a massive expanse houses various microbial communities of obligate anaerobes such as genera *Bacteroides*, *Clostridium*, *Lactobacillus*, *Escherichia* and *Bifidobacterium*, as well as yeasts and other micro-organisms living in reciprocal and dynamic relationships with the human host (Fig. 1). Areas along the digestive tract are colonized by different microbial species with diverse abundance, with the highest microbial counts being found in the colon and distal gut. Indeed, 10^{12} colony-forming units (c.f.u.) ml^{-1} were found in the large intestine and about 10^4 c.f.u. ml^{-1} of bacteria were found in the upstream small intestine (Fig. 1) [2]. These microbial communities carry out a wide range of biochemical activities that affect the human body, including metabolite production, physiological regulation and interaction with the host's cellular response and immunity [3–11]. Moreover, the intestine is uniquely exposed to changing environmental factors such as diet, xeno-antibiotics, pathogens and other conditions relating to life history, e.g. physical activity [12]. Occupying such a variable niche, the intestinal microbiota responds to both environment and host status following the principles of biological adaptation and contributes in turn to the host's fitness and homeostasis.

Microbial diversity is thought to contribute a functional reservoir of the microbiota–human entity and effectively expand the metabolic capabilities of the human host beyond those encoded by its own genome [13]. The gut microbiota may also participate in non-cell-autonomous developmental processes [14]. Moreover, the host genetics has been found to influence microbiota composition; thus, each individual may potentially be regarded as a unique ecosystem. Dynamic changes within the same individual, on the other hand, may be observed within healthy states in response to varying conditions (e.g. dietary changes [15–17]), while disturbances of the gut microbiota called dysbioses have been found in multiple diseased states. Well-studied disease-associated dysbioses include obesity [18, 19], type 2 and type 1 diabetes

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Keywords: human gut microbiota; microbiota–host interaction; gut ecology; metabolic networks; genetic regulation; diet; food additives; metabolic disease; obesity; type 2 diabetes; atherosclerosis; bariatric surgery; exercise.
Abbreviations: ABC, ATP Binding Cassette; AGB, Adjustable Gastric Banding; AMPK, 5` AMP-activated Protein Kinase; γ-BB, gamma-butyrobetaine; BIB, Bilio-pancreatic diversion with or without duodenal switch; BMI, Body Mass Index; BPD/DS, Bilio-pancreatic diversion with or without duodenal switch; CD, Crohn's Disease; CDI, C. difficile Infection; CFU, colony forming unit; CRC, Colorectal Cancer; CVD, Cardiovascular Disease; FFAR, Free Fatty-Acid Receptor; Fiaf, Fasting-Induced Adipose Factor; FMO, Flavin monooxygenase; FMT, Fecal Matter Transplant; FXR, Farnesoid X-Receptor; GB-IL, Gall Bladder Diversion to the Ileum; GI, Gastrointestinal; GIP, Glucose-dependent Insulinotropic Peptide; GIP, Gastric Inhibitory Peptide; GLP-1, Glucagon-like Peptide-1; GMT, Gut Matter Transplantation; GPCRs, G Protein-Coupled Receptors; GRAS, Generally Regarded as Safe; IL, Interleukin; LPS, Lipopolysaccharide; NAFLD, Non-alcoholic Fatty Liver Disease; NAPEs, N-acyl phosphatidylethanolamines; NASH, Non-alcoholic Steatohepatitis; NNS, non-nutritive Sweeteners; NOD, Non-Obese Diabetic; PYY, Peptide YY; qPCR, Quantitative PCR; r, ribosomal; RYGB, Roux-en-Y Gastric Bypass; SCFA, Short-Chain Fatty Acids; SG, Laparoscopic Sleeve Gastrectomy; SVSG, Vertical Sleeve Gastrectomy; T1D, Type 1 Diabetes; T2D, Type 2 Diabetes; TLRs, Toll-like Receptors; TMAO, Trimethylamine N-oxide; TMAO, Trimethylamine N-oxide; Treg, T regulatory; VBG, Vertically Banded Gastroplasty.

(respectively T2D, T1D) [20, 21], atherosclerosis [22], cirrhosis [23] and cancer [24].

Micro-organisms present along the gastrointestinal (GI) tract support food breakdown and ferment complex carbohydrates and amino acids, produce short-chain fatty acids (SCFAs; e.g. acetate, propionate and butyrate) and contribute to lipid and amino acid metabolism, protein digestion and energy balance [25–37]. For example, *Bifidobacteria* and lactic acid bacteria, *Lactobacilli,* produce essential vitamins that humans cannot synthesize [38]. In the small intestine, species belonging to the genus *Bifidobacterium* utilize carbohydrates and fatty acids to synthesize vitamin K and water-soluble B vitamins *de novo* [38]. The gut microbiota was also found to metabolize potentially toxic compounds such as indoles, derived from tryptophan *in vivo* breakdown. Notably, *Clostridium sporogenes* can convert indole to indole-3-propionic acid, a powerful antioxidant and potential Alzheimer's disease treatment [39]. Therefore, the human gut microbiota is being extensively studied for its deep influence on global physiology and metabolism, for its adaptive potential and for overall effects on host pathophysiology [40–42].

Heritability of the intestinal microbiota

Microbial ancestry, in addition to diet and lifestyle, is thought to affect individual microbial diversity. Analyses of the faecal microbiome of 1126 twin pairs revealed a close relationship between the microbiota and heritable microbial taxa [43]. Moreover, the microbiota from identical twins was more closely related than that of fraternal twins [43–47], corroborating evidence that one's genetic makeup may influence the type and taxonomical composition of the human gut microbiota and despite a small-sample study with contradicting results [48]. Heritable bacteria were similarly abundant among genetically close relatives and included, among others, species belonging to the bacterial family *Christensenellaceae* and archaeal methanogens [49]. Interestingly, the presence of *Christensenellaceae* also distinguishes omnivorous mammals from strict herbivores and carnivores [50]. The micro-organisms themselves actively contribute to shape the consortium by secreting regulatory peptides and molecules influencing the metabolic profile of co-existing species. Species cross-talk is thought to underlie the observation that individuals with lean body mass index (BMI) harboured anti-correlated abundance of the families *Methanobacteriaceae* and *Dehalobacteriaceae*, *Firmicutes* and *Tenericutes* vs the families *Bacteroidaceae* and *Bifidobacteriaceae* [49].

To minimize the effects of host genetics and test a causal link between microbial consortia and metabolic state, the microbiota of patients with Crohn's disease (CD) has been studied in genetic relatives (twins, parents and non-twin siblings) using DNA fingerprinting [51, 52]. A dysbiotic signature was present in twin CD patients, and absent in unaffected relatives, despite their shared genetic background [51]. CD twins displayed under-represented butyrate-producing bacteria, including *Faecalibacterium prausnitzii* [53], *Bifidobacterium adolescentis* [54], *Dialister invisus* and unknown species of *Clostridium* cluster XIVa, as well as an increase of *Ruminococcus gnavus* [51, 55].Unaffected relatives of CD patients harboured abundant mucin-degrading bacteria phylogenetically related to *Clostridium nexile,* and *Ruminococcus torques* (both belonging to non-butyrate-producing members of *Clostridium* cluster XIVa) and *Clostridium comes* [51]. Clostridia have been previously linked to CD [56, 57], and *C. comes* may contribute to CD pathogenesis through its interaction with host immunity [51, 58]. Thus, a shift in the normal microbial community and altered mucin degradation was found to result in dysbiosis and systemic inflammation, all contributing to the CD presentation.

Microbiota-induced changes of gene expression

Gut colonization by the microbiota was found to elicit transcriptional changes in the intestinal cells. Comparative transcriptomics of fractionated epithelia from the jejunum, ileum and colon derived from germ-free mice and siblings colonized in adulthood showed regional specificity for 86% of 2256 microbiota-responding genes, including metabolic genes in the colon and immune-related genes in the ileum [59]. Upon microbial colonization, functions related to protein biosynthesis became enriched in the crypts – those related to cholesterol and lipid metabolism in the ileum tip and those related to glutathione-S-transferase activity in the colon tip – while amino-acid transport and glycogen metabolism appeared to be reduced, suggesting regional, and likely cell-specific, differences in the response to microbiota [59]. While the proximity of the intestinal mucosa to the microbiota may easily justify reciprocal influence, growing evidence points to deeper physiological connections operating through the host hormonal signalling.

Diet influences the intestinal microbiota

Among the environmental factors interacting with the microbiota, the host's diet can strongly influence the intestinal microbial population. From a biological viewpoint, microbial adaptation to available nutrients may improve the health and survival of the host–microbiota unit in different climates and in response to seasonal fluctuations of food supplies. Indeed, observations of early development have indicated that the gut microbiota responds dynamically to the changing diets of developing infants [45]. Regardless of age and genetic background, diet seemed to contribute to the observed geographical differences in gut microbiota composition among different human populations [19, 60–64]. In adults, fibre-rich diets correlated with the prevalence of *Prevotella*, *Bifidobacteria* and *Lactobacilli*, high-carbohydrate diets with *Methanobrevibacter*, *Prevotella* and *Candida*, and high-fat-and-amino-acid-rich diets with *Bacteroides* [65, 66]. When fed an obesogenic 'lard diet' high in saturated fat, both human and mice subjects gained weight, became insulin-resistant, and displayed inflammation in white adipose tissue and immune activation, as compared to individuals fed identical calories from a diet high in polyunsaturated fats. Western-type diets rich in fatty acids were found to increase the expression of the Toll-like receptors (TLRs) and alter the permeability

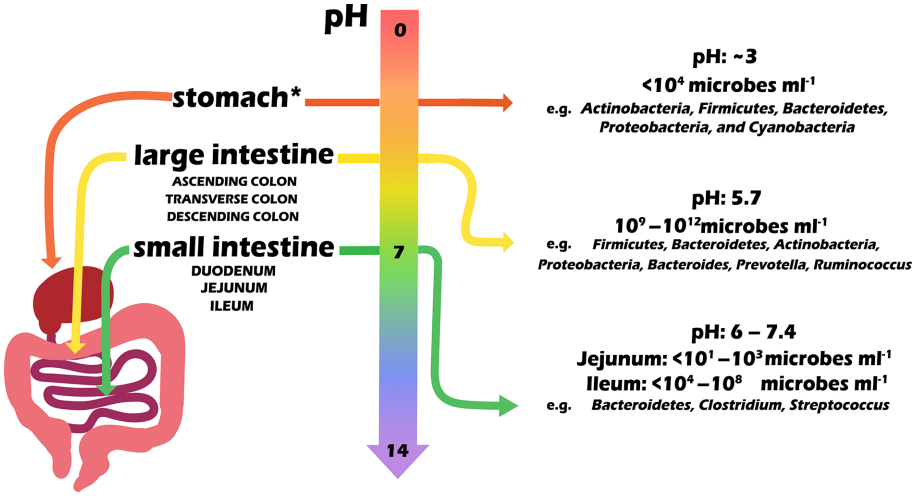

Fig. 1. Bacterial distribution and abundance in the human lower gastrointestinal tract. The GI tract contains environments with distinct conditions that favour colonization by different micro-organisms. This figure highlights the changes of pH in the stomach and intestine with the relative microbial counts and gives examples of abundant resident taxa [332–339].

of the intestinal barrier, promoting inflammation, which has been described in detail elsewhere [63, 67–71].

Food additives

While the global effects of diet on the intestinal microbiota are well recognized, much less is known about the specific effects of single dietary components, including the food additives used in modern-day chemical engineering, food optimization, storage and distribution. As such, there is growing concern for the potential to influence the human microbiome directly or indirectly and harm host metabolism through the use of food additives. Emulsifiers and surfactants such as polysorbate-80 and carboxymethylcellulose, used to texturize and stabilize emulsions in preserved foods for human consumption, are recognized by the United States Food and Drug Administration (US FDA). However, neither additive is found in the US FDA list of generally recognized as safe (GRAS) products for consumption and negative effects were recently identified. In mice, polysorbate-80 and carboxymethylcellulose were found to decrease microbiota diversity and *Bacteroidales* levels, increase the representation of mucolytic bacteria, halve the protective mucus layer of the intestinal epithelium and reduce the production of anti-inflammatory *n*-butyrate [72]. Because these effects were absent in germ-free mice, and could be transferred to other animals via microbiota transplantation, emulsifiers appeared to act through the intestinal micro-organisms [72]. Further investigation of the human microbiota *ex vivo* revealed that emulsifier administration changed the microbiota transcriptional activity, especially increasing expression of the pro-inflammatory *lipopolysaccharide* (LPS) and *flagellin* genes [73]. Higher flagellin expression is predicted to increase bacterial motility and the capacity to penetrate the protective mucus layer of the intestine [73]. Moreover, flagellin activated the host's TLR5-dependent inflammatory response *in vivo*, which in turn induced he secretion of antibacterial peptides, and may contribute to the observed taxonomical shifts in the microbial consortia in polysorbate-80- and carboxymethylcellulose-administered animals [73]. Thus, it appears that emulsifiers cause a cascade of biological effects simultaneously in the microbiota and host, and impinge on the host's genetic resilience to offset detrimental changes and maintain balance. Indeed, wild-type mice displayed low inflammation in response to emulsifiers; however, animals genetically susceptible to intestinal inflammation and carrying interleukin-10 (IL-10) or TLR5 mutations showed extreme disruption of the microbiota composition and developed severe colitis [72].

Non-nutritive sweeteners (NNSs) are non-caloric alternatives to sugars that are relatively indigestible and pass through the digestive system without being assimilated. They are commonly used in diet soft drinks, chewing gum and sugar-free desserts. Despite their long-standing use to offset obesity, it seems that they may, paradoxically, contribute to it and to other metabolic disorders through at least two pathways [74–76]. The first involves disruption of taste perception and energy intake in the host and is independent of the microbiota [77] thus, is not further discussed here. A second pathway, however, appeared to be linked to the gut microbiota. NNS consumption in mice increased fasting glycaemia and glucose intolerance, regardless of diet, effects that could be transferred to other animals via faecal transplantation [78]. Studies in rats have shown that three common NNSs, saccharine, sucralose and aspartame, also affected the microbiota [79–81]. These earlier observations were confirmed in subsequent studies using rodents and swine (reviewed in [82]). In both mice and humans, NNSs altered the taxonomical composition of the intestinal microbiota and changed microbial gene activity and metabolism. Despite attenuating the increase in *Firmicutes*-to-*Bacteroidetes* ratio normally seen as a consequence of high-fat

diets, aspartame consumption heightened total bacteria (especially *Enterobacteriaceae* and *Clostridium leptum*), upregulated genes encoding mono- and oligo-saccharide uptake components, and was also processed into propionate that stimulated gluconeogenesis and increased insulin sensitivity [83]. Saccharin consumption augmented species of the order *Bacteroidales*, reduced members of the genus *Lactobacilli* and differentially altered taxa of the order *Clostridiales* in ways resembling the changes accompanying T2D [82]. Many NNSs are bacteriostatic for several species, including those involved in the aetiology of dental caries [81, 84–88]. Saccharin also decreased the expression of phosphotransferases involved in carbohydrate uptake [82], which may conceivably lessen some bacterial fermentative capabilities. Microbial metabolic changes were associated with the host's higher energy uptake and elevated glucose and lipid synthesis, all recognized obesity risks. Saccharin-induced changes in the microbial populations were reproducible in host-free contexts, suggesting direct effects on the microbial metabolism [82]. Moreover, saccharin-grown microbial cultures induced the above host metabolic changes when transplanted in animals, even without saccharin administration [82]. Human subjects were found to respond to saccharin differentially. In saccharin responders, consuming NNSs clearly altered the gut microbial composition in as little as 4 days, suggesting that higher energy harvest from dietary sources could rapidly increase glycaemic levels and glucose intolerance [78]. The microbiota perturbation appeared reversible upon cessation of saccharin administration, at least in some individuals [82]. Some NNSs can be metabolized by both microbiota and host [89], likely with varying individual efficiency [82, 90]. Corroborating the link between the microbiota and host genetics, the individual capacity to respond to saccharin correlated with the microbiota composition in responders vs non-responders prior to saccharin administration [82]. Given the extreme microbial metabolic diversity and observations of differential metabolic response even in genetically related bacterial strains [81], it is conceivable that specific NNSs may preferentially affect certain taxa and contribute to the observed patterns of dysbiosis. The superposition of such adaptive responses, however, appears to converge into fewer resulting metabolic (possibly diseased) states.

MICROBIAL SCFAS AND THEIR EFFECTS ON THE GUT MICROBIOTA AND HOST METABOLISM

SCFAs are prominent byproducts of the fermentation of indigestible polysaccharides from dietary fibre by the intestinal microbiota. They are found in the large intestine in high tens-of-millimolar concentrations [91, 92]. The taxonomical composition of the gut microbiota is thus expected to determine the fermentation type. SCFAs can be utilized as an energy source by the colonocytes [92–97]. *N*-butyrate, for example, is up-taken by mitochondria and undergoes aerobic fatty acid oxidation to produce acetyl-CoA, which enters the Kreb's cycle [98]. SCFAs can become substrates for cholesterol and long-chain fatty acid synthesis, as well as precursors for gluconeogenesis [28]. SCFAs may serve as building blocks, presumably through their conversion into glucose, although this pathway may be secondary to utilizing glucose from other sources [28]. SCFAs may also function as signalling molecules, possibly via chromatin acetylation, and affect the host's lipid and glucose levels, liver, skeletal muscle and immunity [99–108]. In mice, gut-generated propionate prompted hepatic gluconeogenesis, while butyrate and acetate were lipogenic [28]. Butyrate regulates *claudin 1* and *mucin* gene expression and other tight junction proteins [109]. Functional tight junctions are essential to the intestinal barrier, the integrity of which is important for immune balance to reduce the risk of endotoxemia (the release of toxic pathogen-derived metabolites in the blood), minimize inflammation and reduce adipose cell activation [110, 111].

In dysbiosis, aside from host-related factors e.g. diet and exercise, altered cocktails of microbially-produced SCFAs may influence obesity, insulin sensitivity, weight gain and retention, possible comorbidities, and numerous health risks [28, 35, 107, 112]. Fermenting complex carbohydrates and plant-derived polysaccharides, *Firmicutes* and *Bacteroidetes* produce up to 70% of the total SCFA intake [28]. Firmicutes are the main producers of *n*-butyrate, while *Bacteroidetes* are the main producers of acetate and propionate [28, 113, 114]. SCFAs bind to G protein-coupled receptors (GPCR) in the host cells. Among these, GPR41 (also called free fatty acid receptor, FFAR3) and GPR43 (FFAR2) display 41% of identity at the primary sequence level [115]. GPR41 and GPR43 bind acetate, propionate and butyrate at low affinity (EC_{50}=0.5 mM) and are expressed in many tissues, including white adipose tissue and pancreatic β and α cells, and mediate inflammation and species-specific responses [115–118]. Studies in mice have implicated GPR41 and GPR43 in colitis, asthma and arthritis [113, 119–126]. GPR43 has been directly linked with obesity [113, 124, 125, 127–129].

SCFAs were also found to influence host hormonal signalling [113, 130]. Weight control and energy metabolism are regulated in part by the anorexic peptide hormones glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic peptide (GIP) and pancreatic peptide tyrosine tyrosine (PYY) normally secreted by enteroendocrine cells. GLP-1 and PYY are known to influence levels of satiety and feeding behaviour, generally promoting weight loss and hypoglycaemia, and lowering the diabetes risk [113, 131]. SCFAs stimulated PYY and GLP-1 secretion [132], improved glucose tolerance, increased intestinal gluconeogenesis and decreased weight gain [133, 134]. In lean mice, oral supplementation with butyrate and propionate stimulated pro-anorexic hormones, improved insulin sensitivity, and regulated both satiety and body weight, even when combined with a high-fat diet [135]. Infusion of acetate and butyrate increased GLP-1 and PYY secretion independently of GPR41 and GPR43, suggesting that SCFAs may be utilized as energy sources by colonic enterocytes [107, 135]. In rat colon, GPR43 and GPR41 ligand binding had no effect on GLP-1 secretion and glucose tolerance, although a GPR41 agonist elevated PYY release [94]. In

both humans and mice, GPR41 activation promoted satiety-inducing leptin and PYY production [26, 136, 137], while GPR43 activation suppressed insulin-dependent fat accumulation [125]. When fed high-fat diets, GPR43-deficient mice (*GPR43*$^{-/-}$) displayed higher weight gains than control mice and were also obese on a normal diet [125]. Conversely, adipocyte-specific GPR43 overexpression produced leaner mice than the wild-type, due to suppressed insulin signalling in adipose tissue [125]. Hence, GPR41 and GPR43 have direct effects on body weight and feeding. The response to microbiota-produced SCFAs via GPR41 and GPR43 receptors appeared to be conserved in many mammalian species. Important to control body weight and glycaemia, propionate activated intestinal gluconeogenesis via fatty acid receptor GPR1/FFAR3 signalling, while butyrate instead functioned through cyclic AMP, and succinate functioned through an alternative mechanism [133].

Two intestinal SCFA producers, *Bacteroides thetaiotaomicron* and *Methanobrevibacter smithii*, could affect GPR41 activity [26]. Upon *B. thetaiotaomicron* and *M. smithii* inoculation, germ-free and conventional knockout *GPR41*$^{-/-}$ mice exhibited slower weight gain and significant weight reduction, as compared to the controls, possibly because of decreased nutrient absorption [26]. *GPR43*$^{-/-}$ *GPR41*$^{-/-}$ double knockout mice fed a high-fat diet displayed aspects of global improvement in pancreatic β-cell function, including restored glucose homeostasis and greater insulin secretion, in addition to improved glucose tolerance [113, 138, 139].

MICROBIOTA AND ENVIRONMENT

Host body mass and obesity

Obesity is considered to be a complex and largely preventable condition with increasing prevalence worldwide. Caused by a growth in adipose tissue and increased BMI [140], obesity can lead to additional conditions, including diabetes mellitus, insulin resistance, dyslipidemia, hypertension, atherosclerosis and epigenetic dysregulation [141]. Animal models of obesity have suggested that the gut microbiota composition may influence obesity independently of diet, likely due to the differential capacity of extracting monosaccharides and energy from food in obese vs non-obese individuals and the induction of hepatic lipogenesis [35, 142–144]. Indeed, the presence of a microbiota impacts on body fat, as faecal transplant from conventionally reared mice into germ-free animals of the same genotype increased total body fat [37]. Homozygous mice mutants in the *leptin* gene are a widely used obesity model (C57BL/6J$^{ob/ob}$, herein *ob/ob*). Different compositions of the faecal microbiota were found in the *ob/ob* mice homozygotes, their lean *ob*/+ and +/+ siblings, and their *ob*/+ mothers fed the same chow diet [25]. Specifically, the *ob/ob* mice harboured 50% less *Bacteroidetes* and a greater proportion of *Firmicutes*. Supporting the conclusion that obese mice have distinct metabolic potential and higher lipogenesis, microbial obesity-associated genetic tags were enriched in carbohydrate-degrading enzymes, e.g. glycoside hydrolases, ATP-binding cassette (ABC) transporters and various fermentation enzymes [25]. Similar results were obtained in human cohorts. Terminal restriction fragment length polymorphism and next-generation sequencing analyses of the faecal microbiome from obese and non-obese Japanese subjects revealed that the former harboured less *Bacteroidetes* and increased *Firmicutes* compared to the latter [145]. In contrast, 16S ribosomal (r)RNA sequencing data from the Human Microbiome Project [146] did not show a quantitative association between BMI and the *Firmicutes*-to-*Bacteroidetes* ratio, or the relative abundance of the five major gut bacterial phyla, namely *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Proteobacteria* and *Fusobacteria* [147]. This initial discrepancy suggested that multiple factors may contribute to obesity, some of which may escape detection, depending on host characteristics, co-morbidities and/or analysis sensitivity [148]. Moreover, BMI values may need to be tailored to different ethnicities [149]. Despite discrepancies and possible individual and/or population differences [150], there is accumulating evidence pointing to the obese state being often characterized by altered *Firmicutes*-to-*Bacteroides* ratios [21, 25, 35, 145, 151–156], deviating from the 51.9% *Firmicutes* to 37.68% *Bacteroidetes* ratio generally considered healthy [157]. Metanalyses of the microbiota from lean and obese subjects with inflammatory bowel diseases have indicated a trend for reduced diversity in obese vs lean patients, regardless of the *Firmicutes*-to-*Bacteroidetes* ratio [148].

Obese mice and humans tend to respond to a switch to low-calorie diet inducing weight loss by adjusting their *Firmicutes*-to-*Bacteroidetes* ratio [34]. Moreover, the human obese microbiota can reproduce the obesity profile when transplanted into mice [158]. Diet composition, adiposity and the microbiota, however, appear to interact in many reciprocal ways that are challenging to study. Using a murine model and keeping parameters such as individual weight controlled, dietary fat appeared to influence the microbiota. Both body weight and diet seemed to affect representation of the genus *Allobaculum* [159], *Firmicutes* that respond to dietary fat [110]. Circulating leptin correlated with mucin production by the enterocytes both *in vivo* and in cell culture [160, 161] which reduced representation of the mucus consumers *Akkermansia* and *Allobaculum* and favoured *Mucispirillum*, a group of mucus colonizers [158]. In human obese patients, the genus *Mucor*, normally prevalent in non-obese subjects, increased after diet-induced weight loss [162]. Population analysis of 169 obese and 123 non-obese Danish individuals showed that the former displayed a 'low-gene-count' (<480,000 genes, with an average of 380,000), whereas healthy subjects displayed 'high-gene-counts' (average 640,000) [163], implying that the obese state is associated with differential microbial diversity and reduced diversity. The low-gene-count obese microbiota featured increased species representation from the phyla *Proteobacteria* and *Bacteroidetes*, with potential pro-inflammatory microbes such as *R. gnavus* (which has been linked to IBD) [51, 164], as well as *Parabacteroides*, *Campylobacter*/*Shigella*, *Dialister*, *Porphyromonas*, *Staphylococcus* and *Anaerostipes*. The high-gene-count lean microbiota, on the other hand, included *Verrucomicrobia*, *Actinobacteria*

and *Euryarchaeota*, with abundant anti-inflammatory species such as *F. prausnitzii, Anaerotruncus colihominis, Butyrivibrio crossotus,* and species of the genus *Akkermansia*. The high ratio between *Akkermansia* and *R. torque/gnavus* appeared to favour a resilient microbial ecosystem producing high levels of *n*-butyrate, displaying methanogenic/acetogenic metabolism and high H^+, and producing scarce hydrogen sulfide, associated with overall reduced incidence of metabolic disease and obesity [163]. Compared to the lean high-gene-count one, the low-gene-count obese microbiome had genetic potential to produce noxious metabolites through dissimilatory nitrate reduction and aromatic amino acid degradation, consumed hydrogen to reduce sulfate, and displayed improved oxidative stress response and higher mucolytic capacity, which presumably facilitate its retention in the intestinal environment [163]. Note, high levels of hydrogen sulfide inhibit butyrate oxidation and colonocyte mitochondrial function and favour pathobiont proliferation [165]. In contrast, the high-gene-count lean microbiome had potential for high organic acid and hydrogen production, with the latter being utilized for methano- and aceto-genesis [163] . In human subjects, higher levels of faecal SCFAs were also associated with central obesity (i.e. waist circumference), hypertension, subclinical measures of cardiometabolic disease (e.g. inflammation, glycaemia and dyslipidemia) as well as a measure of gut permeability (i.e. lipopolysaccharide-binding protein) [166]. Inflammation, in turn, promotes insulin resistance and hyperphagia (overeating) [68]. Microbially produced SCFAs may be converted into more complex lipids in the liver [107] and are ultimately deposited into adipose cells, contributing, when in excess, to the pathophysiology of obesity [157]. The prototypic Western diet was observed to support a bloom of *Firmicutes* (e.g. *Eubacterium dolichum*) and mollicutes at the expense of *Bacteroidetes*. Mollicutes can efficiently metabolize simple sugars that are abundant in the distal guts of obese individuals [157], which were found to be proportional to adiposity levels therein [167]. Transplantation of just *E. dolichum* (similar to the entire intestinal microbiota) from obesity-prone rats into healthy normal animals was sufficient to increase markers of adipogenesis and lipogenesis [157, 168]. Finally, bacterial dysbiosis seemed to be only one of the features of the obese microbial consortia. In fact, different proportions of fungi were also found, with *Eurotiomycetes* decreased to less than 1%, increased populations of members of the families *Dipodascaceae* and *Saccharomycetaceae* (class *Saccharomycetes*, phylum *Ascomycota*) and class *Tremellomycetes*, and correlated with poor-quality host glucose and lipid metabolic profiles and metabolic disorders, including insulin resistance, as compared with non-obese counterparts [162]. Conversely, the fungal families *Mucoraceae*, *Nectriaceae*, *Ceratocystidaceae*, *Corticiaceae*, *Debariomycetaceae* and *Hypocraceae*, and the genera *Mucor*, *Penicillium*, *Monilliela* and *Ceratocystis* (classes *Agaricomycetes* and *Eurotiomycetes*, phylum *Zygomycota*) were found to be associated to microbiota protective against metabolic disorders [162]. Knowledge of the response of other components of the microbial consortia (e.g. viruses) is very limited.

Diet, microbiota and obesity

Obese and diabetic individuals were found to have high capacity for dietary lipid absorption and elevated intracellular bile acids, which inhibit the synthesis of hepatic bile acid [169, 170]. The microbiota can affect triglyceride storage and release in response to lipid ingestion and energy demands, and participates in bile acid synthesis. In fact, secondary bile acids are first synthesized by the liver and then microbially processed [171]. *Lactobacillus* and *Bifidobacteria* can produce bile salt hydrolase, the enzyme that catalyzes bile acid deconjugation, reducing lipid emulsification capacity [172], and affects the host systemic lipid metabolism, lowering cholesterol and the uptake of certain lipids [173]. Indeed, changes in microbial consortia were confirmed to alter bile acid metabolism in the ileum [174]. Interestingly, it has been proposed that bile acid metabolism may be an example of microbial long-range communication reminiscent of quorum sensing [171].

In contrast to the lipid and bile acid-related energy storage mechanisms mentioned above, fatty acid oxidation was explored in a lean mouse phenotype to investigate how gut microbes affect energy harvest. Germ-free mice were less likely to become obese when fed an obesogenic diet and were found to have higher-than-normal phosphorylated (active) 5′ AMP-activated protein kinase (AMPK) in muscle and liver cells [175]. AMPK, upon sensing low energy charge, stimulated cellular catabolism and fatty acid oxidation, while simultaneously inactivating anabolism [176]. Moreover, obese mice, both germ-free and conventional, displayed reduced capacity for enzymatic fatty acid oxidation and higher levels of lipogenic factors, including fasting-induced adipose factor (Fiaf), an inhibitor of lipoprotein lipase that promotes fatty acid uptake and oxidation in adipocytes [175, 177]. Recolonization of the intestine of germ-free mice with *B. thetaiotaomicron* inhibited *Fiaf* gene expression and increased both lipoprotein lipase activity and triglyceride storage in adipocytes [37, 178].

N-acyl phosphatidylethanolamines (NAPEs) are lipidic precursors that are normally synthesized by the enterocytes in the proximal intestine in response to feeding and are hydrolyzed to N-acyl-ethanolamides. Endogenous or administered NAPEs accumulate in the hypothalamus and function as anorexigenics, modulating food intake and reducing adiposity, insulin resistance and hepatic lipid accumulation [179–181]. Enterocytes from obese patients do not produce sufficient NAPEs. Additionally, high-fat diets may inhibit NAPE secretion [180, 182, 183]. Thus, bacteria from commensal strain *Escherichia coli* Nissle 1971 were engineered to produce heterologous NAPEs from *Arabidopsis thaliana* to test the remedy potential of boosting NAPE production by means of the gut microbiota. Oral administration of NAPE-producing *E. coli* to C57BL/6J mice fed an obesogenic diet promoted the maintenance of body weight and decreased adiposity compared to controls, as long as the processing enzymes were present [181]. Remarkably, the positive effects persisted for up to 4 weeks after the last administration and were slowly reversed, with the eventual return to the obese state [181]. While these

results have proven, in principle, the potential of manipulating the microbiota to manage diet-induced obesity and associated metabolic diseases, adaptive microbial flexibility may in practice challenge the use of this strategy in therapeutic settings. Potential hurdles may reside in the distinct metabolic differences of the engineered bacteria once they occupy the human intestinal niche, where the availability of synthesis building blocks may vary and substrate or micro-organism competition may be present. A cautionary tale came from the realization that NAPE-producing *E. coli* yielded different profiles of NAPE compounds in laboratory growth conditions vs in animals, indicating that some biosynthetic capabilities may be context-dependent [184].

Antibiotics and obesity

Antibiotics can modulate the microbial gut communities in the short and, possibly, the medium to long term [185, 186]. Antibiotic administration was often found to be obesogenic to the treated mice [187]. Indeed, low-dose antibiotics have been used to boost livestock growth [188]. Compositional changes of the microbiota in response to low-dose penicillin treatment in early life were found to be transient, and the microbiota gradually renormalized after cessation of administration. However, the associated metabolic changes included altered ileal expression of obesity-promoting genes and were, instead, long lasting [187]. In a different model, cefoperazone administration rapidly reshaped both the microbial community and its activity (measured as concentrations of sugar alcohols, SCFAs and bile acids), which eventually reached conditions of high carbohydrate and low SCFAs, which favoured spore germination and colonization of pathogenic *Clostridium difficile* [189]. Microbiota composition improved 6 weeks post-treatment, approaching a metabolic profile resembling untreated age-matched animals. Underscoring that antibiotic treatment can permanently alter the intestinal ecosystem, remodel the microbiota structure and modify its metabolic potential, the new consortium was no longer susceptible to *C. difficile* infection and both microbiome and metabolomic analyses of post-treatment animals remained distinct from both the age-matched control and the pretreatment status [189].

Co-morbidities

Obese individuals are more prone to develop T2D and colorectal cancer (CRC) than non-obese subjects [111, 190]. Onset of sporadic colorectal cancer may be facilitated by the activity of colonic microbiota [191–193], while certain intestinal microbiota compositions may be protective [24]. Several potential micro-organism targets may be relevant to CRC, including *Bacteroides fragilis* (associated with tumorigenesis, producing DNA-damaging genotoxins), and other pathogenic bacteria, commonly present in adenomas and CRC, such as *Fusobacterium nucleatum*, *Porphyromonas asaccharolytica*, *Parvimonas micra*, *Prevotella intermedia*, *Alistipes finegoldii* and *Thermanaerovibrio acidaminovorans* [193–195]. One study demonstrated that compositional changes of gut bacteria paired with cell stress from innate immunity activation promoted tumour growth in the colon [196].

MICROBIOTA AND DIABETES

T2D is a rising disease for which genomic studies have indicated decreased diversity and functional shifts of the gut microbiota [197, 198]. With a presentation of high glycaemia, altered lipid metabolism and high blood pressure that link it to obesity, and numerous metabolic dysfunctions, T2D affects almost 350 million people worldwide [143, 198–201] and is predicted to become one of the top 10 causes of death by 2030 [202]. T2D patients also display immunological abnormalities, including reduced T regulatory (T_{reg}) cells and chronic inflammation, similar to diet-induced obese mice [203, 204]. T2D subjects and mice models often present increased intestinal permeability with bacteraemia and activation of the inflammatory response [204–206]. Studies of small and larger cohorts suggested that, like obesity, T2D is associated with changes in the microbial consortia that are reminiscent of, yet distinct from, those found in obese subjects. Analyses of 60 000 T2D-associated gut microbial markers from a metagenomic linkage group of 368 Chinese T2D patients and control individuals revealed moderate dysbiosis with reduced butyrate producers, e.g. species belonging to the genera *Roseburia* and *Faecalibacterium*, *Eubacterium* and *Clostridiales* sp. SS3/4, and concurrent expansion of non-butyrate-producing *Haemophilus parainfluenzae,* which may have an uncharacterized antagonistic relationship with a T2D-enriched bacteria related to the genus *Subdoligranulum*, and variable opportunistic pathogens with mucin-degrading and sulfate-reducing properties, e.g. *Akkermansia muciniphila* and *Desulfovibrio* sp. 3_1_syn3 [197]. The bacterial metagenome displayed enhanced capability for sugar and amino acid transport, sulfate reduction and xenobiotic processing, and reduced *n*-butyrate synthesis, vitamin and cofactor metabolism, and motility [197]. Notably, genes expressing membrane transporters and markers of oxidative stress resistance were also activated, which implies that the gut environment of T2D patients stimulates bacterial defence mechanisms and is consistent with the observed persistent low-grade inflammation found in diabetic patients [111, 197]. A small study of 18 T2D and 18 normal middle-aged male patients showed that the total intestinal bacterial count was indistinguishable in the two groups, with increased *Firmicutes* in the normal reference group and a trend toward increased *Proteobacteria* and *Bacteroides* in the T2D group [20]. The increased *Bacteroides*-to *Firmicutes* ratio, however, did not correlate with BMI, as one could have predicted on the basis of the obesity studies, indicating that the 'T2D microbiota' and the 'obese microbiota' are distinct [20]. A study of post-menopausal women with 53 T2D patients, 49 with pre-diabetic state and 43 normal subjects, found similar gene counts in all groups (unlike what was found in the obese microbiota), with elevated *Lactobacillus* species, and a decrease in five *Clostridium* species, with no association with BMI [207].

Underscoring the versatility of the intestinal microbiota, the Chinese and European T2D cohorts revealed common metabolic potential and potential discriminating power in the abundance of *Roseburia* and *Faecalibacterium prausnitzii*, yet enough species-levels differences to produce distinct clusters [207]. While such differences may relate to a combination of genetic and lifestyle factors, sex and medications, the metabolic commonalities appeared to be more predictive of T2D than other parameters, including BMI [21]. In particular, the use of anti-diabetic medications was not thoroughly considered, which, in light of recent findings concerning their impact on the microbiota (discussed below), may account for some of the observed differences. The anti-inflammatory action and improved insulin sensitivity associated with the butyrate producers could be transferred to male metabolic syndrome patients via faecal transplantation that increased composition diversity, boosted *Roseburia intestinalis* levels 2.5-fold and prevented the decrease of *Eubacterium hallii* levels observed in the controls [21, 208]. When identifying specific strains to decrease T2D and/or insulin sensitivity, *Lactobacillus reuteri* GMNL-263 was found to decrease T2D morbidity [209].

A particularly well-studied relationship is that of T2D and *A. muciniphila*, a mucin-degrading Gram-negative intestinal bacterium that inhabits many animals and is involved in the biological processes implicated in T2D and obesity [210, 211]. *A. muciniphila* growth conditions are relatively permissive, with temperatures ranging from 20 to 40 °C, and pH from 5.5 to 8, enabling these bacteria to adapt effectively and co-evolve with their host [212, 213]. An astounding 11% of *A. muciniphila* proteins are involved in mucin degradation for energy, carbon and nitrogen acquisition [212], supporting growth and colonization of the intestinal environment even under stress, when nutrition from the host dwindles [214]. As a byproduct of mucin degradation, *A. muciniphila* can form acetate and propionate that benefit neighbouring bacteria, promoting a healthy intestinal barrier [212]. Adhesion of administered *A. muciniphila* improved intestinal permeability and reduced the low-grade inflammation and LPS-induced endotoxaemia typical of T2D and obesity and was positively correlated with gut and systemic health improvements *in vivo* [110, 111, 210, 211]. Consistently, reduced faecal counts of *A. muciniphila* were found in mouse models of obesity and T2D, featuring a thinner mucus layer, intestinal dysbiosis, disrupted gut barrier function and altered glucose homeostasis [211]. Note that *A. muciniphila* elicited interleukin-8 production, a marker of inflammation, albeit at levels 100 times lower than *E. coli,* possibly because of its benign LPS composition that does not cause endotoxaemia [210, 215]. The T2D dysbiosis is thought to reduce GPR signalling because of an altered SCFA profile, thereby favouring lipid accumulation and obesity.

T2D pharmacology and the intestinal microbiota

The anti-diabetic metformin was strikingly found to accumulate in the intestinal mucosa at 300 times higher than hematic levels [216]. Consistently, metformin modulated the microbiota, increased both the abundance and activity of *Akkermansia* [217, 218], improved the *Bacteroidetes*-to-*Firmicutes* ratio [218, 219] and reduced markers of inflammation interleukin-6 and interleukin-1β in adipose tissue, suggesting that at least part of metformin effects are mediated by the microbiota [218]. Metagenomic analyses revealed that mice fed a high-fat diet harboured significantly decreased *Akkermansia* and *Alistupes* populations and increased proportions of species from the genera *Anaerotruncus*, *Lactococcus*, *Parabacteroides*, *Odoribacter*, *Lawsonia*, *Blautia* and *Lactonifactor* [217, 220]. Metformin administration normalized these differences, supported health-promoting *Akkermansia* [217] and stimulated the microbial expression of metalloproteins and transporters [221]. More pronounced shifts were observed in animals fed high-fat diets, suggesting that metformin may affect the microbiota as a function of diet [217]. Metformin stimulated the proliferation of mucin-producing goblet cells that contribute to intestinal barrier integrity and promote immunomodulating T_{reg} cell production [217, 220]. Metformin treatment and oral administration of *Akkermansia* were shown to restore T_{reg} cell population in mice [217], thus increasing the capacity to quell inflammation and oxidative stress in T1D and T2D diabetic models [217, 222]. In human diabetic patients, metformin similarly shifted microbiota taxonomic composition [220, 223]. However, the metagenomics of patient datasets from different countries indicated substantial differences that will have to be investigated [223]. Recognition of the outer membrane protein Amuc_100 by TLR2 was recently found to recapitulate the *Akkermansia*-dependent effects [224]. While the mechanistic details of *Akkermansia* response to metformin remain largely unknown and may be complex [225], improved microbiota parameters and the overall condition of T2D patients suggest that microbiota manipulation may be beneficial in T2D.

Antibiotic effect on insulin sensitivity and obesity

Antibiotic-induced dysbioses appeared to increase the likelihood of developing T1D in non-obese diabetic (NOD) mice. In addition to genetics, T1D has a recognized environmental component [226]. Among genetically susceptible infants, those who develop T1D have an unstable prediabetic microbiota characterized by reduced diversity and expanded *Bacteroides* representation compared to those who do not develop the disease [227]. Conceivably, antibiotics may precipitate the condition towards T1D. Commonly used antibiotics, including vancomycin and neomycin (discussed below), preferentially target SCFA-producing Gram-positive bacteria, including beneficial *Firmicutes*. Vancomycin- and neomycin-induced diabetogenic microbiota was established early in NOD mice, with each antibiotic producing distinct alterations of the SCFA profile, likely reflecting differential remodelling of the microbial community. The dysbiotic status itself (rather than particular microbial species) appeared to be pro-inflammatory and drive autoimmunity [228]. Male patients treated with a vancomycin analogue to remedy infective endocarditis (a bacterial infection localized to the inner surface of the heart) significantly gained weight following a

6-week intravenous treatment [229]. Vancomycin impaired peripheral insulin sensitivity in obese men, likely because of its targeting of *n*-butyrate-producing bacteria (e.g. *Firmicutes*, *E. hallii* and *F. prausznitzii*), promoting a reciprocal increase in Gram-negative *Proteobacteria* (e.g. *Lactobacillus plantarum*) and altered bile acid profile [230]. Antibiotic administration to young mice resulted in increased lipogenesis and gastric inhibitory peptide (GIP), a hormone that induces insulin production and also affects bone remodelling [32, 231]. Thus, diabetogenic microbiomes may develop because of antibiotic exposure [171, 228]. The epidemiology of human obesity and its possible relationship with antibiotic use has been extensively discussed [32, 232–234].

PROATHEROSCLEROSIS, ATHEROSCLEROSIS AND THE HUMAN MICROBIOTA

Atherosclerosis is a clinically silent chronic vascular disease in which plaques of accumulated cholesterol, fat and calcium form inside the arteries and attract macrophages. Atherosclerotic plaques contain microbial DNA, suggesting that the plaque microbiota may be due to the relocation of micro-organisms from the oral or gut communities to the arterial walls, where they initiate an inflammatory response and promote the development of atherosclerotic lesions (atheromas) [235]. 16S rRNA pyrosequencing and quantitative (q)PCR comparison of the microbiomes from several body sites with the atherosclerotic plaques revealed that the *Firmicutes Veillonella* and *Streptococcus*, common members of dental plaques and gut colonizers, and *Chlamydia,* were similarly found in atherosclerotic plaques [235–237]. *Pseudomonas luteola* (previously *Chryseomonas*), already implicated in endocarditis, was only found in plaques [235]. Notably, *Streptococcus* abundance appeared to correlate with LDL cholesterol and total cholesterol, which are common risk indicators for atherosclerosis [235]. A twofold increase of *C. pneumoniae* was identified in aortic tissue of patients suffering from cardiovascular disease (CVD) [235, 238]. The origin of the plaque microbial DNA is still debated and may derive in part from the phagocytic activity of macrophages [235].

Microbial involvement in atherosclerosis may be direct, via relocation or the metabolism of cholesterol, lipids and dietary components that may contribute, at least in part, to the accumulation of pro-atherosclerotic metabolites favoring plaque formation. Among the latter, trimethylamine N-oxide (TMAO) is thought to activate immunity and lead to plaque build-up, possible arterial rupture and atherosclerosis [239–242]. The capacity to produce TMAO from dietary components was introduced in an apolipoprotein E-deficient mouse model (*ApoE*$^{-/-}$) by faecal transplantation and found to result in atherosclerosis [243–245]. Thus, dysbiotic microbiota overproducing TMAO may contribute to disease progression [243, 246]. TMAO is synthesized in two distinct pathways from dietary trimethylamine (TMA) molecules formed by microbial degradation of choline, phosphatidylcholine and L-carnitine found naturally in red meat, eggs and nowadays in some energy drinks [239, 242, 247–250]. Although harmful in large quantities, these nutrients are essential: choline is a building block for neurotransmitters and is crucial for liver metabolism; phosphatidylcholine supports the structural integrity of cell membranes and facilitates cell–cell communication; L-carnitine, although conditionally essential, participates in energy production [251]. TMA is normally excreted with urine, while TMAO is a cardiovascular risk predictor that contributes to inflammation, plaque formation and atherosclerosis [242, 251]. In the direct pathway of TMAO synthesis, TMA molecules are transported to the liver, oxidized primarily by flavin monooxygenase 3 (FMO3) [239, 247], and transformed into TMAO. Likely more relevant for atherosclerosis, in the indirect pathway L-carnitine is first converted into gamma-butyrobetaine (γ-BB) [252], then into TMA and eventually into TMAO by hepatic FMO3 [242, 243, 247]. Multiple steps of the indirect pathway appear to rely on the intestinal microbiota. Bacteria of the phyla *Firmicutes* and *Proteobacteria* were found to influence the initial conversion of L-carnitine to γ-BB [242, 247–250]. Microbial dysbiosis can lead to increased TMAO synthesis [253]. Bacteria (e.g. *C. pneumoniae*, *Staphylococcus* spp., *Streptococcus* spp., *K. pneumoniae, P. vulgaris, Burkholderia* and *Pseudomonas aeruginosa*) have been implicated in accelerating CVD progression [254]. In one study, 8 out of 79 species from the dominant phyla *Firmicutes* and *Proteobacteria* were found to metabolize choline to produce TMA. These included *Anaerococcus hydrogenalis*, *Clostridium asparagiforme*, *Clostridium hathewayi*, *Clostridium sporogenes*, *Escherichia fergusonii*, *Proteus penneri*, *Providencia rettger* and strains of *Edwardsiella tarda* that may have acquired this capability through horizontal gene transfer [255]. Germ-free mice, on the other hand, displayed greatly reduced levels of TMA and TMAO [242, 255].

Attempted microbial manipulations to contrast atherosclerotic disease progression include faecal transplantation, narrow-spectrum antibiotics, probiotics, prebiotics and diets [239, 256]. Probiotics and prebiotics have shown potential for reducing the atherosclerotic plaques [257]. A probiotic mixture known as VSL#3, composed of *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Lactobacillus acidophilus*, *L. plantarum, Lactobacillus paracasei*, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* administered to *ApoE*$^{-/-}$ mice reduced atherosclerosis and improved microbial diversity [257]. Alternative strategies aim at transforming TMA into biologically inert molecules, such as methane [258], through biochemical processes carried out by the archaea *Methanosarcina barkeri* normally found in ruminators [259]. Candidate archaea to carry out such a supplementary function include *Methanobrevibacter smithii*, *Methanobrevibacter stadtmanae* and the recently identified *Methanomassiliicoccus luminyensis*, all known inhabitants of the human intestine [260, 261]. Moreover, rumen-resident *M. luminyensis* B10, which uses hydrogen to reduce methanol, was confirmed to consume the byproducts of TMA catabolism [258]. Thus, therapeutic 'archaebiotics' may potentially limit the accumulation of pro-atherosclerotic metabolites and retard atherosclerotic progression. However, some technical

challenges must first be overcome before archaebiotics can be considered to be of therapeutic value. For instance, *M. luminyensis* is oxygen-sensitive, which reduces its efficacy of colonization upon supplementation [258].

TOWARDS TARGETED MICROBIOTA MANIPULATION

Bariatric surgery

In contrast to caloric restriction and exercise alone, bariatric surgery is considered to be an effective treatment (or co-treatment) for obesity and morbid obesity that may remedy related comorbidities by markedly reducing adiposity for years after the procedure [13, 155, 262–268]. Genetic, physiological, environmental, psychological, social, economic and political factors (e.g. food tax [269]) contribute to the development of obesity to varying degrees. Co-occurring psychiatric conditions such as anxiety and mood disorders [270], as well as T2D, sleep apnea and CVD may also lead to obesity persistence [271]. Strategically reducing and restructuring the gut anatomy, bariatric surgery affects the feeding process (Table 1). For example, in the Roux-en-Y gastric bypass (RYBG), the stomach is reduced and connected to the jejunum, bypassing the duodenum. In vertical sleeve gastrectomy (VSG), the stomach is instead reduced lengthwise. However, while bariatric surgery has traditionally been thought to affect weight loss by reducing stomach size, altering food absorption, and by other postprandial or metabolic effects [272], growing evidence strongly suggests that restriction and malabsorption may instead be secondary [273–275]. The efficacy of bariatric surgery, in fact, appeared to be largely due to its effects on the intestinal microbiota [276]. The impact on host health and the remodelling of the human microbiome observed following bariatric surgery are summarized in Table 1. Obese patients who had undergone gastric bypass surgery featured an increased *Firmicutes*-to-*Bacteroidetes* ratio approaching the microbial profile and species richness structure of lean subjects (as measured by the Shannon index) [21, 25, 145, 152–156, 277–280].

The anatomical reshaping and bypassing created by these surgeries were found to alter the composition, genetic content and fermentation profiles of microbes in the gut, promoting decreased overall adiposity, rapidly improved glucose metabolism and remission of obesity comorbidities (Table 1) [271, 274, 277]. The faecal microbiome from patients having undergone RYBG and VSG revealed expanded *Proteobacteria* populations including *Escherichia*, *Klebsiella* and *Pseudomonas*, and reduced representation of species from the phylum *Firmicutes*, e.g. *C. difficile*, *Clostridium hiranonis* and *Gemella sanguinis* [271]. Confirming that the physiological changes observed post-bariatric surgery depended on the microbiota, mice colonized with microbiota from RYBG- and VSG-treated patients maintained a lower weight than those colonized with the obese microbiota withdrawn prior to the surgical procedure [267, 271]. Additional studies have shown that bariatric surgery alone was insufficient for remission of obesity and its symptoms, without key metabolites contributing to weight maintenance. The nuclear bile acid receptor, farnesoid X-receptor (FXR), involved in lipid–glucose metabolism [281, 282] appeared to be a required mediator, because FXR knockout mice having undergone VSG were unable to regulate bile acids and did not lose weight when overfed [264]. In light of these observations, it is tempting to speculate that the changed anatomy may alter the microbial environment in ways conducive to the host's health and reminiscent of a previous example of environmental normalization [19]. Perhaps more important, digestion may be substantially different, especially after RYBG, because of the duodenum bypass, which is expected to change the composition of the digested food arriving in the jejunum, conceivably affecting members of the microbial communities differentially.

To better understand the effect of bile acid-mediated weight loss following bariatric surgery, wild-type C57BL/6J mice, and mutant GLP-1 knockout (*GLP-1r*$^{-/-}$), FXR-null (*FXR*$^{\Delta/E}$) and *Tgr5*$^{-/-}$ mice were fed *ad libitum* on lean or high-fat chow and subjected to gall bladder diversion to the ileum (GB-IL) [283]. *Farnesoid X-receptor*, but not *Tgr5* loss of function, stimulated weight loss in obese GB-IL mice, while among lean mice improvements in glucose tolerance were observed, independent of changes in body weight, *habitus* (body build) or food intake [283]. GB-IL lean mice likewise displayed improved glucose tolerance, which was conceivably attained through improved hepatic insulin sensitivity, better GLP-1-mediated bile acid circulation and FXR functionality [283–287]. In response to surgery, the faecal microbiome of GB-IL mice had improved abundances of *A. muciniphila*, *Clostridiales*, *Oxalobacteraceae, Streptococcaceae* and *Ruminococcaceae*, although *Lactobacillaceae* and *Lachnospiraceae* members (including the genus *Roseburia*) were reduced [283]. Because these studies strongly suggest that bariatric surgery can remodel the microbiota and substantially improve obesity in the long term, surgery is regarded as an effective means to remedy cases of extreme obesity. However, it is also an invasive procedure with associated risks. Therefore, non-surgical manipulations of the microbiota are preferable low-risk alternatives to treat moderate obesity and its associated symptoms.

Non-surgical manipulations of the microbiota

Faecal microbiota transplant (FMT) has been successful in treating *C. difficile* infections (CDIs), achieving a success rate of 80–90% in patients of different ages [256, 288], and this has inspired optimism about attempting to manipulate the 'obese' microbiota [289–291]. FMT of normal heterologous microbiota into obese patients was found to promote insulin sensitivity as a result of a 2.5-fold increase of *n*-butyrate-producing intestinal microbes such as *R. intestinalis* [208]. Persistence for a minimum of 6 weeks following FMT suggested that a gut microbiota transplant (GMT) may offer an alternative to bariatric surgery [208]. Currently, the use of FMT or GMT as a treatment for any disease other than CDI requires approval by the USA Food and Drug Administration (FDA) with legitimization of an approved investigational new drug permit [292, 293] and may require better understanding of

Table 1.

Restrictive bariatric surgeries				
Name	**Description**	**Health impact**	**Effects on the human microbiome**	**References**
Vertical sleeve gastric bypass (VSG)	Gastric resection of the fundus, creating a tubular gastric pouch that connects the esophagus with the duodenum	Remission of T2D, nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH). Improved glucose tolerance. Improved BMI	Increased circulation of bile acids, leading to increased farnesoid X-receptor (FXR) signalling that improves gut environment, and in turn microbiota diversity	[264, 340–342]
Vertically banded gastroplasty (VBG)	A small stomach pouch is stapled out. A metallic band is secured slightly below the pouch to slow the transit of food into the lower stomach	Sensation of early satiety (i.e. quickly filled stomach, triggers satiety and empties slowly). Increased pressure on the proximal pouch reduces food intake	Significant increase in the number of circulating bile acids and metabolites (e.g. glycochenodeoxycholic, glycodeoxycholic, glycocholic and taurodeoxycholic acids). Improved insulin sensitivity, incretin secretion and postprandial glycaemia. Remission of NAFLD and significant improvement of liver enzymes and liver triglyceride levels	[343–347]
Laparoscopic sleeve gastrectomy (SG)	Outer stomach is removed while preserving the integrity of the pylorus. No intestinal bypass (see also RYGB below)	Decreased weight and BMI. Euglycaemia via restored fasting plasma glucose, and glycosylated haemoglobin levels. Restored insulin tolerance. T2D remission of independent of oral antidiabetics. Reduced perioperative morbidity and recovery time, as compared to RYGB	Increased *Bacteroidetes*-to-*Firmicutes* ratio at 1 and 3 months post-surgery (increased *Bacteroidetes* and unchanged *Firmicutes*). Increased order *Lactobacillales*	[262, 348–354]
Adjustable gastric banding (AGB)	A saline-filled silicon band is fitted around the stomach, near the esophageal junction, and imposes gastric restriction. Band resizing is achieved by adding or removing saline through a port	Sustainable weight loss and T2D remission	Microbiome effects not yet described	[273, 355]

Continued

Table 1. Continued

Restrictive and malabsorptive bariatric surgeries				
Roux-en-Y gastric bypass (RYGB)	A large portion of the stomach and duodenum is surgically removed. Nutrients are redirected through a small stomach pouch, and into a lower section of the small intestine. The remaining stomach and duodenum are reattached further down, changing the point at which bile acids enter the small intestine. Thus, RYGB restricts gastric volume, and diverts ingested nutrients away from the proximal small intestine	Improvements in weight loss and metabolism through the physical rerouting of the gut (decreased macronutrient absorption). Demonstrated changes in food preferences, increased satiety combined with release of pro-satiety hormones [glucagon-like peptide 1 (GLP-1) and peptide YY (PYY)] in the gut. Improved gastric emptying, bile acid metabolism via increased signalling through the bile acid receptor FXR. T2D remission. Improved BMI	Change in the abundance and composition of gut microbes. Decreased *Firmicutes*-to-*Bacteroidetes* ratio 3–6 months post-surgery. Expansion of *Proteobacteria* (e.g. *Enterobacteriaceae*) communities, and a decrease in *Firmicute* (e.g. *Clostridium*). Improved effects of probiotic supplementation because of reduced stomach (low-pH environment, unfavourable to microbiota)	[153, 262, 264, 356–361]
Bilio-pancreatic diversion with or without duodenal switch (BPD/DS)	A segment of the duodenum is sectioned (or bypassed to a distal portion of the stomach). The small intestine is transected to the Treitz and ileocecal valve, plus RYGB from the gastric pouch to the distal bowel loop. The resulting alimentary limb and an attached biliopancreatic limb form a channel. A duodenal switch can be augmented by the preservation of the lesser curvature, antrum, pylorus and opening of the duodenum, as well as by lengthening the common channel from 50 cm to 100 cm in length	Combines nutrient malabsorption and restriction, causing significant weight loss. T2D remission. High perioperative mortality	Microbiome effects not yet described	[362–364]
Bilio-intestinal bypass (BIB)	A shunt is inserted between the beginning and end of the small bowel, thereby disabling a large portion of the absorptive surfaces. The disabled small bowel is connected to the gall bladder via a sling	Improved circulation of bile acids, significant reduction in BMI and body weight 6 months following surgery. Decrease in circulating glucose and insulin	Significantly increased genera *Lactobacillus*, *Megasphaera* and *Acidaminococcus* and family *Enterobacteriaceae*. Altered SCFAs in faecal samples (reduced acetate and propionate, increased valerate and hexanoate)	[278, 365]

GMT, as well as a consensus on the definition of a *healthy* lean donor. Interestingly, GMT has a long history. In the fourth century, Chinese patients suffering from severe diarrhoea were administered oral–faecal suspensions [294]. Likewise, in the sixteenth century, stool was used to treat diarrhoea, fever, vomiting and constipation [294]. Additionally, in the 1950s, faecal enemas were used to treat human pseudo-membranous colitis [295]. In mice, transplantation of ω3-modified faecal microbiome protected the recipients against diet-induced obesity [296]. Despite these successes, only murine models have successfully shown that obesity can be modified through microbiota manipulation [35], which restricts the use of GMT therapeutically until further evidence of its efficiency is gained in patients.

Pre- and probiotics

Supplementation with pro- and prebiotics may help to manipulate the microbiota beneficially. Probiotic consumption in mice promoted *Roseburia* growth [297–299]. *Roseburia* also increased post-VSG in the ceca of WT-VSG mice and directly correlated with weight loss, independently of caloric intake [264]. Additionally, *Roseburia* appeared to reduce glycaemia, which may underlie observed weight loss effects, and may slow down the progression to T2D [8, 300, 301]. Compounding such effects, prebiotics improved microbial abundance in the gut and reduced the feeling of hunger [302].

Exercise

Exercise has recently been added to the list of environmental factors contributing to gut microbial plasticity. In healthy animals, physical activity was found to alter the microbiota taxonomic composition [303–306]. However, the search for changes in the *Firmicutes*-to-*Bacteroidetes* ratio have yielded discordant results, finding an increase [12, 306, 307], a decrease [304, 305, 308, 309] and no change [303, 310]. In rats, species from the genera *Pseudomonas* and *Lactobacillus* increased significantly following exercise. The association of *Lactobacillus* with the mucosa of the small and large intestine, where lactic acid, CO_2, acetate and ethanol are produced, may lead to a health-promoting acidic environment [12]. *Lactobacillus* and *Bifidobacteria*, also augmented after exercise, can further transform lactate into *n*-butyrate [304, 311]. Despite

differences of experimental models and analyses, the reported taxonomic changes post-exercise seem to promote *n*-butyrate-producing groups [303]. Another murine model responded to voluntary exercise with increased representation of members of the order *Bacteroidales* and *n*-butyrate producers of the phylum *Firmicutes*, order *Clostridiales*, families *Clostridiaceae*, *Lachnospiraceae* and *Ruminococcaceae* [305]. Other species responding to exercise, such as *R. gnavus*, may protect against pathogens [312].

Human studies of elite athletes [313, 314], and one of sedentary women who exercised at the minimum levels recommended by the World Health Organization [315] suggested that, similar to animal models, exercise may shape the intestinal microbiota, favouring health-promoting species, e.g. genera *Prevotella*, *Coprococcus* (a butyrate producer and protector from irritable bowel disease), *Bifidobacterium* and species *F. prausnitzii*, *R. homini* and *A. muciniphila* [313, 315]. Athletes also displayed altered *Firmicutes*-to-*Bacteriodetes* ratio [315]. The effects of exercise were found to be transient, reversible and affected by multiple factors, including diet, age (taxonomical composition varies during life), body composition (lean vs obese) and type of exercise (low vs high intensity) [316]. Consistent with the high inter-individual variability of the human intestinal microbiota, in a cohort of overweight women, only 50% of individuals responded to exercise [317]. Similarly, a study including obese women and men only found a trend toward variations of the microbiota composition, with no significant changes [318]. A recent longitudinal study that controlled for diet and type of exercise among various variables highlighted that exercise may be more effective in lean than in obese subjects [316]. The link between exercise and microbiota is tantalizing and complex. Potential mechanisms include short- and long-range effects of *n*-butyrate. *N*-butyrate has numerous beneficial effects, including stimulating the synthesis of protective mucin [319], supporting the enterocytic energy metabolism and immune balancing. For the latter, intra-epithelial lymphocytes in the gut-associated lymphoid tissue were found to respond to *n*-butyrate by producing cytokines that are conducive to the creation of an anti-inflammatory environment that is likely to influence the microbiota due to its proximity [320–323]. While exciting, the link between exercise and microbiota must be given proper perspective. The dietary changes that often distinguish active and sedentary lifestyles are likely to strongly affect the microbiota consortia. This seems to be the case for the optimized diet of elite athletes, together with individual physiology and age [12, 306, 307, 309]. Exercise type also seems to differentially affects host physiology, with low to moderate exercise stimulating various, generally positive, responses, including faster intestinal transit, and high-intensity training instead negatively affecting the gut barrier and blood circulation to the intestine, and slowing intestinal transit [324–326]. All such changes are likely to impact on the microbiota. Ongoing research will address the open questions of what effect(s) exercise has on both host and microbiota, including, beyond bacteria, the archaea, fungi and viruses, that have not been reported to date.

Conclusions

The microbial communities dwelling in the mammalian intestinal tract were found to enhance their host's metabolism while demonstrating a high degree of resilience and adaptability to the rapidly changing conditions of their environment. The quantity and quality of ingested food may vary in both the short and long term in response to food supply and seasons, with the microbiota responding dynamically to such changes, while simultaneously integrating several physiological cues from the host. The taxonomic composition of the microbiota is in part shaped by genetic factors [19]. Although common consortia traits and organismal relationships may be conserved in close host species (e.g. *Firmicutes* and *Bacteroides* abundance in both mice and humans), others appear to be species-specific and must be considered when extrapolating results from rodent models. In humans, the gut microbiota displays large individual-to-individual variations [25] and, simultaneously, enough shared similarities to allow clustering of individuals into categories with shared similarities. Environmental factors, such as diet, life history and chemical exposure, all influence microbiota composition, although the weight of their relative contributions has not been completely elucidated, except for a substantial contribution of diet [327]. Exercise also appeared to affect the gut microbiota through multiple, perhaps partly indirect, effects and the collected data are still controversial. Interestingly, germ-free mice displayed higher locomotor activity, which may also impact on adiposity in addition to controlling insulin metabolism and regulating anorexigenic molecules [175]. Medical procedures such as surgery and pharmacological treatments also affect the gut microbiota, as was found in the case of T2D, endocarditis, antibiotic therapy and most recently chemotherapy (reviewed in [328]).

The human intestinal microbiota modulates nutrient availability and absorption for the host and, through changes of gene expression, influences hormonal and cytokine signalling, as well as immunity, which in turn reflects on the microbiota. In both patients and rodents, dysbiosis characterized by decreased microbial diversity is found in cases of diabetes, obesity and atherosclerosis, with both direct and indirect repercussions on host metabolism, immunity and behaviour, which reciprocally affect the microbial communities. Despite strong associations between certain dysbiotic patterns and disease, the causality between microbiota composition, especially at the species level, and specific host conditions remains unknown. The host genetics and physiology may favour colonization by certain species or, conversely, the microbial community may directly regulate the host's carbohydrate metabolism and energy production in mitochondria and lipogenesis, among other things. The bacterial family *Lachnospiraceae* was shown to protect the host from colitis [329] and future studies will likely identify more of these relationships. An interesting aspect of the microbiota–host web is its constant dynamic adaptation, whereby the host's response is individual and may change adaptively over time. A high-fat/high-sucrose diet in mice yielded a rapid increase of body fat in most animals. However, as the animals

were adapting to the dietary changes, the patterns of gene expression changed and eventually levelled, except for three amylase genes [142]. The precise species composition of the intestinal microbiota appears to be a continuum and multiple dysbiotic consortia converge into fewer diseased states. Intentional shifts in the microbiota can be caused, at least in the short term, by changing diet and lifestyle, and via he administration of pre-, pro- and antibiotics. The effectiveness of the gastric bypass in reducing obesity may largely be due to its effects on the microbiota, rather than to mere anatomical gastric remodelling. Finally, FMT, and, in mice and other animals, also coprophagy, all contribute to shape the gut microbial communities. Thus, it has become common practice to co-house all mice used experimentally to minimize biological variability [330]. The mounting enthusiasm at the therapeutic possibilities of manipulating the microbiota to promote healthy states is tempered by the realization that our knowledge is largely limited to the luminal (faecal) bacteria and archaebacteria, likely missing important contributions of the mucosa-associated micro-organisms. Non-bacterial groups, e.g. fungi and viruses, remain largely unknown. Possible differences along the regions of the lower gastrointestinal tract are also suspected. A set of common guidelines for conducting studies of the microbiota *in vivo* is being advocated to enable comparisons between studies. The staggering complexity of the interactions between the microbiota and the host also cautions that attempts to manipulate the microbiota in patients may become harmful [331]. Ongoing efforts towards defining the microbiota–host metabolic networks in obesity, diabetes and atherosclerosis will improve our potential to ameliorate and possibly resolve these and other metabolic diseases.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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