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REVIEW

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Metabolic networks of the human gut microbiota

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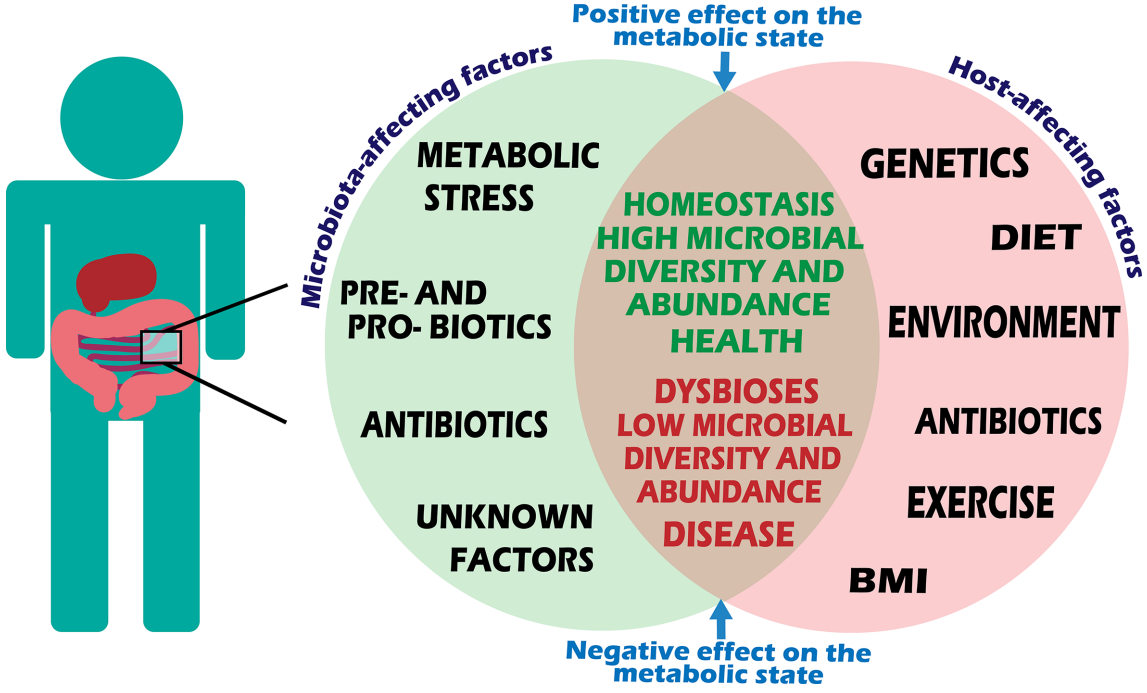

Graphical abstract

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

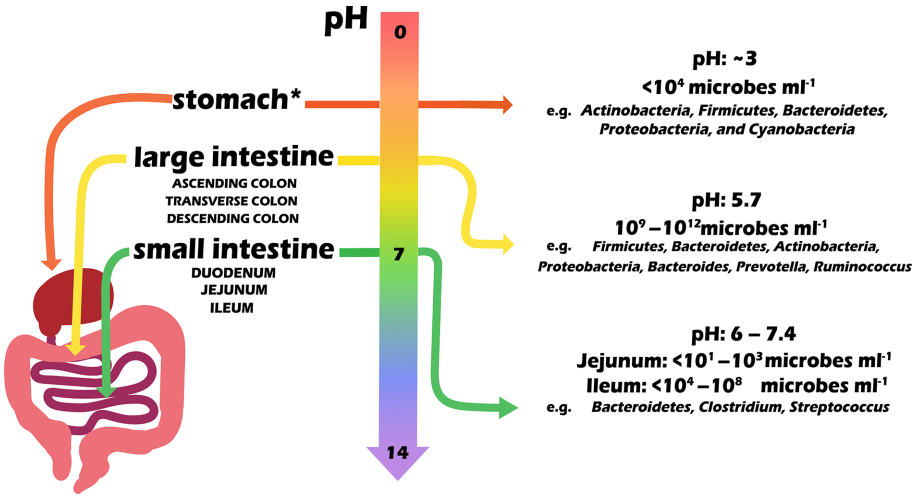

Fig. 1. Bacterial distribution and abundance in the human lower gastrointestinal tract. The GI tract contains environments with distinct conditions that favour colonization by different micro-organisms. This figure highlights the changes of pH in the stomach and intestine with the relative microbial 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.

References

1. Helander HF, Fändriks L. Surface area of the digestive tract-revisited. *Scand J Gastroenterol* 2014;49:681–689.
2. Kocełak P, Zak-Gołąb A, Zahorska-Markiewicz B, Aptekorz M, Zientara M *et al*. Resting energy expenditure and gut microbiota in obese and normal weight subjects. *Eur Rev Med Pharmacol Sci* 2013;17:2816–2821.
3. De Paepe K, Verspreet J, Verbeke K, Raes J, Courtin CM *et al*. Introducing insoluble wheat bran as a gut microbiota niche in an *in vitro* dynamic gut model stimulates propionate and butyrate production and induces colon region specific shifts in the luminal and mucosal microbial community. *Environ Microbiol* 2018;20:3406–3426.
4. Walujkar SA, Kumbhare SV, Marathe NP, Patangia DV, Lawate PS *et al*. Molecular profiling of mucosal tissue associated microbiota in patients manifesting acute exacerbations and remission stage of ulcerative colitis. *World J Microbiol Biotechnol* 2018;34:76.
5. Hannigan GD, Duhaime MB, Koutra D, Schloss PD. Biogeography and environmental conditions shape bacteriophage-bacteria networks across the human microbiome. *PLoS Comput Biol* 2017;14:e1006099.
6. Bergeron N, Williams PT, Lamendella R, Faghihnia N, Grube A *et al*. Diets high in resistant starch increase plasma levels of trimethylamine-*N*-oxide, a gut microbiome metabolite associated with CVD risk. *Br J Nutr* 2016;116:2020–2029.
7. Arrieta M-C, Stiemsma LT, Amenyogbe N, Brown EM, Finlay B. The intestinal microbiome in early life: health and disease. *Front Immunol* 2014;5.
8. Zhang X, Shen D, Fang Z, Jie Z, Qiu X *et al*. Human gut microbiota changes reveal the progression of glucose intolerance. *PLoS One* 2013;8:e71108.
9. Gevers D, Knight R, Petrosino JF, Huang K, McGuire AL *et al*. The human microbiome project: a community resource for the healthy human microbiome. *PLoS Biol* 2012;10:e1001377.
10. Mirande C, Kadlecikova E, Matulova M, Capek P, Bernalier-Donadille A *et al*. Dietary fibre degradation and fermentation by two xylanolytic bacteria Bacteroides xylanisolvens XB1A and Roseburia intestinalis XB6B4 from the human intestine. *J Appl Microbiol* 2010;109:451–460.
11. Tong M, Li X, Wegener Parfrey L, Roth B, Ippoliti A *et al*. A modular organization of the human intestinal mucosal microbiota and its association with inflammatory bowel disease. *PLoS One* 2013;8:e80702.
12. Petriz BA, Castro AP, Almeida JA, Gomes CP, Fernandes GR *et al*. Exercise induction of gut microbiota modifications in obese, non-obese and hypertensive rats. *BMC Genomics* 2014;15:511.
13. Khan MT, Nieuwdorp M, Bäckhed F. Microbial modulation of insulin sensitivity. *Cell Metab* 2014;20:753–760.
14. Gordon JI, Hooper LV, McNevin MS, Wong M, Bry L. Epithelial cell growth and differentiation. III. promoting diversity in the intestine: conversations between the microflora, epithelium, and diffuse GALT. *Am J Physiol* 1997;273:G565–G570.

15. Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J *et al*. Succession of microbial consortia in the developing infant gut microbiome. *Proc Natl Acad Sci USA* 2011;108:4578–4585.
16. McNulty NP, Yatsunenko T, Hsiao A, Faith JJ, Muegge BD *et al*. The impact of a consortium of fermented milk strains on the gut microbiome of gnotobiotic mice and monozygotic twins. *Sci Transl Med* 2011;3:106ra106.
17. McNulty NP, Wu M, Erickson AR, Pan C, Erickson BK *et al*. Effects of diet on resource utilization by a model human gut microbiota containing Bacteroides cellulosilyticus WH2, a symbiont with an extensive glycobiome. *PLoS Biol* 2013;11:e10001637.
18. Tremaroli V, Bäckhed F. Functional interactions between the gut microbiota and host metabolism. *Nature* 2012;489:242–249.
19. Ussar S, Griffin NW, Bezy O, Fujisaka S, Vienberg S *et al*. Interactions between gut microbiota, host genetics and diet modulate the predisposition to obesity and metabolic syndrome. *Cell Metab* 2015;22:516–530.
20. Larsen N, Vogensen FK, van den Berg FWJ, Nielsen DS, Andreasen AS *et al*. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS One* 2010;5:e9085.
21. Karlsson F, Tremaroli V, Nielsen J, Bäckhed F. Assessing the human gut microbiota in metabolic diseases. *Diabetes* 2013;62:3341–3349.
22. Biros E, Karan M, Golledge J. Genetic variation and atherosclerosis. *Curr Genomics* 2008;9:29–42.
23. Federico A, Dallio M, Loguercio C. Silymarin/Silybin and chronic liver disease: a marriage of many years. *Molecules* 2017;22:191.
24. Frezza EE, Wachtel MS, Chiriva-Internati M. Influence of obesity on the risk of developing colon cancer. *Gut* 2006;55:285–291.
25. Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD *et al*. Obesity alters gut microbial ecology. *Proc Natl Acad Sci USA* 2005;102:11070–11075.
26. Samuel BS, Shaito A, Motoike T, Rey FE, Bäckhed F *et al*. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, GPR41. *Proc Natl Acad Sci USA* 2008;105:16767–16772.
27. Dai ZL, Wu G, Zhu WY. Amino acid metabolism in intestinal bacteria: links between gut ecology and host health. *Front Biosci* 2011;16:1768–1786.
28. den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ *et al*. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res* 2013;54:2325–2340.
29. Macfarlane GT, Macfarlane S. Bacteria, colonic fermentation, and gastrointestinal health. *J AOAC Int* 2012;95:50–60.
30. Macfarlane GT, Cummings JH, Allison C. Protein degradation by human intestinal bacteria. *Microbiology* 1986;132:1647–1656.
31. Swann JR, Want EJ, Geier FM, Spagou K, Wilson ID *et al*. Systemic gut microbial modulation of bile acid metabolism in host tissue compartments. *Proc Natl Acad Sci USA* 2011;108:4523–4530.
32. Cho I, Yamanishi S, Cox L, Methé BA, Zavadil J *et al*. Antibiotics in early life alter the murine colonic microbiome and adiposity. *Nature* 2012;488:621–626.
33. Hashimoto T, Perlot T, Rehman A, Trichereau J, Ishiguro H *et al*. ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. *Nature* 2012;487:477–481.
34. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Human gut microbes associated with obesity. *Nature* 2006;444:1022–1023.
35. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER *et al*. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006;444:1027–1031.
36. Vinjé S, Stroes E, Nieuwdorp M, Hazen SL. The gut microbiome as novel cardio-metabolic target: the time has come! *Eur Heart J* 2014;35:883–887.
37. Bäckhed F, Ding H, Wang T, Hooper LV, Koh GY *et al*. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci USA* 2004;101:15718–15723.
38. LeBlanc JG, Milani C, de Giori GS, Sesma F, van Sinderen D *et al*. Bacteria as vitamin suppliers to their host: a gut microbiota perspective. *Curr Opin Biotechnol* 2013;24:160–168.
39. Wikoff WR, Anfora AT, Liu J, Schultz PG, Lesley SA *et al*. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc Natl Acad Sci USA* 2009;106:3698–3703.
40. Selber-Hnatiw S, Rukundo B, Ahmadi M, Akoubi H, Al-Bizri H *et al*. Human gut microbiota: toward an ecology of disease. *Front Microbiol* 2017;8:1265.
41. Chassaing B, Vijay-Kumar M, Gewirtz AT. How diet can impact gut microbiota to promote or endanger health. *Curr Opin Gastroenterol* 2017;33:417–421.
42. Kim S, Jazwinski SM. The gut microbiota and healthy aging: a mini-review. *Gerontology* 2018;64:513–520.
43. Goodrich JK, Davenport ER, Beaumont M, Jackson MA, Knight R *et al*. Genetic determinants of the gut microbiome in UK twins. *Cell Host Microbe* 2016;19:731–743.
44. Turnbaugh PJ, Quince C, Faith JJ, McHardy AC, Yatsunenko T *et al*. Organismal, genetic, and transcriptional variation in the deeply sequenced gut microbiomes of identical twins. *Proc Natl Acad Sci USA* 2010;107:7503–7508.
45. Murphy TM, Wong CCY, Arseneault L, Burrage J, Macdonald R *et al*. Methylomic markers of persistent childhood asthma: a longitudinal study of asthma-discordant monozygotic twins. *Clin Epigenetics* 2015;7:130.
46. Xie H, Guo R, Zhong H, Feng Q, Lan Z *et al*. Shotgun Metagenomics of 250 adult twins reveals genetic and environmental impacts on the gut microbiome. *Cell Syst* 2016;3:572–584.
47. Zhou M, He J, Shen Y, Zhang C, Wang J *et al*. New frontiers in genetics, gut microbiota, and immunity: a Rosetta stone for the pathogenesis of inflammatory bowel disease. *Biomed Res Int* 2017;2017:8201672
48. Dong Q, Xin Y, Wang L, Meng X, Yu X *et al*. Characterization of gastric microbiota in twins. *Curr Microbiol* 2017;74:224–229.
49. Goodrich JK, Waters JL, Poole AC, Sutter JL, Koren O *et al*. Human genetics shape the gut microbiome. *Cell* 2014;159:789–799.
50. Muegge BD, Kuczynski J, Knights D, Clemente JC, González A *et al*. Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. *Science* 2011;332:970–974.
51. Joossens M, Huys G, Cnockaert M, De Preter V, Verbeke K *et al*. Dysbiosis of the faecal microbiota in patients with Crohn's disease and their unaffected relatives. *Gut* 2011;60:631–637.
52. Baumgart DC, Sandborn WJ. Crohn's disease. *The Lancet* 2012;380:1590–1605.
53. Sokol H, Seksik P, Furet JP, Firmesse O, Nion-Larmurier I *et al*. Low counts of Faecalibacterium prausnitzii in colitis microbiota. *Inflamm Bowel Dis* 2009;15:1183–1189.
54. Favier C, Neut C, Mizon C, Cortot A, Colombel JF *et al*. Fecal beta-D-galactosidase production and bifidobacteria are decreased in Crohn's disease. *Dig Dis Sci* 1997;42:817–822.
55. Prindiville T, Cantrell M, Wilson KH. Ribosomal DNA sequence analysis of mucosa-associated bacteria in Crohn's disease. *Inflamm Bowel Dis* 2004;10:824–833.
56. Martinez-Medina M, Aldeguer X, Gonzalez-Huix F, Acero D, Garcia-Gil LJ. Abnormal microbiota composition in the ileocolonic mucosa of Crohn's disease patients as revealed by polymerase chain reaction-denaturing gradient gel electrophoresis. *Inflamm Bowel Dis* 2006;12:1136–1145.
57. Van de Merwe JP, Schröder AM, Wensinck F, Hazenberg MP. The obligate anaerobic faecal flora of patients with Crohn's disease and their first-degree relatives. *Scand J Gastroenterol* 1988;23:1125–1131.

58. Van de Merwe JP, Stegeman JH. Binding of Coprococcus comes to the Fc portion of IgG. A possible role in the pathogenesis of Crohn's disease? *Eur J Immunol* 1985;15:860–863.
59. El Aidy S, Hooiveld G, Tremaroli V, Bäckhed F, Kleerebezem M. The gut microbiota and mucosal homeostasis. *Gut Microbes* 2013;4:118–124.
60. Walker AW, Ince J, Duncan SH, Webster LM, Holtrop G *et al*. Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *ISME J* 2011;5:220–230.
61. Martínez I, Lattimer JM, Hubach KL, Case JA, Yang J *et al*. Gut microbiome composition is linked to whole grain-induced immunological improvements. *ISME J* 2013;7:269–280.
62. Salonen A, Lahti L, Salojärvi J, Holtrop G, Korpela K *et al*. Impact of diet and individual variation on intestinal microbiota composition and fermentation products in obese men. *ISME J* 2014;8:2218–2230.
63. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE *et al*. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2014;505:559–563.
64. O'Keefe SJD, Li JV, Lahti L, Ou J, Carbonero F *et al*. Fat, fibre and cancer risk in African Americans and rural Africans. *Nat Commun* 2015;6:6342.
65. Hoffmann C, Dollive S, Grunberg S, Chen J, Li H *et al*. Archaea and fungi of the human gut microbiome: correlations with diet and bacterial residents. *PLoS One* 2013;8:e66019.
66. Graf D, Di Cagno R, Fåk F, Flint HJ, Nyman M *et al*. Contribution of diet to the composition of the human gut microbiota. *Microb Ecol Health Dis* 2015;26:26164.
67. Lee JY, Sohn KH, Rhee SH, Hwang D. Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through Toll-like receptor 4. *J Biol Chem* 2001;276:16683–16689.
68. Hildebrandt MA, Hoffmann C, Sherrill-Mix SA, Keilbaugh SA, Hamady M *et al*. High-Fat diet determines the composition of the murine gut microbiome independently of obesity. *Gastroenterology* 2009;137:1716–1724.
69. Calder PC. Fatty acids and inflammation: the cutting edge between food and pharma. *Eur J Pharmacol* 2011;668:S50–S58.
70. Pendyala S, Walker JM, Holt PR. A high-fat diet is associated with endotoxemia that originates from the gut. *Gastroenterology* 2012;142:1100–1101.
71. Huang S, Rutkowsky JM, Snodgrass RG, Ono-Moore KD, Schneider DA *et al*. Saturated fatty acids activate TLR-mediated proinflammatory signaling pathways. *J Lipid Res* 2012;53:2002–2013.
72. Chassaing B, Koren O, Goodrich JK, Poole AC, Srinivasan S *et al*. Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. *Nature* 2015;519:92–96.
73. Chassaing B, Van de Wiele T, De Bodt J, Marzorati M, Gewirtz AT. Dietary emulsifiers directly alter human microbiota composition and gene expression ex vivo potentiating intestinal inflammation. *Gut* 2017;66:1414–1427.
74. Pepino MY. Metabolic effects of non-nutritive sweeteners. *Physiol Behav* 2015;152:450–455.
75. Glendinning JI. Do low-calorie sweeteners promote weight gain in rodents? *Physiol Behav* 2016;164:509–513.
76. Nettleton JE, Reimer RA, Shearer J. Reshaping the gut microbiota: impact of low calorie sweeteners and the link to insulin resistance? *Physiol Behav* 2016;164:488–493.
77. Wang Q-P, Lin YQ, Zhang L, Wilson YA, Oyston LJ *et al*. Sucralose promotes food intake through NPY and a neuronal fasting response. *Cell Metab* 2016;24:75–90.
78. Suez J, Korem T, Zeevi D, Zilberman-Schapira G, Thaiss CA *et al*. Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature* 2014;514:181–186.
79. Anderson RL, Kirkland JJ. The effect of sodium saccharin in the diet on caecal microflora. *Food Cosmet Toxicol* 1980;18:353–355.
80. Abou-Donia MB, El-Masry EM, Abdel-Rahman AA, McLendon RE, Schiffman SS. Splenda alters gut microflora and increases intestinal P-glycoprotein and cytochrome P-450 in male rats. *J Toxicol Environ Health A* 2008;71:1415–1429.
81. Wang QP, Browman D, Herzog H, Neely GG. Non-Nutritive sweeteners possess a bacteriostatic effect and alter gut microbiota in mice. *PLoS One* 2018;13:e0199080.
82. Suez J, Korem T, Zilberman-Schapira G, Segal E, Elinav E. Non-caloric artificial sweeteners and the microbiome: findings and challenges. *Gut Microbes* 2015;6:149–155.
83. Palmnäs MSA, Cowan TE, Bomhof MR, Su J, Reimer RA *et al*. Low-Dose aspartame consumption differentially affects gut Microbiota-Host metabolic interactions in the diet-induced obese rat. *PLoS One* 2014;9:e10984.
84. Pfeffer M, Ziesenitz SC, Siebert G. Acesulfame K, cyclamate and saccharin inhibit the anaerobic fermentation of glucose by intestinal bacteria. *Z Ernährungswiss* 1985;24:231–235.
85. Young DA, Bowen WH. The influence of sucralose on bacterial metabolism. *Am J Gastroenterol* 1990;107:1755.
86. Bowen WH, Pearson SK. The effects of sucralose, xylitol and sorbitol on remineralization of caries lesions in rats. *J Dent Res* 1992;71:1166–1168.
87. Prashant GM, Patil RB, Nagaraj T, Patel VB. The antimicrobial activity of the three commercially available intense sweeteners against common periodontal pathogens: an *in vitro* study. *J Contemp Dent Pract* 2012;13:749–752.
88. Deniņa I, Semjonovs P, Fomina A, Treimane R, Linde R. The influence of stevia glycosides on the growth of *Lactobacillus reuteri* strains. *Lett Appl Microbiol* 2014;58:278–284.
89. Oppermann JA. Aspartame metabolism in animals. In: Filer LJ and Stegink F (editors). *Aspartame: Physiology and Biochemistry*. New York: Marcel Dekker Inc; 1984.
90. Keller SE, Newberg SS, Krieger TM, Shazer WH. Degradation of aspartame in yogurt related to microbial growth. *J Food Sci* 1991;56:21–23.
91. Cummings JH, Pomare EW, Branch WJ, Naylor CP, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* 1987;28:1221–1227.
92. Bloemen JG, Venema K, van de Poll MC, Olde Damink SW, Buurman WA *et al*. Short chain fatty acids exchange across the gut and liver in humans measured at surgery. *Clin Nutr* 2009;28:657–661.
93. Cummings JH, Macfarlane GT. Role of intestinal bacteria in nutrient metabolism. *JPEN J Parenter Enteral Nutr* 1997;21:357–365.
94. Christiansen CB, Gabe MBN, Svendsen B, Dragsted LO, Rosenkilde MM *et al*. The impact of short-chain fatty acids on GLP-1 and PYY secretion from the isolated perfused rat colon. *Am J Physiol Gastrointest Liver Physiol* 2018;315:G53–G65.
95. Layden BT, Angueira AR, Brodsky M, Durai V, Lowe WL. Short chain fatty acids and their receptors: new metabolic targets. *Transl Res* 2013;161:131–140.
96. Pouteau E, Meirim I, Métairon S, Fay LB. Acetate, propionate and butyrate in plasma: determination of the concentration and isotopic enrichment by gas chromatography/mass spectrometry with positive chemical ionization. *J Mass Spectrom* 2001;36:798–805.
97. Roediger WE. Utilization of nutrients by isolated epithelial cells of the rat colon. *Gastroenterology* 1982;83:424–429.
98. Donohoe DR, Garge N, Zhang X, Sun W, O'Connell TM *et al*. The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metab* 2011;13:517–526.
99. Candido EP, Reeves R, Davie JR. Sodium butyrate inhibits histone deacetylation in cultured cells. *Cell* 1978;14:105–113.
100. Sealy L, Chalkley R. The effect of sodium butyrate on histone modification. *Cell* 1978;14:115–121.

101. Vidali G, Boffa LC, Bradbury EM, Allfrey VG. Butyrate suppression of histone deacetylation leads to accumulation of multiacetylated forms of histones H3 and H4 and increased DNase I sensitivity of the associated DNA sequences. *Proc Natl Acad Sci USA* 1978;75:2239–2243.
102. Boffa LC, Vidali G, Mann RS, Allfrey VG. Suppression of histone deacetylation *in vivo* and *in vitro* by sodium butyrate. *J Biol Chem* 1978;253:3364–3366.
103. Hinnebusch BF, Meng S, Wu JT, Archer SY, Hodin RA. The effects of short-chain fatty acids on human colon cancer cell phenotype are associated with histone hyperacetylation. *J Nutr* 2002;132:1012–1017.
104. Wong JMW, de Souza R, Kendall CWC, Emam A, Jenkins DJA. Colonic health: fermentation and short chain fatty acids. *J Clin Gastroenterol* 2006;40:235–243.
105. Roy CC, Kien CL, Bouthillier L, Levy E. Short-Chain fatty acids: ready for prime time? *Nutr Clin Pract* 2006;21:351–366.
106. Vinolo MAR, Rodrigues HG, Nachbar RT, Curi R. Regulation of inflammation by short chain fatty acids. *Nutrients* 2011;3:858–876.
107. Canfora EE, Jocken JW, Blaak EE. Short-Chain fatty acids in control of body weight and insulin sensitivity. *Nat Rev Endocrinol* 2015;11:577–591.
108. Cani PD. Human gut microbiome: hopes, threats and promises. *Gut* 2018;67:1716–1725.
109. Cheng D, Xu JH, Li JY, Wang SY, Wu TF *et al*. Butyrate ameliorated-NLRC3 protects the intestinal barrier in a GPR43-dependent manner. *Exp Cell Res* 2018;368:101–110.
110. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM *et al*. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* 2008;57:1470–1481.
111. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C *et al*. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 2007;56:1761–1772.
112. Fernandes J, Su W, Rahat-Rozenbloom S, Wolever TMS, Comelli EM. Adiposity, gut microbiota and faecal short chain fatty acids are linked in adult humans. *Nutr Diabetes* 2014;4:e121.
113. Tolhurst G, Heffron H, Lam YS, Parker HE, Habib AM *et al*. Short-Chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. *Diabetes* 2012;61:364–371.
114. Macfarlane S, Macfarlane GT. Regulation of short-chain fatty acid production. *Proc Nutr Soc* 2003;62:67–72.
115. Ang Z, Ding JL. GPR41 and GPR43 in Obesity and Inflammation - Protective or Causative? *Front Immunol* 2016;7:28.
116. Brown AJ, Goldsworthy SM, Barnes AA, Eilert MM, Tcheang L *et al*. The orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J Biol Chem* 2003;278:11312–11319.
117. Karaki SI, Tazoe H, Hayashi H, Kashiwabara H, Tooyama K *et al*. Expression of the short-chain fatty acid receptor, GPR43, in the human colon. *J Mol Histol* 2008;39:135–142.
118. Tazoe H, Otomo Y, Kaji I, Tanaka R, Karaki SI *et al*. Roles of short-chain fatty acids receptors, GPR41 and GPR43 on colonic function. *J Physiol. Pharmacol Suppl* 2008;2:251–262.
119. Maslowski KM, Vieira AT, Ng A, Kranich J, Sierro F *et al*. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* 2009;461:1282–1286.
120. Sina C, Gavrilova O, Förster M, Till A, Derer S *et al*. G protein-coupled receptor 43 is essential for neutrophil recruitment during intestinal inflammation. *J Immunol* 2009;183:7514–7522.
121. Kim MH, Kang SG, Park JH, Yanagisawa M, Kim CH. Short-Chain fatty acids activate GPR41 and GPR43 on intestinal epithelial cells to promote inflammatory responses in mice. *Gastroenterology* 2013;145:396–406.
122. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA *et al*. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science* 2013;341:569–573.
123. Masui R, Sasaki M, Funaki Y, Ogasawara N, Mizuno M *et al*. G protein-coupled receptor 43 moderates gut inflammation through cytokine regulation from mononuclear cells. *Inflamm Bowel Dis* 2013;19:2848–2856.
124. Bjursell M, Admyre T, Göransson M, Marley AE, Smith DM *et al*. Improved glucose control and reduced body fat mass in free fatty acid receptor 2-deficient mice fed a high-fat diet. *Am J Physiol Endocrinol Metab* 2011;300:E211–E220.
125. Kimura I, Ozawa K, Inoue D, Imamura T, Kimura K *et al*. The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. *Nat Commun* 2013;4:1829.
126. Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N *et al*. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat Med* 2014;20:159–166.
127. Ge H, Li X, Weiszmann J, Wang P, Baribault H *et al*. Activation of G protein-coupled receptor 43 in adipocytes leads to inhibition of lipolysis and suppression of plasma free fatty acids. *Endocrinology* 2008;149:4519–4526.
128. McNelis JC, Lee YS, Mayoral R, van der Kant R, Johnson AMF *et al*. Gpr43 potentiates β-cell function in obesity. *Diabetes* 2015;64:3203–3217.
129. Priyadarshini M, Villa SR, Fuller M, Wicksteed B, Mackay CR *et al*. An Acetate-Specific GPCR, FFAR2, regulates insulin secretion. *Mol Endocrinol* 2015;29:1055–1066.
130. Psichas A, Sleeth ML, Murphy KG, Brooks L, Bewick GA *et al*. The short chain fatty acid propionate stimulates GLP-1 and PYY secretion via free fatty acid receptor 2 in rodents. *Int J Obes* 2015;39:424–429.
131. Caylak E. Anorexigenic peptides in health and d isease. *eLS*. Chichester: John Wiley & Sons Ltd; 2012.
132. Puddu A, Sanguineti R, Montecucco F, Viviani GL. Evidence for the gut microbiota short-chain fatty acids as key pathophysiological molecules improving diabetes. *Mediators Inflamm* 2014;2014:162021
133. De Vadder F, Kovatcheva-Datchary P, Goncalves D, Vinera J, Zitoun C *et al*. Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. *Cell* 2014;156:84–96.
134. Flint HJ, Scott KP, Duncan SH, Louis P, Forano E. Microbial degradation of complex carbohydrates in the gut. *Gut Microbes* 2012;3:289–306.
135. Lin HV, Frassetto A, Kowalik EJ, Nawrocki AR, Lu MM *et al*. Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms. *PLoS One* 2012;7:e35240.
136. Inoue D, Tsujimoto G, Kimura I. Regulation of energy homeostasis by GPR41. *Front Endocrinol* 2014;5:81.
137. Xiong Y, Miyamoto N, Shibata K, Valasek MA, Motoike T *et al*. Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. *Proc Natl Acad Sci USA* 2004;101:1045–1050.
138. Pais R, Gribble FM, Reimann F. Stimulation of incretin secreting cells. *Ther Adv Endocrinol Metab* 2016;7:24–42.
139. Tang C, Ahmed K, Gille A, Lu S, Gröne HJ *et al*. Loss of FFA2 and FFA3 increases insulin secretion and improves glucose tolerance in type 2 diabetes. *Nat Med* 2015;21:173–177.
140. National heart, Lung, and Blood Institute. 2019. Classification of overweight and obesity by BMI, waist circumference, and associated disease risks. https://www.nhlbi.nih.gov/health/educational/lose_wt/BMI/bmi_dis.htm
141. Redinger RN. The pathophysiology of obesity and its clinical manifestations. *Gastroenterol Hepatol* 2007;3:856–863.

142. Parks BW, Nam E, Org E, Kostem E, Norheim F *et al*. Genetic control of obesity and gut microbiota composition in response to high-fat, high-sucrose diet in mice. *Cell Metab* 2013;17:141–152.
143. Musso G, Gambino R, Cassader M. Obesity, diabetes, and gut microbiota: the hygiene hypothesis expanded? *Diabetes Care* 2010;33:2277–2284.
144. Ferraris RP, Vinnakota RR. Intestinal nutrient transport in genetically obese mice. *Am J Clin Nutr* 1995;62:540–546.
145. Kasai C, Sugimoto K, Moritani I, Tanaka J, Oya Y *et al*. Comparison of the gut microbiota composition between obese and non-obese individuals in a Japanese population, as analyzed by terminal restriction fragment length polymorphism and next-generation sequencing. *BMC Gastroenterol* 2015;15:100.
146. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature* 2012;486:207–214.
147. Finucane MM, Sharpton TJ, Laurent TJ, Pollard KS. A taxonomic signature of obesity in the microbiome? getting to the guts of the matter. *PLoS One* 2014;9:e84689.
148. Walters WA, Xu Z, Knight R. Meta-analyses of human gut microbes associated with obesity and IBD. *FEBS Lett* 2014;588:4223–4233.
149. WHO. 2019. Obesity and overweight. http://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight
150. Blaut M, Klaus S. Intestinal Microbiota and Obesity. In: Joost HG (editor). *Appetite Control. Handbook of Experimental Pharmacology*, 209. Heidelberg: Springer; 2012.
151. DiBaise JK, Frank DN, Mathur R. Impact of the gut microbiota on the development of obesity: current concepts. *Am J Gastroenterol Suppl* 2012;1:22–27.
152. Shen J, Obin MS, Zhao L. The gut microbiota, obesity and insulin resistance. *Mol Aspects Med* 2013;34:39–58.
153. Sweeney TE, Morton JM. The human gut microbiome. *JAMA Surg* 2013;148:563–569.
154. Barlow GM, Yu A, Mathur R. Role of the gut microbiome in obesity and diabetes mellitus. *Nutr Clin Pract* 2015;30:787–797.
155. Villanueva-Millán MJ, Pérez-Matute P, Oteo JA. Gut microbiota: a key player in health and disease. A review focused on obesity. *J Physiol Biochem* 2015;71:509–525.
156. Koliada A, Syzenko G, Moseiko V, Budovska L, Puchkov K *et al*. Association between body mass index and Firmicutes/Bacteroidetes ratio in an adult Ukrainian population. *BMC Microbiol* 2017;17:120.
157. Turnbaugh PJ, Bäckhed F, Fulton L, Gordon JI. "Marked alterations in the distal gut microbiome linked to diet-induced obesity.". *Cell Host Microbe* 2008;3:213–223.
158. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE *et al*. Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science* 2013;341:1241214.
159. Ravussin Y, Koren O, Spor A, LeDuc C, Gutman R *et al*. Responses of gut microbiota to diet composition and weight loss in lean and obese mice. *Obesity* 2012;20:738–747.
160. Homsi ME, Ducroc R, Claustre J, Jourdan G, Gertler A *et al*. Leptin modulates the expression of secreted and membrane-associated mucins in colonic epithelial cells by targeting PKC, PI3K, and MAPK pathways. *Am J Physiol Gastrointest Liver Physiol* 2007;293:G365–G373.
161. Plaisancie P, Ducroc R, Homsi ME, Tsocas A, Guilmeau S *et al*. Luminal leptin activates mucin-secreting goblet cells in the large bowel. *Am J Physiol Gastrointest Liver Physiol* 2006;290:G805–G812.
162. Mar Rodríguez M, Pérez D, Javier Chaves F, Esteve E, Marin-Garcia P *et al*. Obesity changes the human gut mycobiome. *Sci Rep* 2015;5:14600.
163. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F *et al*. Richness of human gut microbiome correlates with metabolic markers. *Nature* 2013;500:541–546.
164. Swidsinski A, Weber J, Loening-Baucke V, Hale LP, Lochs H. Spatial organization and composition of the mucosal flora in patients with inflammatory bowel disease. *J Clin Microbiol* 2005;43:3380–3389.
165. Leschelle X, Goubern M, Andriamihaja M, Blottière HM, Couplan E *et al*. Adaptative metabolic response of human colonic epithelial cells to the adverse effects of the luminal compound sulfide. *Biochimica et Biophysica Acta (BBA) - General Subjects* 2005;1725:201–212.
166. de la Cuesta-Zuluaga J, Mueller N, Álvarez-Quintero R, Velásquez-Mejía E, Sierra J *et al*. Higher fecal short-chain fatty acid levels are associated with gut microbiome dysbiosis, obesity, hypertension and cardiometabolic disease risk factors. *Nutrients* 2019;11:E51.
167. Yang J, Summanen PH, Henning SM, Hsu M, Lam H *et al*. Xylooligosaccharide supplementation alters gut bacteria in both healthy and prediabetic adults: a pilot study. *Front Physiol* 2015;6.
168. Duca FA, Sakar Y, Lepage P, Devime F, Langelier B *et al*. Replication of obesity and associated signaling pathways through transfer of microbiota from Obese-Prone rats. *Diabetes* 2014;63:1624–1636.
169. Houten SM, Watanabe M, Auwerx J. Endocrine functions of bile acids. *EMBO J* 2006;25:1419–1425.
170. Watanabe M, Houten SM, Mataki C, Christoffolete MA, Kim BW *et al*. Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature* 2006;439:484–489.
171. Nicholson JK, Holmes E, Wilson ID. Gut microorganisms, mammalian metabolism and personalized health care. *Nat Rev Microbiol* 2005;3:431–438.
172. Smet ID, Boever PD, Verstraete W. Cholesterol lowering in pigs through enhanced bacterial bile salt hydrolase activity. *Br J Nutr* 1998;79:185–194.
173. Xiao JZ, Kondo S, Takahashi N, Miyaji K, Oshida K *et al*. Effects of milk products fermented by Bifidobacterium longum on blood lipids in rats and healthy adult male volunteers. *J Dairy Sci* 2003;86:2452–2461.
174. Martin François-Pierre J, Dumas Marc-Emmanuel, Wang Y, Legido-Quigley C, Yap IKS *et al*. A top-down systems biology view of microbiome-mammalian metabolic interactions in a mouse model. *Mol Syst Biol* 2007;3:112.
175. Bäckhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proc Natl Acad Sci U S A* 2007;104:979–984.
176. Carling D. The AMP-activated protein kinase cascade – a unifying system for energy control. *Trends Biochem Sci* 2004;29:18–24.
177. Vega RB, Huss JM, Kelly DP. The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor alpha in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. *Mol Cell Biol* 2000;20:1868–1876.
178. Preiss-Landl K, Zimmermann R, Hämmerle G, Zechner R. Lipoprotein lipase: the regulation of tissue specific expression and its role in lipid and energy metabolism. *Curr Opin Lipidol* 2002;13:471–481.
179. Fu J, Astarita G, Gaetani S, Kim J, Cravatt BF *et al*. Food intake regulates oleoylethanolamide formation and degradation in the proximal small intestine. *J Biol Chem* 2007;282:1518–1528.
180. Gillum MP, Zhang D, Zhang X-M, Erion DM, Jamison RA *et al*. N-Acylphosphatidylethanolamine, a Gut- derived circulating factor induced by fat ingestion, inhibits food intake. *Cell* 2008;135:813–824.
181. Chen Z, Guo L, Zhang Y, Walzem RL, Pendergast JS *et al*. Incorporation of therapeutically modified bacteria into gut microbiota inhibits obesity. *J Clin Invest* 2014;124:3391–3406.
182. Diep TA, Madsen AN, Krogh-Hansen S, Al-Shahwani M, Al-Sabagh L *et al*. Dietary non-esterified oleic acid decreases the jejunal levels of anorectic *N*-acylethanolamines. *PLoS One* 2014;9:e100365.

183. Diep TA, Madsen AN, Holst B, Kristiansen MM, Wellner N *et al*. Dietary fat decreases intestinal levels of the anorectic lipids through a fat sensor. *The FASEB Journal* 2011;25:765–774.

184. Dosoky NS, Guo L, Chen Z, Feigley AV, Davies SS. Dietary Fatty Acids Control the Species of *N* -Acyl-Phosphatidylethanolamines Synthesized by Therapeutically Modified Bacteria *in* the Intestinal Tract. *ACS Infect. Dis.* 2018;4:3–13.

185. Dethlefsen L, Huse S, Sogin ML, Relman DA. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biol* 2008;6:e280.

186. Antonopoulos DA, Huse SM, Morrison HG, Schmidt TM, Sogin ML *et al*. Reproducible community dynamics of the gastrointestinal microbiota following antibiotic perturbation. *Infect Immun* 2009;77:2367–2375.

187. Cox LM, Yamanishi S, Sohn J, Alekseyenko AV, Leung JM *et al*. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. *Cell* 2014;158:705–721.

188. Cromwell GL. Why and how antibiotics are used in swine production. *Anim Biotechnol* 2002;13:7–27.

189. Theriot CM, Koenigsknecht MJ, Carlson PE, Hatton GE, Nelson AM *et al*. Antibiotic-induced shifts in the mouse gut microbiome and metabolome increase susceptibility to Clostridium difficile infection. *Nat Commun* 2014;311:5.

190. Kahn BB, Flier JS. Obesity and insulin resistance. *J Clin Invest* 2000;106:473–481.

191. Ou J, Carbonero F, Zoetendal EG, DeLany JP, Wang M *et al*. Diet, microbiota, and microbial metabolites in colon cancer risk in rural Africans and African Americans. *Am J Clin Nutr* 2013;98:111–120.

192. Scanlan PD, Shanahan F, Clune Y, Collins JK, O'Sullivan GC *et al*. Culture-Independent analysis of the gut microbiota in colorectal cancer and polyposis. *Environ Microbiol* 2008;10:789–798.

193. Marchesi JR, Dutilh BE, Hall N, Peters WHM, Roelofs R *et al*. Towards the human colorectal cancer microbiome. *PLoS One* 2011;6:e20447.

194. Dai Z, Coker OO, Nakatsu G, Wu WKK, Zhao L *et al*. Multi-Cohort analysis of colorectal cancer metagenome identified altered bacteria across populations and universal bacterial markers. *Microbiome* 2018;6:70.

195. Marchesi JR, Adams DH, Fava F, Hermes GDA, Hirschfield GM *et al*. The gut microbiota and host health: a new clinical frontier. *Gut* 2016;65:330–339.

196. Coleman OI, Lobner EM, Bierwirth S, Sorbie A, Waldschmitt N *et al*. Activated ATF6 induces intestinal dysbiosis and innate immune response to promote colorectal tumorigenesis. *Gastroenterology* 2018;155:1539–1552.

197. Qin J, Li Y, Cai Z, Li S, Zhu J *et al*. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 2012;490:55–60.

198. Wen L, Duffy A. Factors influencing the gut microbiota, inflammation, and type 2 diabetes. *J Nutr* 2017;147:1468S–1475.

199. Fonseca VA. Defining and characterizing the progression of type 2 diabetes. *Diabetes Care* 2009;32:S151–S156.

200. Roglic G, Varghese C, Thamarangsi T. Diabetes in south-east Asia: burden, gaps, challenges and ways forward. *WHO South-East Asia J Public Health* 2016;5:1–4.

201. Agarwal AK, Ahirwar G, Marskole P, Bhagwat AK. A community based study to assess the validity of Indian diabetic risk score, among urban population of North central India. *IJCMPH* 2016;4:2101–2106.

202. Kelly T, Yang W, Chen CS, Reynolds K, He J. Global burden of obesity in 2005 and projections to 2030. *Int J Obes* 2008;32:1431–1437.

203. Cipolletta D, Kolodin D, Benoist C, Mathis D. Tissular Tregs: a unique population of adipose-tissue-resident Foxp3+CD4+ T cells that impacts organismal metabolism. *Semin Immunol* 2011;23:431–437.

204. Ding S, Lund PK. Role of intestinal inflammation as an early event in obesity and insulin resistance. *Curr Opin Clin Nutr Metab Care* 2011;14:328–333.

205. Horton F, Wright J, Smith L, Hinton PJ, Robertson MD. Increased intestinal permeability to oral chromium (51 Cr) -EDTA in human type 2 diabetes. *Diabet Med* 2014;31:559–563.

206. Xiao S, Fei N, Pang X, Shen J, Wang L *et al*. A gut microbiota-targeted dietary intervention for amelioration of chronic inflammation underlying metabolic syndrome. *FEMS Microbiol Ecol* 2014;87:357–367.

207. Karlsson FH, Tremaroli V, Nookaew I, Bergström G, Behre CJ *et al*. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* 2013;498:99–103.

208. Vrieze A, Van Nood E, Holleman F, Salojärvi J, Kootte RS *et al*. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* 2012;143:913–916.

209. Hsieh FC, Lee CL, Chai CY, Chen WT, Lu YC *et al*. Oral administration of Lactobacillus reuteri GMNL-263 improves insulin resistance and ameliorates hepatic steatosis in high fructose-fed rats. *Nutr Metab* 2013;10:35.

210. Reunanen J, Kainulainen V, Huuskonen L, Ottman N, Belzer C *et al*. Akkermansia muciniphila adheres to enterocytes and strengthens the integrity of the epithelial cell layer. *Appl Environ Microbiol* 2015;81:3655–3662.

211. Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C *et al*. Cross-Talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. *Proc Natl Acad Sci U S A* 2013;110:9066–9071.

212. Belzer C, de Vos WM. Microbes inside--from diversity to function: the case of Akkermansia. *ISME J* 2012;6:1449–1458.

213. Derrien M, Vaughan EE, Plugge CM, de Vos WM. Akkermansia muciniphila gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. *Int J Syst Evol Microbiol* 2004;54:1469–1476.

214. Sonoyama K, Fujiwara R, Takemura N, Ogasawara T, Watanabe J *et al*. Response of gut microbiota to fasting and hibernation in Syrian hamsters. *Appl Environ Microbiol* 2009;75:6451–6456.

215. Ring C, Klopfleisch R, Dahlke K, Basic M, Bleich A *et al*. Akkermansia muciniphila strain ATCC BAA-835 does not promote short-term intestinal inflammation in gnotobiotic interleukin-10-deficient mice. *Gut Microbes* 2018;25:1–16.

216. Minamii T, Nogami M, Ogawa W. Mechanisms of metformin action: in and out of the gut. *J Diabetes Investig* 2018;9:701–703.

217. Shin N-R, Lee J-C, Lee H-Y, Kim M-S, Whon TW *et al*. An increase in the *Akkermansia* spp. population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. *Gut* 2014;63:727–735.

218. Hur KY, Lee M-S. New mechanisms of metformin action: focusing on mitochondria and the gut. *J Diabetes Investig* 2015;6:600–609.

219. Lee H, Ko G. Effect of metformin on metabolic improvement and gut microbiota. *Appl Environ Microbiol* 2014;80:5935–5943.

220. Rodriguez J, Hiel S, Delzenne NM. Metformin: old friend new ways of action-implication of the gut microbiome? *Curr Opin Clin Nutr Metab Care* 2018;21:294–301.

221. Wu H, Esteve E, Tremaroli V, Khan MT, Caesar R *et al*. Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. *Nat Med* 2017;23:850–858.

222. Zhang L, Qin Q, Liu M, Zhang X, He F *et al*. Akkermansia muciniphila can reduce the damage of gluco/lipotoxicity, oxidative stress and inflammation, and normalize intestine microbiota in streptozotocin-induced diabetic rats. *Pathog Dis* 2018;76.

223. Forslund K, Hildebrand F, Nielsen T, Falony G, Le Chatelier E *et al*. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature* 2015;528:262–266.

224. Plovier H, Everard A, Druart C, Depommier C, Van Hul M *et al*. A purified membrane protein from Akkermansia muciniphila or

the pasteurized bacterium improves metabolism in obese and diabetic mice. *Nat Med* 2017;23:107–113.

225. Pascale A, Marchesi N, Govoni S, Coppola A, Gazzaruso C. The role of gut microbiota in obesity, diabetes mellitus, and effect of metformin: new insights into old diseases. *Curr Opin Pharmacol* 2019;49:1–5.
226. Akerblom HK, Knip M. Putative environmental factors in type 1 diabetes. *Diabetes Metab Rev* 1998;14:31–68.
227. Giongo A, Gano KA, Crabb DB, Mukherjee N, Novelo LL *et al*. Toward defining the autoimmune microbiome for type 1 diabetes. *ISME J* 2011;5:82–91.
228. Brown K, Godovannyi A, Ma C, Zhang Y, Ahmadi-Vand Z *et al*. Prolonged antibiotic treatment induces a diabetogenic intestinal microbiome that accelerates diabetes in NOD mice. *ISME J* 2016;10:321–332.
229. Thuny F, Richet H, Casalta J-P, Angelakis E, Habib G *et al*. Vancomycin treatment of infective endocarditis is linked with recently acquired obesity. *PLoS One* 2010;5:e9074.
230. Vrieze A, Out C, Fuentes S, Jonker L, Reuling I *et al*. Impact of oral vancomycin on gut microbiota, bile acid metabolism, and insulin sensitivity. *J Hepatol* 2014;60:824–831.
231. Yamada Y, Miyawaki K, Tsukiyama K, Harada N, Yamada C *et al*. Pancreatic and extrapancreatic effects of gastric inhibitory polypeptide. *Diabetes* 2006;55:S86–S91.
232. Hwang I, Park YJ, Kim Y-R, Kim YN, Ka S *et al*. Alteration of gut microbiota by vancomycin and bacitracin improves insulin resistance *via* glucagon-like peptide 1 in diet-induced obesity. *The FASEB Journal* 2015;29:2397–2411.
233. Miele L, Giorgio V, Alberelli MA, De Candia E, Gasbarrini A *et al*. Impact of gut microbiota on obesity, diabetes, and cardiovascular disease risk. *Curr Cardiol Rep* 2015;17:120.
234. Sharma S, Tripathi P. Gut microbiome and type 2 diabetes: where we are and where to go? *J Nutr Biochem* 2019;63:101–108.
235. Koren O, Spor A, Felin J, Fåk F, Stombaugh J *et al*. Human oral, gut, and plaque microbiota in patients with atherosclerosis. *Proc Natl Acad Sci U S A* 2011;108:4592–4598.
236. Conti S, Santos SSFdos, Koga-Ito CY, Jorge AOC. Enterobacteriaceae and Pseudomonadaceae on the dorsum of the human tongue. *J. Appl. Oral Sci.* 2009;17:375–380.
237. Campbell LA, Kuo CC. Chlamydia pneumoniae — an infectious risk factor for atherosclerosis? *Nat Rev Microbiol* 2004;2:23–32.
238. Smieja M, Mahony J, Petrich A, Boman J, Chernesky M. Association of circulating Chlamydia pneumoniaeDNA with cardiovascular disease: a systematic review. *BMC Infect Dis* 2002;2:21.
239. Peng J, Xiao X, Hu M, Zhang X. Interaction between gut microbiome and cardiovascular disease. *Life Sci* 2018;214:153–157.
240. Stock J. Gut microbiota: an environmental risk factor for cardiovascular disease. *Atherosclerosis* 2013;229:440–442.
241. Jonsson AL, Bäckhed F. Role of gut microbiota in atherosclerosis. *Nat Rev Cardiol* 2017;14:79–87.
242. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS *et al*. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* 2011;472:57–63.
243. Canyelles M, Tondo M, Cedó L, Farràs M, Escolà-Gil JC *et al*. Trimethylamine N-oxide: a link among diet, gut microbiota, gene regulation of liver and intestine cholesterol homeostasis and HDL function. *Int J Mol Sci* 2018;19:3228.
244. Gregory JC, Buffa JA, Org E, Wang Z, Levison BS *et al*. Transmission of atherosclerosis susceptibility with gut microbial transplantation. *J. Biol. Chem.* 2015;290:5647–5660.
245. Lindskog Jonsson A, Caesar R, Akrami R, Reinhardt C, Fåk Hållenius F *et al*. Impact of Gut Microbiota and Diet on the Development of Atherosclerosis in *Apoe-/-* Mice. *Arterioscler Thromb Vasc Biol* 2018;38:2318–2326.
246. Cho CE, Caudill MA. Trimethylamine-N-Oxide: Friend, foe, or simply caught in the cross-fire? *Trends Endocrinol Metab* 2017;28:121–130.
247. Bennett BJ, de Aguiar Vallim TQ, Wang Z, Shih DM, Meng Y *et al*. Trimethylamine-N-Oxide, a metabolite associated with atherosclerosis, exhibits complex genetic and dietary regulation. *Cell Metab* 2013;17:49–60.
248. Koeth RA, Levison BS, Culley MK, Buffa JA, Wang Z *et al*. γ-Butyrobetaine is a proatherogenic intermediate in gut microbial metabolism of L -carnitine to TMAO. *Cell Metab* 2014;20:799–812.
249. Tang WHW, Wang Z, Kennedy DJ, Wu Y, Buffa JA *et al*. Gut Microbiota-dependent trimethylamine *N* -oxide (TMAO) pathway contributes to both development of renal insufficiency and mortality risk in chronic kidney disease. *Circ Res* 2015;116:448–455.
250. Tang WHW, Wang Z, Levison BS, Koeth RA, Britt EB *et al*. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N Engl J Med* 2013;368:1575–1584.
251. Falony G, Vieira-Silva S, Raes J. Microbiology meets big data: the case of gut microbiota–derived trimethylamine. *Annu Rev Microbiol* 2015;69:305–321.
252. Zhu Y, Jameson E, Crosatti M, Schäfer H, Rajakumar K *et al*. Carnitine metabolism to trimethylamine by an unusual Rieske-type oxygenase from human microbiota. *Proc Natl Acad Sci USA* 2014;111:4268–4273.
253. Al-Rubaye H, Perfetti G, Kaski JC. The role of microbiota in cardiovascular risk: focus on trimethylamine oxide. *Curr Probl Cardiol* 2019;44:182–196.
254. Ott SJ, El Mokhtari NE, Musfeldt M, Hellmig S, Freitag S *et al*. Detection of diverse bacterial signatures in atherosclerotic lesions of patients with coronary heart disease. *Circulation* 2006;113:929–937.
255. Romano KA, Vivas EI, Amador-Noguez D, Rey FE. Intestinal microbiota composition modulates choline bioavailability from diet and accumulation of the proatherogenic metabolite trimethylamine-N-oxide. *MBio* 2015;6:e2481–14.
256. Kelly CR, Khoruts A, Staley C, Sadowsky MJ, Abd M *et al*. Effect of Fecal Microbiota Transplantation on Recurrence in Multiply Recurrent *Clostridium difficile* Infection. *Ann Intern Med* 2016;165:609–616.
257. Chan YK, El-Nezami H, Chen Y, Kinnunen K, Kirjavainen PV. Probiotic mixture VSL#3 reduce high fat diet induced vascular inflammation and atherosclerosis in ApoE–/– mice. *AMB Express* 2016;6:61.
258. Brugère JF, Borrel G, Gaci N, Tottey W, O'Toole PW *et al*. Archaebiotics: proposed therapeutic use of archaea to prevent trimethylaminuria and cardiovascular disease. *Gut Microbes* 2014;5:5–10.
259. Neill AR, Grime DW, Dawson RMC. Conversion of choline methyl groups through trimethylamine into methane in the rumen. *Biochem. J.* 1978;170:529–535.
260. Mihajlovski A, Alric M, Brugère J-F. A putative new order of methanogenic archaea inhabiting the human gut, as revealed by molecular analyses of the mcrA gene. *Res Microbiol* 2008;159:516–521.
261. Dridi B, Fardeau M-L, Ollivier B, Raoult D, Drancourt M. *Methanomassiliicoccus luminyensis* gen. nov., sp. nov., a methanogenic archaeon isolated from human faeces. *Int J Syst Evol Microbiol* 2012;62:1902–1907.
262. Li JV, Ashrafian H, Bueter M, Kinross J, Sands C *et al*. Metabolic surgery profoundly influences gut microbial-host metabolic cross-talk. *Gut* 2011;60:1214–1223.
263. Liou AP, Paziuk M, Luevano J-M, Machineni S, Turnbaugh PJ *et al*. Conserved shifts in the gut microbiota due to gastric bypass reduce host weight and adiposity. *Sci Transl Med* 2013;5:178ra41.
264. Ryan KK, Tremaroli V, Clemmensen C, Kovatcheva-Datchary P, Myronovych A *et al*. Fxr is a molecular target for the effects of vertical sleeve gastrectomy. *Nature* 2014;509:183–188.

265. Sjöström L, Narbro K, Sjöström CD, Karason K, Larsson B *et al*. Effects of bariatric surgery on mortality in Swedish obese subjects. *N Engl J Med* 2007;357:741–752.

266. Sjöström L, Peltonen M, Jacobson P, Sjöström CD, Karason K *et al*. Bariatric surgery and long-term cardiovascular events. *JAMA* 2012;307:56–65.

267. Werling M, Fändriks L, Björklund P, Maleckas A, Brandberg J *et al*. Long-term results of a randomized clinical trial comparing Roux-en-$\hbox{Y}$ gastric bypass with vertical banded gastroplasty. *Br J Surg* 2013;100:222–230.

268. Pories WJ. Bariatric surgery: risks and rewards. *J Clin Endocrinol Metab* 2008;93:s89–s96.

269. Aronne LJ, Nelinson DS, Lillo JL. Obesity as a disease state: a new paradigm for diagnosis and treatment. *Clin Cornerstone* 2009;9:9–29.

270. Valderas JM, Starfield B, Sibbald B, Salisbury C, Roland M. Defining comorbidity: implications for understanding health and health services. *The Annals of Family Medicine* 2009;7:357–363.

271. Tremaroli V, Karlsson F, Werling M, Ståhlman M, Kovatcheva-Datchary P *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* 2015;22:228–238.

272. Hughes V. Weight-Loss surgery: a gut-wrenching question. *Nature* 2014;511:282–284.

273. Stefater MA, Wilson-Pérez HE, Chambers AP, Sandoval DA, Seeley RJ. All bariatric surgeries are not created equal: insights from mechanistic comparisons. *Endocr Rev* 2012;33:595–622.

274. Chambers AP, Wilson-Perez HE, McGrath S, Grayson BE, Ryan KK *et al*. Effect of vertical sleeve gastrectomy on food selection and satiation in rats. *Am J Physiol Endocrinol Metab* 2012;303:E1076–E1084.

275. Karmali S, Schauer P, Birch D, Sharma AM, Sherman V. Laparoscopic sleeve gastrectomy: an innovative new tool in the battle against the obesity epidemic in Canada. *Can J Surg* 2010;53:126–132.

276. Peck BCE, Seeley RJ. How does 'metabolic surgery' work its magic? New evidence for gut microbiota. *Curr Opin Endocrinol Diabetes Obes* 2018;25:81–86.

277. Guo Y, Huang Z-P, Liu C-Q, Qi L, Sheng Y *et al*. Modulation of the gut microbiome: a systematic review of the effect of bariatric surgery. *Eur J Endocrinol* 2018;178:43–56.

278. Patrone V, Vajana E, Minuti A, Callegari ML, Federico A *et al*. Postoperative changes in fecal bacterial communities and fermentation products in obese patients undergoing Bilio-Intestinal bypass. *Front Microbiol* 2016;7:200.

279. Palleja A, Kashani A, Allin KH, Nielsen T, Zhang C *et al*. Roux-En-Y gastric bypass surgery of morbidly obese patients induces swift and persistent changes of the individual gut microbiota. *Genome Med* 2016;8:67.

280. Basso N, Soricelli E, Castagneto-Gissey L, Casella G, Albanese D *et al*. Insulin resistance, microbiota, and fat distribution changes by a new model of vertical sleeve gastrectomy in obese rats. *Diabetes* 2016;65:2990–3001.

281. Ma K, Saha PK, Chan L, Moore DD. Farnesoid X receptor is essential for normal glucose homeostasis. *Journal of Clinical Investigation* 2006;116:1102–1109.

282. Zhang Y, Lee FY, Barrera G, Lee H, Vales C *et al*. Activation of the nuclear receptor FXR improves hyperglycemia and hyperlipidemia in diabetic mice. *Proc Natl Acad Sci U S A* 2006;103:1006–1011.

283. Albaugh VL, Banan B, Antoun J, Xiong Y, Guo Y *et al*. Role of bile acids and GLP-1 in mediating the metabolic improvements of bariatric surgery. *Gastroenterology* 2019;156:1041–1051.

284. Kohli R, Setchell KDR, Kirby M, Myronovych A, Ryan KK *et al*. A surgical model in male obese rats uncovers protective effects of bile acids Post-Bariatric surgery. *Endocrinology* 2013;154:2341–2351.

285. Pournaras DJ, Glicksman C, Vincent RP, Kuganolipava S, Alaghband-Zadeh J *et al*. The role of bile after Roux-en-Y gastric bypass in promoting weight loss and improving glycaemic control. *Endocrinology* 2012;153:3613–3619.

286. Breitman I, Isbell JM, Saliba J, Jabbour K, Flynn CR *et al*. Effects of proximal gut bypass on glucose tolerance and insulin sensitivity in humans. *Diabetes Care* 2013;36:e57.

287. Pathak P, Xie C, Nichols RG, Ferrell JM, Boehme S *et al*. Intestine farnesoid X receptor agonist and the gut microbiota activate G-protein bile acid receptor-1 signaling to improve metabolism. *Hepatology* 2018;68:1574–1588.

288. Agrawal M, Aroniadis OC, Brandt LJ, Kelly C, Freeman S *et al*. The long-term efficacy and safety of fecal microbiota transplant for recurrent, severe, and complicated Clostridium difficile infection in 146 elderly individuals. *J Clin Gastroenterol* 2016;50:403–407.

289. Jayasinghe TN, Chiavaroli V, Holland DJ, Cutfield WS, O'Sullivan JM. The new era of treatment for obesity and metabolic disorders: evidence and expectations for gut microbiome transplantation. *Front Cell Infect Microbiol* 2016;6:15.

290. Aroniadis OC, Brandt LJ. Intestinal microbiota and the efficacy of fecal microbiota transplantation in gastrointestinal disease. *Gastroenterol Hepatol* 2014;10:230–237.

291. Smits LP, Bouter KEC, de Vos WM, Borody TJ, Nieuwdorp M. Therapeutic potential of fecal microbiota transplantation. *Gastroenterology* 2013;145:946–953.

292. Moore T, Rodriguez A, Bakken JS. Fecal microbiota transplantation: a practical update for the infectious disease specialist. *Clinical Infectious Diseases* 2014;58:541–545.

293. Kelly CR, Kahn S, Kashyap P, Laine L, Rubin D *et al*. Update on fecal microbiota transplantation 2015: indications, methodologies, mechanisms, and outlook. *Gastroenterology* 2015;149:223–237.

294. Zhang F, Luo W, Shi Y, Fan Z, Ji G. Should we standardize the 1,700-year-old fecal microbiota transplantation? *Am J Gastroenterol* 2012;107:1755–1756.

295. Eiseman B, Silen W, Bascom GS, Kauvar AJ. Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. *Surgery* 1958;44:854–859.

296. Bidu C, Escoula Q, Bellenger S, Spor A, Galan M *et al*. The transplantation of ω3 PUFA–Altered gut microbiota of fat-1 mice to wild-type littermates prevents obesity and associated metabolic disorders. *Diabetes* 2018;67:1512–1523.

297. Lee HL, Shen H, Hwang IY, Ling H, Yew WS *et al*. Targeted approaches for in situ gut microbiome manipulation. *Genes* 2018;9:E351.

298. Rivière A, Selak M, Lantin D, Leroy F, De Vuyst L. Bifidobacteria and butyrate-producing colon bacteria: importance and strategies for their stimulation in the human gut. *Front Microbiol* 2016;7:979.

299. Dewulf EM, Cani PD, Claus SP, Fuentes S, Puylaert PGB *et al*. Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. *Gut* 2013;62:1112–1121.

300. Wu X, Ma C, Han L, Nawaz M, Gao F *et al*. Molecular characterisation of the faecal microbiota in patients with type II diabetes. *Curr Microbiol* 2010;61:69–78.

301. Tamanai-Shacoori Z, Smida I, Bousarghin L, Loreal O, Meuric V *et al*. *Roseburia* spp.: a marker of health? *Future Microbiol* 2017;12:157–170.

302. Barczynska R, Bandurska K, Slizewska K, Litwin M, Szalecki M *et al*. Intestinal microbiota, obesity and prebiotics. *Pol J Microbiol* 2015;64:93–100.

303. Matsumoto M, Inoue R, Tsukahara T, Ushida K, Chiji H *et al*. Voluntary running exercise alters microbiota composition and increases n-butyrate concentration in the rat cecum. *Biosci Biotechnol Biochem* 2008;72:572–576.

304. Queipo-Ortuño MI, Seoane LM, Murri M, Pardo M, Gomez-Zumaquero JM *et al*. Gut microbiota composition in male rat

models under different nutritional status and physical activity and its association with serum leptin and ghrelin levels. *PLoS One* 2013;8:e65465.

305. Evans CC, LePard KJ, Kwak JW, Stancukas MC, Laskowski S *et al*. Exercise prevents weight gain and alters the gut microbiota in a mouse model of high fat diet-induced obesity. *PLoS One* 2014;9:e92193.

306. Kang SS, Jeraldo PR, Kurti A, Miller ME, Cook MD *et al*. Diet and exercise orthogonally alter the gut microbiome and reveal independent associations with anxiety and cognition. *Mol Neurodegener* 2014;9:36.

307. Lambert JE, Myslicki JP, Bomhof MR, Belke DD, Shearer J *et al*. Exercise training modifies gut microbiota in normal and diabetic mice. *Appl Physiol Nutr Metab* 2015;40:749–752.

308. Denou E, Marcinko K, Surette MG, Steinberg GR, Schertzer JD. High-Intensity exercise training increases the diversity and metabolic capacity of the mouse distal gut microbiota during diet-induced obesity. *Am J Physiol Endocrinol Metab* 2016;310:E982–E993.

309. Mika A, Van Treuren W, González A, Herrera JJ, Knight R *et al*. Exercise is more effective at altering gut microbial composition and producing stable changes in lean mass in juvenile versus adult male F344 rats. *PLoS One* 2015;10:e0125889.

310. Campbell SC, Wisniewski PJ, Noji M, McGuinness LR, Häggblom MM *et al*. The effect of diet and exercise on intestinal integrity and microbial diversity in mice. *PLoS One* 2016;11:e0150502.

311. Brown CT, Davis-Richardson AG, Giongo A, Gano KA, Crabb DB *et al*. Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. *PLoS One* 2011;6:e25792.

312. Dabard J, Bridonneau C, Phillipe C, Anglade P, Molle D *et al*. Ruminococcin A, a new lantibiotic produced by a *Ruminococcus gnavus* strain isolated from human feces. *Appl Environ Microbiol* 2001;67:4111–4118.

313. Clarke SF, Murphy EF, O'Sullivan O, Lucey AJ, Humphreys M *et al*. Exercise and associated dietary extremes impact on gut microbial diversity. *Gut* 2014;63:1913–1920.

314. Murtaza N, Burke L, Vlahovich N, Charlesson B, O' Neill H *et al*. The effects of dietary pattern during intensified training on stool microbiota of elite race walkers. *Nutrients* 2019;11:pii:E261.

315. Bressa C, Bailén-Andrino M, Pérez-Santiago J, González-Soltero R, Pérez M *et al*. Differences in gut microbiota profile between women with active lifestyle and sedentary women. *PLoS One* 2017;12:e0171352.

316. Allen JM, Mailing LJ, Niemiro GM, Moore R, Cook MD *et al*. Exercise alters gut microbiota composition and function in lean and obese humans. *Medicine & Science in Sports & Exercise* 2018;50:747–757.

317. Munukka E, Ahtiainen JP, Puigbó P, Jalkanen S, Pahkala K *et al*. Six-Week endurance exercise alters gut metagenome that is not reflected in systemic metabolism in Over-weight women. *Front Microbiol* 2018;9:2323.

318. Cronin O, Barton W, Skuse P, Penney NC, Garcia-Perez I *et al*. A prospective metagenomic and metabolomic analysis of the impact of exercise and/or whey protein supplementation on the gut microbiome of sedentary adults. *mSystems* 2018;3:pii: e00044-18.

319. Peng L, Li Z-R, Green RS, Holzman IR, Lin J. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. *J Nutr* 2009;139:1619–1625.

320. Packer N, Hoffman-Goetz L. Exercise training reduces inflammatory mediators in the intestinal tract of healthy older adult mice. *Can. J. Aging* 2012;31:161–171.

321. Hoffman-Goetz L, Pervaiz N, Guan J. Voluntary exercise training in mice increases the expression of antioxidant enzymes and decreases the expression of TNF-α in intestinal lymphocytes. *Brain Behav Immun* 2009;23:498–506.

322. Hoffman-Goetz L, Pervaiz N, Packer N, Guan J. Freewheel training decreases pro- and increases anti-inflammatory cytokine expression in mouse intestinal lymphocytes. *Brain Behav Immun* 2010;24:1105–1115.

323. Ismail AS, Severson KM, Vaishnava S, Behrendt CL, Yu X *et al*. Gammadelta intraepithelial lymphocytes are essential mediators of host-microbial homeostasis at the intestinal mucosal surface. *Proc Natl Acad Sci USA* 2011;108:8743–8748.

324. Rowell LB, Brengelmann GL, Blackmon JR, Twiss RD, Kusumi F. Splanchnic blood flow and metabolism in heat-stressed man. *J Appl Physiol* 1968;24:475–484.

325. van Wijck K, Lenaerts K, van Loon LJC, Peters WHM, Buurman WA *et al*. Exercise-Induced splanchnic hypoperfusion results in gut dysfunction in healthy men. *PLoS One* 2011;6:e222366.

326. Otte JA, Oostveen E, Geelkerken RH, Groeneveld ABJ, Kolkman JJ. Exercise induces gastric ischemia in healthy volunteers: a tonometry study. *J Appl Physiol* 2001;91:866–871.

327. Carmody RN, Gerber GK, Luevano JM, Gatti DM, Somes L *et al*. Diet dominates host genotype in shaping the murine gut microbiota. *Cell Host Microbe* 2015;17:72–84.

328. Ma W, Mao Q, Xia W, Dong G, Yu C *et al*. Gut microbiota shapes the efficiency of cancer therapy. *Front Microbiol* 2019;10:1050.

329. Surana NK, Kasper DL. Erratum: moving beyond microbiome-wide associations to causal microbe identification. *Nature* 2018;554:392–247.

330. McCoy KD, Geuking MB, Ronchi F. Gut microbiome standardization in control and experimental mice. *Curr Protoc Immunol* 2017;117:1–13.

331. Atıcı S, Soysal A, Karadeniz Cerit K, Yılmaz Şerife, Aksu B *et al*. Catheter-Related Saccharomyces cerevisiae fungemia following Saccharomyces boulardii probiotic treatment: in a child in intensive care unit and review of the literature. *Med Mycol Case Rep* 2017;15:33–35.

332. d'Hennezel E, Abubucker S, Murphy LO, Cullen TW. Total lipopolysaccharide from the human gut microbiome silences Toll-like receptor signaling. *mSystems* 2017;2:e00046–17.

333. Blaut M, Clavel T. Metabolic diversity of the intestinal microbiota: implications for health and disease. *J Nutr* 2007;137:751S–755.

334. Valentini M, Piermattei A, Di Sante G, Migliara G, Delogu G *et al*. Immunomodulation by gut microbiota: role of Toll-like receptor expressed by T cells. *J Immunol Res* 2014;2014:1–8.

335. Uematsu S, Fujimoto K, Jang MH, Yang BG, Jung YJ *et al*. Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5. *Nat Immunol* 2008;9:769–776.

336. Singh RK, Chang HW, Yan D, Lee KM, Ucmak D *et al*. Influence of diet on the gut microbiome and implications for human health. *J Transl Med* 2017;15:73.

337. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol* 2009;9:313–323.

338. Griffin C, Eter L, Lanzetta N, Abrishami S, Varghese M *et al*. TLR4, TRIF, and MyD88 are essential for myelopoiesis and CD11c$^+$ adipose tissue macrophage production in obese mice. *J Biol Chem* 2018;293:8775–8786.

339. Tun X, Yasukawa K, Yamada K-ichi, Yamada K. Involvement of nitric oxide with activation of Toll-like receptor 4 signaling in mice with dextran sodium sulfate-induced colitis. *Free Radic Biol Med* 2014;74:108–117.

340. Ali M, El Chaar M, Ghiassi S, Rogers AM. American society for metabolic and bariatric surgery updated position statement on sleeve gastrectomy as a bariatric procedure. *Surg Obes Relat Dis* 2017;13:1652–1657.

341. Du J, Tian J, Ding L, Trac C, Xia B *et al*. Vertical sleeve gastrectomy reverses diet-induced gene-regulatory changes impacting lipid metabolism. *Sci Rep* 2017;7:5274.

342. Schauer PR, Kashyap SR, Wolski K, Brethauer SA, Kirwan JP *et al*. Bariatric surgery versus intensive medical therapy in obese patients with diabetes. *N Engl J Med* 2012;366:1567–1576.

343. Hsu LK, Betancourt S, Sullivan SP. Eating disturbances before and after vertical banded gastroplasty: a pilot study. *Int J Eat Disord* 1996;19:23–34.

344. Mistiaen W, Vaneerdeweg W, Blockx P, Van Hee R, Hubens G *et al*. Gastric emptying rate measurement after vertical banded gastroplasty. *Obes Surg* 2000;10:245–249.

345. Patti M-E, Houten SM, Bianco AC, Bernier R, Larsen PR *et al*. Serum bile acids are higher in humans with prior gastric bypass: potential contribution to improved glucose and lipid metabolism. *Obesity* 2009;17:1671–1677.

346. Karcz WK, Krawczykowski D, Kuesters S, Marjanovic G, Kulemann B *et al*. Influence of sleeve gastrectomy on NASH and type 2 diabetes mellitus. *J Obes* 2011;2011:765473–.

347. Myronovych A, Kirby M, Seeley RJ, Kohli R. 50 sleeve gastrectomy in obese mice results in elevated serum bile acids and reduced hepatic steatosis that correlate with weight loss post surgery. *Gastroenterology* 2012;142:S13.

348. Schauer PR, Ikramuddin S, Gourash W, Ramanathan R, Luketich J. Outcomes after laparoscopic Roux-en-Y gastric bypass for morbid obesity. *Ann Surg* 2000;232:515–529.

349. Schauer PR, Burguera B, Ikramuddin S, Cottam D, Gourash W *et al*. "Effect of laparoscopic roux-en-Y gastric bypass on type 2 diabetes mellitus.". *Ann Surg* 2003;238:467–485.

350. Higa KD, Boone KB, Ho T. Complications of the laparoscopic Roux-en-Y gastric bypass: 1,040 patients--what have we learned? *Obes Surg* 2000;10:509–513.

351. DeMaria EJ, Sugerman HJ, Kellum JM, Meador JG, Wolfe LG. Results of 281 consecutive total laparoscopic Roux-en-Y gastric bypasses to treat morbid obesity. *Ann Surg* 2002;235:640–647.

352. Nguyen NT, Ho HS, Palmer LS, Wolfe BM. A comparison study of laparoscopic versus open gastric bypass for morbid obesity. *J Am Coll Surg* 2000;191:149–157.

353. Wittgrove AC, Clark GW. Laparoscopic gastric bypass, Roux-en-Y-500 patients: technique and results, with 3-60 month follow-up. *Obes Surg* 2000;10:233–239.

354. Kikuchi R, Irie J, Yamada-Goto N, Kikkawa E, Seki Y *et al*. The impact of laparoscopic sleeve gastrectomy with duodenojejunal bypass on intestinal microbiota differs from that of laparoscopic sleeve gastrectomy in Japanese patients with obesity. *Clin Drug Investig* 2018;38:545–552.

355. Miller K, Hell E. Laparoscopic surgical concepts of morbid obesity. *Langenbecks Arch Surg* 2003;388:375–384.

356. Li JF, Lai DD, Ni B, Sun KX. Comparison of laparoscopic Roux-en-Y gastric bypass with laparoscopic sleeve gastrectomy for morbid obesity or type 2 diabetes mellitus: a meta-analysis of randomized controlled trials. *Can J Surg* 2013;56:E158–164.

357. Suter M, Donadini A, Romy S, Demartines N, Giusti V. Laparoscopic Roux-en-Y gastric bypass: significant long-term weight loss, improvement of obesity-related comorbidities and quality of life. *Ann Surg* 2011;254:267–273.

358. le Roux CW, Bloom SR. Why do patients lose weight after Roux-en-Y gastric bypass? *The Journal of Clinical Endocrinology & Metabolism* 2005;90:591–592.

359. Miras AD, le Roux CW. Mechanisms underlying weight loss after bariatric surgery. *Nat Rev Gastroenterol Hepatol* 2013;10:575–584.

360. Zhang H, DiBaise JK, Zuccolo A, Kudrna D, Braidotti M *et al*. Human gut microbiota in obesity and after gastric bypass. *Proc Natl Acad Sci USA* 2009;106:2365–2370.

361. Furet J-P, Kong L-C, Tap J, Poitou C, Basdevant A *et al*. Differential adaptation of human gut microbiota to bariatric surgery-induced weight loss: links with metabolic and low-grade inflammation markers. *Diabetes* 2010;59:3049–3057.

362. Mayo Clinic. 2016. Guide to types of weight-loss surgery. http://www.mayoclinic.org/tests-procedures/bariatric-surgery/in-depth/weight-loss-surgery/art-20045334?pg=2

363. Anderson B, Gill RS, de Gara CJ, Karmali S, Gagner M. Biliopancreatic diversion: the effectiveness of duodenal switch and its limitations. *Gastroenterol Res Pract* 2013;2013:974762–.

364. Scopinaro N, Gianetta E, Civalleri D, Bonalumi U, Bachi V. Biliopancreatic bypass for obesity: II. initial experience in man. *Br J Surg* 1979;66:618–620.

365. Eriksson F. Biliointestinal bypass. *Int J Obes* 1981;5:437–447.