



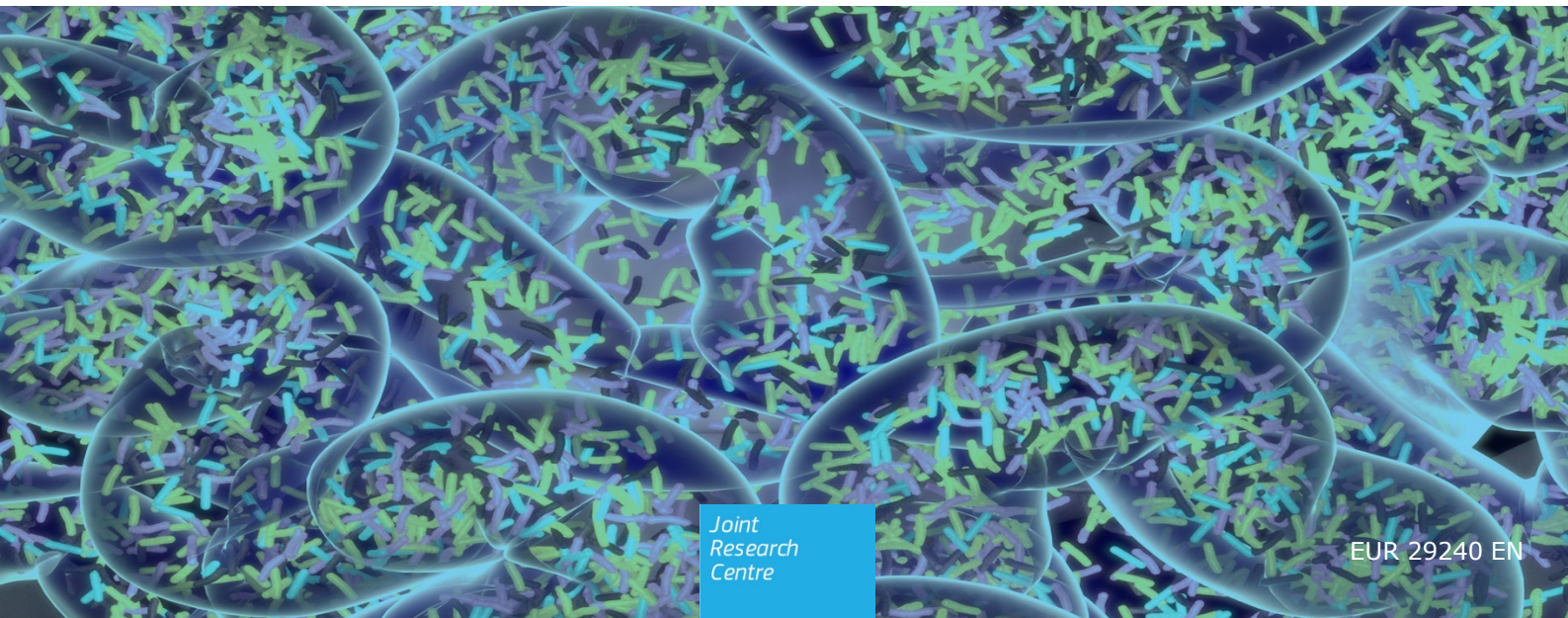
JRC TECHNICAL REPORTS

The Human Gut Microbiota

Overview and analysis of
the current scientific
knowledge and possible
impact on healthcare and
well-being

JRC F7 - Knowledge for Health and
Consumer Safety

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Contact information

Name: Guy Van den Eede
Address: Retieseweg 111 B-2440 Geel/Belgium
Email: guy.van-den-eede@ec.europa.eu
Tel.: +32 14 57 14 81

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Contributors (in alphabetical order)

Alexandre Angers, European Commission Directorate General Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Knowledge for Health and Consumer Safety Unit

Dafni Kagkli, European Commission Directorate General Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Knowledge for Health and Consumer Safety Unit

Alex Patak, European Commission Directorate General Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Knowledge for Health and Consumer Safety Unit

Mauro Petrillo, European Commission Directorate General Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Knowledge for Health and Consumer Safety Unit

Maddalena Querci, European Commission Directorate General Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Knowledge for Health and Consumer Safety Unit

Patrick Rüdelsheim, Perseus BVBA, Kortrijksesteenweg 127, B-9830 Sint Martens Latem, Belgium

Greet Smets, Perseus BVBA, Kortrijksesteenweg 127, 9830 Sint Martens Latem, Belgium

Guy Van den Eede, European Commission Directorate General Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Knowledge for Health and Consumer Safety Unit

1 Foreword

Recent years have seen a fast increase in the analytical capacity to read genetic information and in the ability to understand the link between the genetic information and the functioning of organisms. This has increased the scientific knowledge in previously underexploited fields. One example is the *human microbiota* and the understanding of the vital role that the microbiota plays in the physiological and psychological human health status and well-being. Brain degenerative diseases like Alzheimer and Parkinson are, for example, now considered to be linked to abnormalities in the functioning of the human gut microbiota.

This understanding may have revolutionary impact on (personal) healthcare but this promise has not yet been fully recognized by the general public or the policy community and for example today, microbiota-related policy interventions are mostly restricted to the marketing and health claims of possible probiotic foods and food supplements.

As the JRC is holding the responsibility for the knowledge management of health-related scientific information for policy, we present and discuss here the most recent information available on the vital role of the human gut microbiota and the associated opportunities for human health and well-being.

This report provides the state-of-the-art of scientific progress and details how we are only starting to learn its importance for human health, food and chemicals safety, as well as for our protection against environmental stressors. We also indicate why and how the human gut microbiota is going to have an impact on healthcare, nutrition and well-being and how this may change the way we assess the risks of the food, drugs and chemicals we are in contact with.

2 Executive summary

Starting at birth and throughout its whole life, the human being keeps an intimate interaction with its microbial community for protection, as a filter against aggressions from the environment, and as a supplier of beneficiary essential molecules. These microorganisms are found mostly in the gut, but also in the oral cavity, uterus and vagina and they cover very large areas of the human skin. Taken all together, this microbial community is called "microbiota".

The microbiota co-evolves with and plays an important role in the normal functioning of its host organism. The benefits are mutual: for example, the microorganisms in the gut are supported, survive and grow using the food a person eats and in return play a key role in health throughout human life. However, the microbiota is a living entity, which means that its composition may vary quickly and that, for example, pathogenic microorganisms may eventually exceed the beneficial and innocuous ones, impeding well-being and eventually causing disease.

Currently, the most exciting example of human body – microbiota interactions is the immune response system, as it has been demonstrated that perturbing the equilibrium between the cells of the human body and the gut microbiota results in disturbances of processes related to inflammation, autoimmunity, metabolism and neurodegeneration. Even effects on the development and progression of cancer have been reported.

The ratio between the cells of a human body and the components of its microbiota is generally believed to be between 3 and 10, in other words: for every "human cell" the body carries 3 to 10 microbial cells. Each cell in the human body has generally the same genetic information: for example, a nerve cell differs from a liver cell not in the content of its genetic material, but in the way that this genetic information is used. The microbiota, in contrast, consists of a large multitude of different genomes that thus potentially encode for a multiplicity of characteristics as compared to human body cells. Moreover, it is known that bacteria often exchange very large fragments of genetic material among them, thus vastly increasing the genetic versatility of the microbiota.

Irrespective thus of the actual numbers or ratio, it is essential to recognise that macroscopically the whole human body is a "super-organism" made up of cells that themselves are organised in structures like organs and of which the microbiota is an essential, vital component. Some refer to the human body, together with its microbiota, as a *unity* called "holobiont", a term used to describe a set of different species (in this case the human plus the microbiota) that form an ecological unit. Others refer to the microbiota as an "organ" of its own. This latter definition however is too strict and it should be better to consider it as a "meta-organ", composed of an agglomerate of *different* genomes with genes that are *differently* expressed in *different* microbial cells, and that interact with yet another set of *different* and differently expressed genes of the host genome and *varying* environmental contexts.

It must be stressed that, to date, research on the microbiota is very bacteria-centric and mainly focused on those present in the gut. Very few studies have looked at the viral component (or virome) and bacteriophages, eukaryotes such as protozoa, yeast and fungi, or have looked into other body compartments.

Nevertheless, the results obtained so far provide a strong indication that human gut microbiota are influenced by:

- The host genome and heritability - although they have a limited effect on the microbiota diversity.
- Early development. The gut microbiota is established early in life, even before birth. During the first 2-3 years of life there are significant changes as a result of nutrition and the overall environment.

- Diet. It is one of the key drivers for the differences in gut microbiota between people and across geographies and lifestyles. Food largely determines the intake of commensal, food-associated microbes and the composition of the diet will favour some species and hinder others. Effects of the geographic location can also be linked to differences in dietary patterns and lifestyle in a specific area.
- Diseases and infections. Antibiotic treatment may affect and kill naturally residing beneficial bacteria in the gut, changing the population's profile of the microbiota.
- Aging. Both the physiological modification of human organs and systems as well as changes in lifestyle have effects on the gut microbiota and its interaction with the host.

Furthermore, the gut microbiota may be associated with effects on human health and well-being:

- **Eating behaviour** (the microbiota-gut-brain axis), including preliminary evidence for the role of the gut microbiota in eating patterns, as well as alcohol and substance use disorders.
- **Dysbiosis** (i.e. imbalances or alterations in microbial composition or activity) is implicated in various diseases such as obesity, type 2 diabetes, cancer, mental health issues, coeliac disease, asthma, allergies and inflammatory bowel disease.
- **Infection** - The microbiota directly protects against infections by acting as a "gate-keeper", inhibiting unwanted organisms from colonising the human body. It can also act indirectly, by modulating the body's immune system response.
- **Therapeutic drugs** - The gut microbiota may inactivate therapeutic drugs, rendering them less effective. Alternatively, drugs may be "biotransformed" into different active derivatives that can have unpredicted toxic effects. The composition of the microbiota was also shown to affect vaccine efficacy.
- **Environmental chemicals and pollutants** - As for therapeutic drugs, the microbiota interacts with external chemicals with different, unpredictable consequences (neutralisation or activation of toxic substances, etc.). Conversely, exposure to environmental chemicals can induce microbiota alterations that modulate adverse health effects. Screening environmental chemicals should thus include toxicity end-points for the microbiota.

Whereas several factors that affect the microbiota as well as several phenomena that are associated with certain microbiota profiles have been determined, there is less clarity on how humans can use the microbiota to direct or support improvements in health and well-being. For example, in the gut microbiota context, possible therapeutic options that have been explored include a change of diet, the addition of non-digestible prebiotics, probiotics, and synbiotics to food products, as well as the use of antibiotics and faecal microbiota transplantation. While some of these treatments have been reported to be effective, reviewers in the field have highlighted the need for studies with larger sample sizes (to reach an adequate statistical power), homogeneous patient groups, standardised treatments, the elimination of confounding factors, the inclusion of measurements of biomarkers related to the immune system and intestinal health, to be able to compare results and understand the underlying phenomena.

Commercial applications leveraging the health potential of manipulating the microbiota raise concerns about property rights, accessibility of data, patentability of faecal microbiota profiles, financial benefits, etc. When performed outside of the regulated establishments, there are additional concerns on safety, follow-up, and exaggerated expectations. Today, in many areas such as in faecal transplantation, the clinical practitioners demand an adequate framework for microbiota-derived clinical therapies and applications.

Irrespective of the application, it is evident that the human microbiota is going to impact on healthcare, nutrition and well-being. As these microbes are the closest environment interacting with us, the microbiota is the first and most important barrier and filter between the human body and the environment. The food we eat, the air we breathe, the drugs we ingest and the environmental pollutants that enter our body come first into contact with the microbiota. The growing awareness of this fact and the observation that the human body - microbiota equilibrium may change, or that the microbiota may have a beneficial or harmful role in the conversion of the metabolites it encounters, may impact the future risk assessment of food, chemicals and drugs. Indeed, the core elements of risk assessment as established in the eighties (hazard identification, dose-response assessment, exposure assessment and risk assessment) have remained relatively unchanged and may require revision in the light of the role of the microbiota. Regardless of the approaches used to provide data for various risk assessments (e.g. animal toxicology studies, *in vitro* assays and computational approaches, biomarkers assessment), none has explicitly considered the human microbiota and thus risk assessment in its current approach may mischaracterise the nature of a hazard associated with an exposure to the human body and over- or underestimate the risk. Moreover, since the composition and functioning of the microbiota is both very specific to an individual and variable in time, a new approach of "personalised", "meta-risk assessment" may be required for a comprehensive risk-based approach.

To summarise, the human gut microbiota is not only expected to impact on healthcare, nutrition and well-being, but also on the whole risk assessment framework.

3 Abbreviations

ADME	Absorption, distribution, metabolism, and excretion
ALD	Alcoholic liver disease
CCK	Cholecystokinin
CD	Crohn's disease
CRC	Colorectal cancer
CVD	Cardiovascular disease
DCA	Deoxycholic acid
DNA	Deoxyribonucleic acid
EFSA	European Food Safety Authority
FDA	Food and Drug Administration (USA)
FGF	Fibroblast growth factor
FIAF	Fasting-induced adipocyte factor
FISH	Fluorescent in situ hybridisation
FOS	Fructo-oligosaccharide
FTIR	Fourier transform infrared spectroscopy
FXR	Farnesoid X receptor
GALT	Gut-associated lymphoid tissue
GC	Gas chromatography
GIP	Glucose-dependent insulinotropic polypeptide
GLP	Glucagon-like peptide
GOS	Galacto-oligosaccharide
GPBAR1	G protein-coupled bile acid receptor 1
GPCR	G protein-coupled receptor
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HDAC	Histone deacetylase
HDL	High-density lipoprotein
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
IND	Investigational new drug (USA)
LC	Liquid chromatography
LCFA	Long-chain fatty acid
LDL	Low-density lipoprotein
LPL	Lipoprotein lipase
LPS	Lipopolysaccharides
MALDI-MSI	Matrix-assisted laser desorption/ionisation mass spectrometry imaging
MCFA	Medium-chain fatty acid
mRNA	Messenger RNA

MS	Mass spectrometry
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NMR	Nuclear magnetic resonance
NSP	Non-starch polysaccharides
OA	Osteoarthritis
PCR	Polymerase chain reaction
PsA	Psoriatic arthritis
PYY	Peptide YY, peptide tyrosine tyrosine
qPCR	Quantitative polymerase chain reaction
QPS	Qualified presumption of safety
RA	Rheumatoid arthritis
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
SCFA	Short chain fatty acid
SIBO	Small intestinal bacterial overgrowth
TMA	Trimethylamine
TMAO	Trimethylamine-N-oxide
TLR	Toll-like receptor
Treg	Regulatory T cells
UC	Ulcerative colitis
UV/Vis	Ultraviolet/visible spectroscopy
VLDL	Very low- density lipoprotein
XOS	Xyloseoligosaccharide

4 What is the gut microbiome?

The microbiome can be defined as the community of commensal, symbiotic, and pathogenic microorganisms that inhabit all kinds of multicellular organisms. The term can be used synonymously with microbiota or microflora. The term "microbiome" is also used to describe the collection of genes that are found in those microbial communities. The human microbiome can be considered a counterpart to the human genome.

The community of commensal, symbiotic, and pathogenic microorganisms that inhabit all kinds of multicellular organisms.

The human microbiome has co-evolved with the human being as a unity called holobiont or hologenome (Salvucci, 2016) (Figure 1). The holobiont is a term used to describe an individual host and its microbial community, including viruses and cellular microorganisms. It distinguishes itself by not only recognizing hosts and their obligate symbionts but also emphasizing the diversity of facultative symbionts and their dynamic associations within a host.

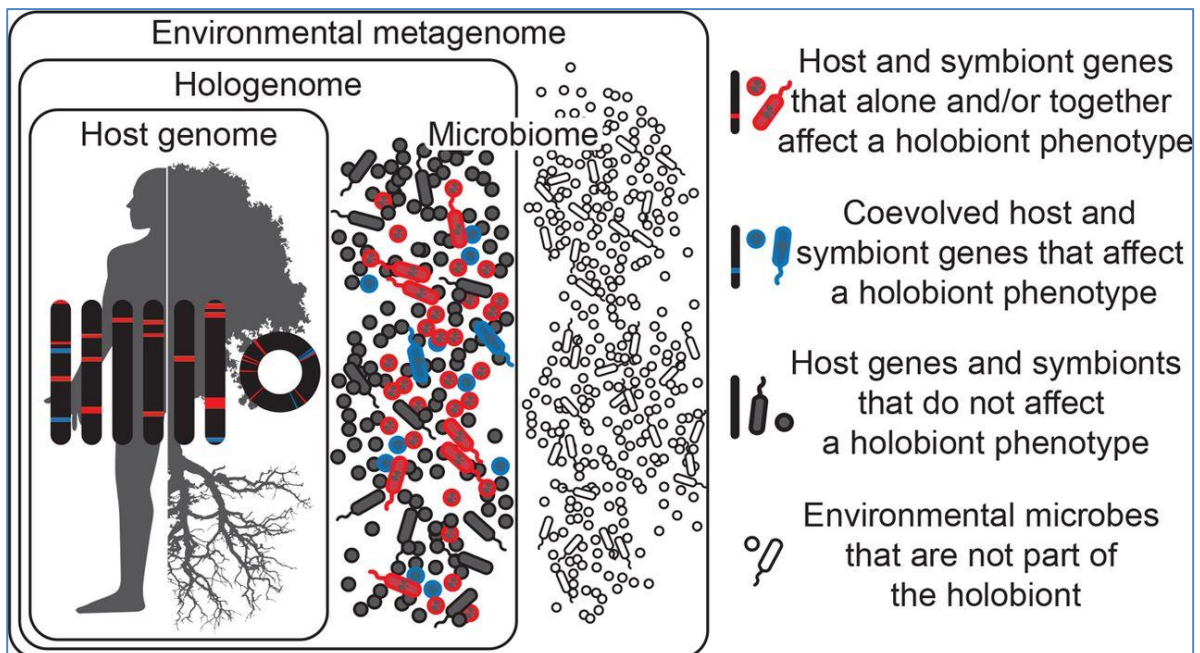


Figure 1. Holobionts are entities comprised of the host and all of its symbiotic microbes, including those which affect the holobiont's phenotype and have coevolved with the host (blue), those which affect the holobiont's phenotype but have not coevolved with the host (red), and those which do not affect the holobiont's phenotype at all (grey). Microbes in the environment are not part of the holobiont (white).

(© Theis/ASM *mSystems*, source: Theis et al., 2016)

In the human microbiome, one can make a distinction between the skin, mouth, nose, digestive tract and vagina microbiomes. This study is focussed in the human gut microbiome.

Microorganisms are found throughout the length of the human gastrointestinal tract from the mouth to the rectum. The density and composition vary according to anatomical site and various impacting factors as will be explained further on. Due to the low pH the abundance in the stomach is low. In the large intestine conditions are favourable for a dense microbial community. Most of the microorganisms are anaerobic organisms.

The microbiota includes bacteria, fungi, protozoa and viruses. The human gut microbiota is estimated to encompass 10^{13} to 10^{14} resident microorganisms. This number is often quoted as 10 times higher than the number of human body cells, however, more recently the ratio is set to be closer to 1:1 (Sender et al., 2016).

The human microbiota is composed primarily of bacteria from either phylum Bacteroidetes (mostly *Bacteroides* or *Prevotella* species), that are gram negative, or Firmicutes (mostly *Clostridium* and *Lactobacillus* species), that are gram positive (Consortium, 2012). The majority are strict anaerobes (97 %), mostly belonging to the phyla Firmicutes (64 %), Bacteroidetes (23 %), Proteobacteria (8 %), and Actinobacteria (3 %); low numbers of the phyla Fusobacteria, Verrucomicrobia, and TM7 (2 %) are also present. Fungi and archaea comprise less than 1 % of the total gut microbiota (Cardinelli et al., 2015).

The Bacteroidetes use a very wide range of substrates and are major producers of propionate. Among the Firmicutes are species that produce butyrate and that are specialist degraders of indigestible polysaccharides. Actinobacteria (that include *Bifidobacterium* spp.), Proteobacteria (including *Escherichia coli*), and Verrucomicrobia (including *Akkermansia mucinophila*) are typically present in smaller numbers in the healthy gut microbiota. Gut microbiota differ in composition between individuals and within individuals with age and development (Consortium, 2012; Yatsunenکو et al., 2012). More than 1000 species are identified, while a person on average carries 160 species (Simpson and Campbell, 2015). The anaerobic bacteria exceed by two or three orders of magnitude the facultative anaerobic and aerobic bacteria. Certain bacteria tend to be adherent to the mucosal surface, while others are predominant in the lumen. The establishment of the human gut microbiota starts early in life before birth.

The gut microbiota has co-evolved with the host. The gut microbiota plays an important role in the normal functioning of the host organism. The benefits are mutual: the microorganisms are supported by the food humans eat and play a key role in health throughout human life. Next to digestion they are involved in establishing the immune system, the defence against pathogens, the endocrine system and mental health. Disruption of the normal equilibrium may induce metabolic and brain related disease. Most microorganisms reside in the distal part of the human gut (colon). As they play a role in the digestion of residual substrates, they contribute to their host in the synthesis of vitamins (vitamins K and B12, thiamine, and riboflavin and folate) and essential amino acids. Fermentation products of dietary fibres and carbohydrates such as butyrate, propionate, and acetate (short-chain fatty acids, SCFAs) act as a major energy source for intestinal epithelial cells and may therefore strengthen the mucosal barrier (Simpson and Campbell, 2015; Singh et al., 2017). Other metabolites include secondary bile acids converted from primary bile acids; metabolites generated from meat-derived choline and L-carnitine; and other lipids including conjugated fatty acids and cholesterol (Abdollahi-Roodsaz et al., 2016). Inflammatory bowel disease, obesity, type 2 diabetes, cardiovascular disease, and cancer are correlated with changes in the composition of the gut microbiota.

The emergence of techniques such as pyrosequencing of 16S rRNA, quantitative polymerase chain reaction (qPCR) and fluorescent *in situ* hybridisation (FISH) have helped a great deal in studying mechanisms of the symbiotic relationship between host and microbiota. The ability to identify and quantify bacterial genera in the gut in studies deliberately altering a certain component makes it possible to go from correlation to causation.

5 Techniques for the study of the microbiome

Thriving in the human gut, a large portion of the microbiota is difficult -or even likely impossible- to isolate, identify and culture, providing significant bias to any of the results and conclusions obtained with this approach.

More recent techniques, such as pyrosequencing of 16S rRNA, quantitative polymerase chain reaction, fluorescent *in situ* hybridisation and genomics have overcome these difficulties but have each their own advantages and limitations.

Given the variation of the microbiome -even in the different parts of the gut of one individual- data gathering must be rigorously standardised in order to allow comparison.

Given the technical challenges, it is uncertain if the species that are identified so far can serve as a marker function (i.e. they represent a typical broader group of organisms reacting in the same way) or if they should be seen as independent species with no correlation in their abundance or reaction/influence on certain factors.

An alternative approach, which is less concerned with the actual species, may be to look at metabolic functions and characterise the microbiome in function of its activity.

5.1 Sampling

Fresh faecal samples are often used as they are relatively easy to obtain. The method is non-invasive and can be carried out privately by study participants (Fu et al., 2016). However, bacteria residing in the lumen of the intestine that end up in the stool are different from the ones residing in the mucosa. Mucosa-associated bacteria might be more important, in which case mucosal biopsy samples are required (Leung et al., 2016). Microbial populations also differ depending on the location along the gastrointestinal tract. Also, in stool samples variation, both longitudinally and radially, might exist. On top of that, day-to-day rhythms may interfere.

5.2 Detection/identification

5.2.1 Culture-based methods

Combinations of plating techniques and staining techniques, i.e., Gram, based on physiological and biochemical properties, were the first methods to describe the human microbiota (Hiergeist et al., 2015). The biggest disadvantage is that only species that survive this laboratory setting are identified. Bacterial culture misses around 80% of the bacteria detectable with next generation pyrosequencing (Marrs and Flohr, 2016). Slow-growing or stressed species are outcompeted by fast-growing species. Inappropriate conditions regarding pH, redox state, temperature, or absence of essential nutrient molecules may hinder others. Interdependency is another cause of failure.

However, further developments in high-throughput culture-based methods made it possible to increasingly identify more species (microbial culturomics). Still species are identified that do not appear in 16S rDNA-targeted approach, possibly because of an inefficient DNA extraction protocol (Hiergeist et al., 2015). As such, culture-based approaches may complement other methods.

5.2.2 DNA-based methods

Fluorescent *in situ* hybridisation (FISH) probes extracted DNA of a microbial community are used to study certain genes of interest (Hiergeist et al., 2015). Also, polymerase chain reaction (PCR) is used to amplify genes of interest, clone them in *E. coli* and subsequently sequence them. Sequencing itself has gone through an evolution from the slow and costly Sanger method to next-generation sequencing and third-generation sequencing (Daliri et al., 2017).

16S ribosomal RNA (rRNA) sequencing technique is based on the fact that the 16S rRNA gene is highly conserved between taxa of bacteria and archaea. This gene has highly conserved and hypervariable sequences (regions V1 to V9). Universal PCR primers can be used to match the conserved sections and the variable sequences are used to classify bacterial taxa. The method starts with the extraction of genomic DNA, the construction of appropriate sequencing libraries, then next-generation sequencing, followed by bioinformatic analysis including quality control, and finally the comparison to reference databases. The accuracy of the analysis and covered taxa depend on the choice of the primers, which may introduce bias. Comparison of results requires amplification of the same region. Also, dormant, dead and quiescent bacteria are picked-up. The bacterial diversity that this technique can study is limited.

Whole metagenome shotgun sequencing (WMS) comprises the whole genetic diversity including all kingdoms (also viral, fungal, and protozoan organisms). It has a much better resolution of bacteria at the species level and allows for annotation of bacterial gene clusters and pathways based on direct sequencing of bacterial genes (Kurilshikov et al., 2017). This technique may be used to define the functional capacity of the microbiome (Fu et al., 2016). Knowledge of the bacterial genes allows for a better understanding of their roles in human health (Singh et al., 2017). Metagenomics follows the same steps of analysis but it's costlier and more time consuming than 16S rRNA sequencing. Moreover, it depends on the availability of reference genome databases (inability to analyse genomes absent in the reference databases or genes with unrecognised function). Contamination by host DNA is another challenge when biopsy or mucosal material is being collected.

Both methods, PCR based and WMS, may have difficulties in detecting low-abundant organisms (Hiergeist et al., 2015). The isolation of highly purified DNA from a wide variety of specimens is a challenge and may introduce bias. Contamination in the PCR procedure is another burden. Comparing research results is only possible applying standardised and quality controlled methods for collecting and sampling (including the time of collection), transport, preservation, pre-analytical manipulations, and DNA-extraction (Fu et al., 2016).

Sequence-based analyses provide no information on the absolute abundance of bacterial cells in a gut sample (Flint et al., 2017). Absolute numbers are estimated most accurately by techniques such as fluorescent *in situ* hybridisation.

Identifying taxa may not tell the whole story. Often different taxa perform the same function. Differences in found taxa between individuals may nevertheless have the same outcome in metabolic functions (Betrapally et al., 2016). Betrapally et al. describe analysis strategies to cope with this.

5.2.3 Other techniques

Metatranscriptomics, sequencing microbial rRNA or messenger RNA (mRNA), can be used to gain insight into gene expression patterns (Simpson and Campbell, 2015). Instability of the mRNA and the lack of reference data are a problem. Moreover, the analysis gives a transient picture of the microbial community.

Metabolomics and metaproteomics are also being developed. They result in dynamic metabolic or protein profiles of the microbiota. Extracting total protein may be challenging due to interfering compounds and membrane/matrix-bound proteins. Liquid chromatography (LC), gas chromatography (GC), mass spectrometry (MS), LC-MS, GC-MS, ultraviolet/visible spectroscopy (UV/Vis), Fourier transform infrared spectroscopy (FT-IR), Matrix-assisted laser desorption/ionisation mass spectrometry imaging (MALDI-MSI) and nuclear magnetic resonance (NMR) spectroscopy allow sensitive identification of microbial and host cell metabolites (Daliri et al., 2017). The metabolome is influenced by a lot of factors and therefore it might be difficult to compare between individuals and treatments. Furthermore, it may be difficult to differentiate between host and microbial metabolite profiles.

6 Effects of/on nutrition

The diet is regarded as one of the key drivers for the differences in gut microbiota between people and across geographies and lifestyles.

As microorganisms are specialised in fermenting certain substrates, even some which are indigestible for human enzymes, the composition of the diet will favour some species and strains and hinder others.

Whole diets as well as food components (protein, fat, carbohydrates, polyphenols), influence the total bacteria count as well as the relative abundance of certain species. Food processing and preservation reduces the intake of commensal, food-associated microbes, whereas fermented foods enrich specific bacteria that transiently colonise the gut.

Prebiotics ("a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health") induce enhancement in gut mucosal barrier integrity and function, increased host mucosal immunity, increased SCFA production and an associated reduction in mucosal interaction of opportunistic enteric pathogens.

Probiotic effects are very strain specific and cannot be generalised. The probiotics can be ingested as such or as part of fermented foods. Since most probiotics do not colonise the host's gut, continuous consumption often is necessary to achieve lasting effects.

Gut microbiota affects the host in his eating behaviour (the microbiota-gut-brain axis). There is preliminary evidence for the role of the gut microbiota in eating and alcohol and substance use disorders.

The diet is regarded as one of the key drivers for the differences in gut microbiota between people and across geographies and lifestyles (De Filippo et al., 2010; Graf et al., 2015; Yatsunenکو et al., 2012). Food components, which are indigestible for human enzymes, provide substrates for the intestinal microbial metabolism. As microorganisms are specialised in fermenting certain substrates, the composition of the diet will favour some species and strains and hinder others. To demonstrate the cause effect relation between diet and microbiome composition, studies have been undertaken where the diet has deliberately been changed (Flint et al., 2017; Graf et al., 2015).

6.1 Metabolic capacity

The gut microbiota is responsible for substrate breakdown, production of vitamins, signalling molecules and anti-microbial compounds, etc. (Daliri et al., 2017). They transform complex indigestible molecules such as dietary fibres and mucin into short chain fatty acids (SCFAs).

The main SCFAs are acetate, propionate and butyrate. They have an important physiological function. The highest levels of SCFA are found in the cecum and proximal colon, declining toward the distal colon (Koh et al., 2016). Most butyrate is used as energy source by the colonic epithelial cells. Butyrate induces the differentiation of regulatory T (Treg) cells. Propionate is absorbed and metabolised in the liver. Hepatocyte cells use propionate for gluconeogenesis. Acetate can cross the blood-brain barrier and reduce appetite via a central homeostatic mechanism. Acetate stimulates the colonic epithelium to improve epithelial integrity. Propionate and butyrate affect peripheral organs indirectly by activation of hormonal and nervous systems. SCFAs decrease colonic pH, decrease circulating cholesterol, inhibit the growth of pathogens, stimulate water and sodium absorption, provide energy to the colonic epithelial cells, and prevent high-fat diet induced obesity by stimulating fat oxidation (Daliri et al., 2017).

Bacterial species responsible for these products are listed in

Table 1. Changes in the composition of the microbiota induces changes in metabolites that affect the hosts' physiology and disease. SCFAs act via two principal mechanisms: by signalling through G protein-coupled receptors (GPCRs), and by inhibiting histone deacetylases.

The metabolic activities of gut microbiota as a whole are influenced by diet and diet-driven changes in microbiota composition. To understand and explain the shifts in metabolite composition it is necessary to identify substrate degrading enzymes in species, to confirm that the degraded products can be utilised, and to demonstrate that the specific species can compete with others in the intestines (Flint et al., 2017). *In vitro* fermentation experiments supplying either inulin or pectin as non-digestible carbohydrate have demonstrated a specific stimulation of several *Bacteroides* species (Chung et al., 2016).

Table 1. Short Chain Fatty Acids (SCFAs) Production by microbes in the gut.

(© Koh / Elsevier, Source: Koh et al., 2016)

SCFAs	Pathways/Reactions	Producers
Acetate	from pyruvate via acetyl-CoA	most of the enteric bacteria, e.g., <i>Akkermansia mucinophila</i> , <i>Bacteroides</i> spp., <i>Bifidobacterium</i> spp., <i>Prevotella</i> spp., <i>Ruminococcus</i> spp.
	Wood-Ljungdahl pathway	<i>Blautia hydrogenotrophica</i> , <i>Clostridium</i> spp., <i>Streptococcus</i> spp.
Propionate	succinate pathway	<i>Bacteroides</i> spp., <i>Phascolarctobacterium succinatutens</i> , <i>Dialister</i> spp., <i>Veillonella</i> spp.
	acrylate pathway	<i>Megasphaera elsdenii</i> , <i>Coprococcus catus</i>
	propanediol pathway	<i>Salmonella</i> spp., <i>Roseburia inulinivorans</i> , <i>Ruminococcus obeum</i>
Butyrate	phosphotransbutyrylase/ butyrate kinase route	<i>Coprococcus comes</i> , <i>Coprococcus eutactus</i>
	butyryl-CoA:acetate CoAtransferase route	<i>Anaerostipes</i> spp. (A, L), <i>Coprococcus catus</i> (A), <i>Eubacterium rectale</i> (A), <i>Eubacterium hallii</i> (A, L), <i>Faecalibacterium prausnitzii</i> (A), <i>Roseburia</i> spp. (A)

6.2 Whole diets

Walker and colleagues studied the microbiome of obese volunteers over time receiving subsequently a different diet (Walker et al., 2011). Targeted qPCR revealed that, although the composition was clearly individual specific, samples showed abundance/peaks in specific bacterial groups occurring rapidly after a dietary change. The diets only differed in the non-digestible carbohydrate type. The type of non-digestible carbohydrate substrates is also responsible for a low or high microbiome diversity. The low diversity microbiomes tended to be dominated by *Bacteroides*. Wu et al. investigated the influence of a short-term intervention on different long-term diets (Wu et al., 2011). Long-term diet low in fat and high in dietary fibre was associated with higher Firmicutes, but diet high in fat was more highly associated with Actinobacteria and

Bacteroides. The intervention changed the microbiota composition within 24 hours, but the magnitude of the effect did not overcome inter-subject variations in the intestinal microbiota.

More drastic shifts were noted when a diet based on animal-derived food versus plant-based food was compared (David et al., 2014b). Here too, the change in microbiome composition was seen within days. The animal-based diet increased the abundance of bile-tolerant microorganisms (*Alistipes*, *Bilophila* and *Bacteroides*) and decreased the levels of Firmicutes that metabolise dietary plant polysaccharides (*Roseburia* spp., *Eubacterium rectale* and *Ruminococcus bromii*). This is consistent with observations that high fat intake causes secretion of more bile acids. The same group made a time series for two persons (David et al., 2014a). They showed that overall microbial communities are stable for months, but sudden changes may alter them. One person travelled from the USA to a developing country and was exposed to a novel diet and environment. The analysis of stools showed that the Bacteroidetes to Firmicutes ratio increased from 0.37 (pre-travel) to 0.71 (mid-travel).

De Filippis and colleagues examined the effect of Mediterranean diet that is characterised by a high-level consumption of cereals, fruit, vegetables and legumes (De Filippis et al., 2016). A significant association was detected between consumption of vegetable-based diets and increased levels of faecal SCFAs, *Prevotella* and some fibre-degrading Firmicutes. Several studies investigated the influence of whole grain breakfast cereals or flakes on gut microbiota composition. The proportion of *Bifidobacterium* spp. and the *Lactobacillus/Enterococcus* group was increased compared to the control (Graf et al., 2015). The influence of fruit consumption, especially berries, is characterised by an increase in *Bifidobacterium* spp. The daily consumption of red wine polyphenol for 4 weeks significantly increased the number of *Enterococcus*, *Prevotella*, *Bacteroides*, *Bifidobacterium*, *Bacteroides uniformis*, *Eggerthella lenta*, and *Blautia coccoides-Eubacterium rectale* groups (Queipo-Ortuno et al., 2012). The consumption of chickpeas containing significant levels of oligosaccharides had no effect on the taxonomic composition or diversity of gut microbiota (Fernando et al., 2010). There was also no effect on SCFA concentrations. A study with overweight and obese men drinking soy milk showed a decrease in Firmicutes to Bacteroidetes compared to baseline values (Fernandez-Raudales et al., 2012). On the influence of nuts, the consumption of pistachios had a stronger impact on microbiota composition than the consumption of almonds with a higher production of butyrate (Ukhanova et al., 2014).

A high-protein and moderate-carbohydrate diet was compared with a high-protein and low-carbohydrate diet in obese men (Russell et al., 2011). Both diets resulted in increased proportions of branched-chain fatty acids and concentrations of phenylacetic acid and N-nitroso compounds compared to control diet. *Roseburia/Eubacterium rectale* group of bacteria were reduced resulting in a decrease of the proportion of butyrate in faecal SCFA concentrations. Another study with overweight and obese volunteers examined the effect of an 8 - week energy-restricted diet of low-carbohydrate, high fat compared to a high-carbohydrate, low fat diet (Brinkworth et al., 2009). In the low-carbohydrate diet, the amount of bifidobacteria dropped and the SCFA levels were lower compared to the starting point. Other studies confirmed that with a reduction in dietary carbohydrate intake, the abundance of *Roseburia* spp., *Eubacterium rectale* and *Bifidobacterium* spp. decrease, and total SCFA reduced in response to this (Simpson and Campbell, 2015).

6.3 Processed food

Food processing also has an effect on the intestinal microbiota (Graf et al., 2015). Raw food, vegetables and fruit, have their own microbiota that is affected by the processing method. Highly processed and preserved foods reduce the intake of commensal, food-associated microbes. Fermented foods like cheese are enriched in lactic acid bacteria that transiently colonise the gut (David et al., 2014b).

6.4 Single components

Singh and colleagues performed a systematic literature review on the influence of diet on gut microbiota and human health (Singh et al., 2017). They discussed the effect of the main food components.

6.4.1 Protein

Protein consumption positively correlates with overall microbial diversity.

Consumption of whey and pea protein extract has been reported to increase gut-commensal *Bifidobacterium* and *Lactobacillus*, while whey additionally decreases the pathogenic *Bacteroides fragilis* and *Clostridium perfringens*. Pea proteins lead to an increase in intestinal SCFA levels. Consuming more animal protein enriches *Bacteroides* and *Alistipes* in the microbiota and reduces faecal SCFAs. *Bifidobacterium* spp., *Lactobacillus* spp., *Roseburia* spp., *Eubacterium* spp. and *Faecalibacterium prausnitzii* are associated with the increased production of SCFA that are considered anti-inflammatory and important for maintenance of the mucosal barrier.

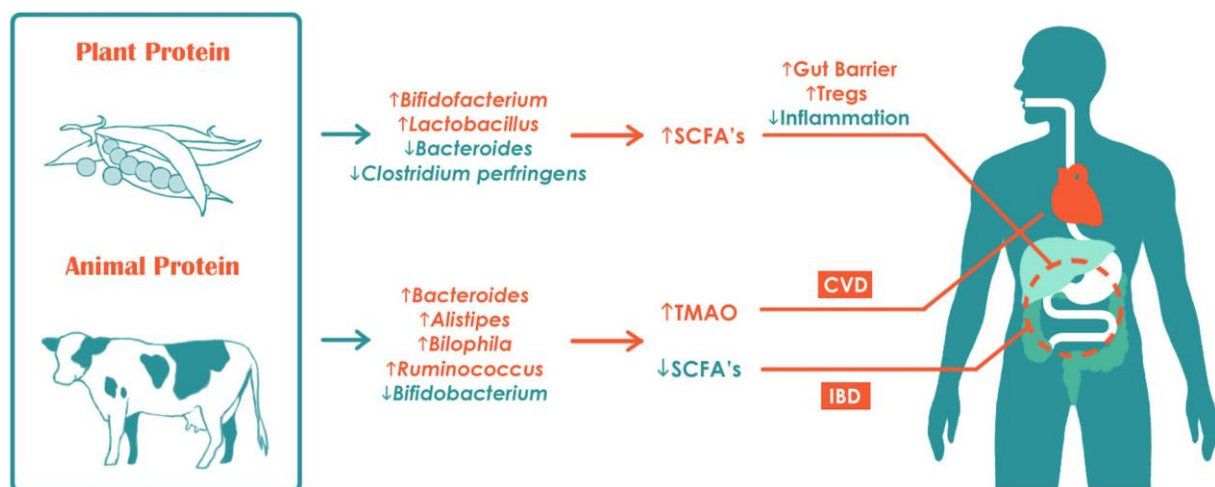


Figure 2. Impact of dietary protein on intestinal microbiota and health outcomes. SCFA (short chain fatty acids), TMAO (trimethylamine N-oxide), Tregs (T regulatory cells), CVD (cardiovascular disease); IBD (inflammatory bowel disease).

(© Singh/BMC, source: Singh et al., 2017)

Red meat consumption is associated with increased levels of trimethylamine-N-oxide (TMAO), a proatherogenic compound that increases risk of cardiovascular disease. However, an animal protein-based diet usually also means a higher fat intake. It still needs to be investigated what influence each constituent has.

6.4.2 Fat

Human studies indicate increases in total anaerobic microflora and amount of *Bacteroides* in a high-fat diet. Rats feeding on high-fat feed show less *Lactobacillus intestinalis* and disproportionately more propionate and acetate producing species, including *Clostridiales*, *Bacteroides*, and *Enterobacteriales*. A low-fat diet increases human faecal abundance of *Bifidobacterium* at the same time reducing fasting glucose and total cholesterol. A high saturated fat diet shows a relative higher proportion of *Faecalibacterium prausnitzii*. No shifts in the relative abundance of any bacterial genera is seen with high monounsaturated fat consumption, as in salmon which is high in mono and polyunsaturated fats. Lard-fed mice proved to have more *Bacteroides* and *Bilophila*, while fish-oil-fed mice revealed to have more *Actinobacteria* (*Bifidobacterium* and *Adlercreutzia*), lactic acid bacteria (*Lactobacillus* and *Streptococcus*), and *Verrucomicrobia* (*Akkermansia muciniphila*). A saturated lipid diet promotes local intestinal immunity through its effects on toll-like receptor (TLR) expression.

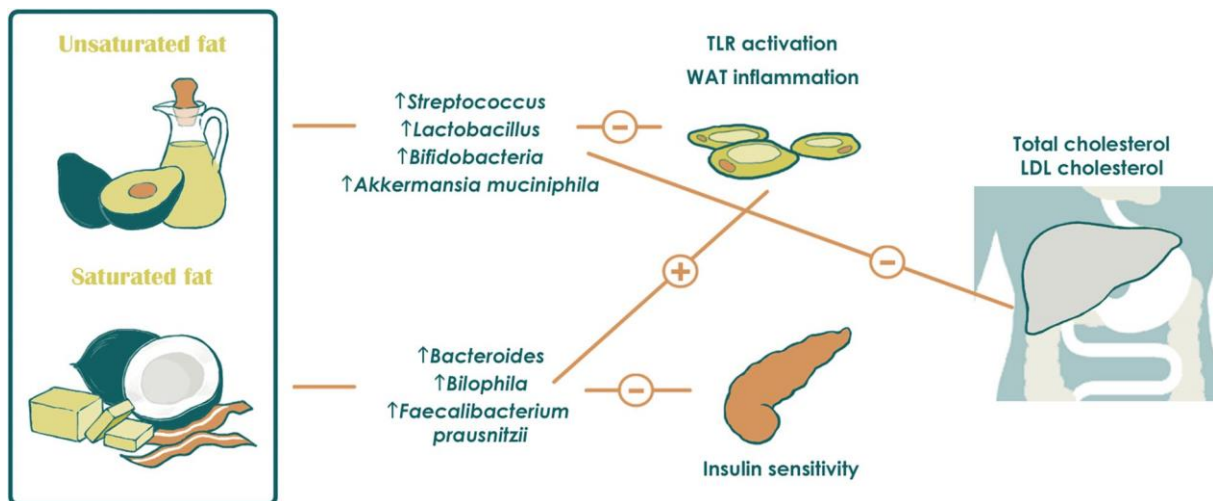


Figure 3. Impact of dietary fats on intestinal microbiota and host metabolism. TLR: toll-like receptor, WAT: white adipose tissue, LDL: low-density lipoprotein
(© Singh/BMC, source: Singh et al., 2017)

6.4.3 Carbohydrates

A distinction is made between digestible carbohydrates (starch, sugars) and non-digestible carbohydrates (fibre). Digestible carbohydrates are enzymatically degraded in the small intestine, while non-digestible carbohydrates are fermented in the large intestine by microorganisms. Sugars like glucose, lactose, fructose and sucrose increase the relative abundance of *Bifidobacteria*, and reduce the number of *Bacteroides*. Lactose is also decreasing *Clostridium* species. The opposite effect is seen in a mouse study that used artificial sweetener saccharin. This suggests that artificial sweeteners may actually be healthier to consume than natural sugars.

Non-digestible carbohydrates when not sufficiently present in the diet reduce total bacterial abundance. Addition of non-digestible carbohydrate as in whole grain and wheat bran induces an increase in gut *Bifidobacteria* and *Lactobacilli*. Resistant starch and whole grain barley, appear to also increase abundance of *Ruminococcus*, *Eubacterium rectale*, and *Roseburia*. Fructooligosaccharides, polydextrose and arabinooligosaccharides are shown to reduce *Clostridium* and *Enterococcus* species. The property of these fibres to induce shifts in the microbiome provides their additional designation as prebiotics. Prebiotics also induce shifts in immune markers: reductions in the pro-inflammatory cytokine IL-6 and anti-inflammatory cytokine IL-10. Also, metabolites change: reduction in serum triglycerides, total cholesterol, low-density lipoprotein (LDL)-cholesterol, and haemoglobin A1c.

6.4.4 Polyphenols

Polyphenols are found in fruits, seeds, vegetables, tea, cocoa products, and wine. Consumption of these foods increases *Bifidobacterium* and *Lactobacillus*, and for wine in particular, relative abundance of *Bacteroides* is observed, and reduction of the numbers of *Clostridium perfringens* and *Clostridium histolyticum*. Fruit polyphenols work against the enteropathogens *Staphylococcus aureus* and *Salmonella typhimurium*. Cocoa-derived polyphenols significantly increase plasma high-density lipoproteins and significantly reduce plasma triacylglycerol and C-reactive protein concentrations.

Singh et al. also investigated the impact of Western, gluten-free, omnivore, vegetarian, vegan, and Mediterranean diets (Singh et al., 2017).

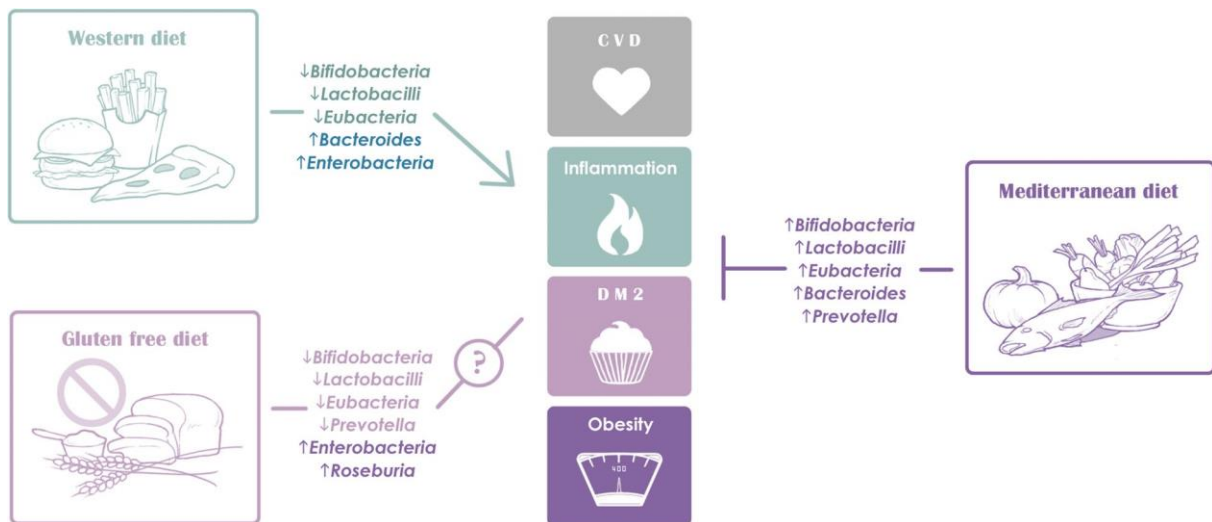


Figure 4. Impact of popular diets on intestinal microbiota and cardiometabolic disease. CVD cardiovascular disease, DM2 type 2 diabetes mellitus
 (© Singh/BMC, source: Singh et al., 2017)

Studies reveal that Western diet (high in animal protein and fat, low in fibre) leads to a marked decrease in total bacteria counts and beneficial *Bifidobacterium* spp. and *Eubacterium* spp. Gluten-free diets allow for the proliferation of *E. coli* and total *Enterobacteriaceae*, which may include further opportunistic pathogens, and *Victivallaceae* and *Clostridiaceae*. Furthermore, it decreases the number of beneficial *Bifidobacterium*, *Lactobacillus*, *Ruminococcus bromii* and *Roseburia faecis*. For vegan and vegetarian diets study results are not consistent due to differences in methods of analysis, reference diets and host genetics. In reviewing studies that compared vegetarians to omnivores. Graf et al. came to the same conclusion (Graf et al., 2015). Apart from the study by De Filippis et al. mentioned above, other studies described the impact of Mediterranean diet as improving obesity, the lipid profile, and inflammation. Diet-derived increases in *Lactobacillus*, *Bifidobacterium*, and *Prevotella*, and decreases in *Clostridium* may be the cause.

6.5 Prebiotics

A prebiotic is "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health" (de Vrese and Schrezenmeir, 2008). Food delivering prebiotics are soybean, chicory roots, raw oats, unrefined wheat, unrefined barley etc. Dietary fibre includes carbohydrates such as cellulose, lignin, and non-starch polysaccharides (NSP) such as hemicelluloses. Prebiotic oligosaccharides comprise fructo-oligosaccharide (FOS), galacto-oligosaccharides (GOS) and xyloseoligosaccharide (XOS) inulin and pectin. They are not digested in the small intestine but are fermented in the large intestine by anaerobic colonic microbiota to SCFAs.

A selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health

Prebiotics confer benefits to the host including enhancement in gut mucosal barrier integrity and function, increased host mucosal immunity, increased SCFA production and an associated reduction in mucosal interaction of opportunistic enteric pathogens (Simpson and Campbell, 2015).

Insoluble non-digestible substrates are difficult to break down. Only a few species are able to degrade them and provide other species with soluble breakdown products (Flint

et al., 2017). Absence of these primary degrading species means that some substrates remain integral with an effect on subsequent degrading species. In rural agrarian societies, a high level of faecal SCFA is seen whereas higher consumption of fermentable substrate in vegans did not result in such an increase in a dietary intervention in a US population (Wu et al., 2016). This may be due to the absence of the primary degraders whose activities are required to initiate degradation of these recalcitrant substrates. In this way inter-individual differences in gut microbiota composition before a dietary intervention can affect responses to dietary change.

Rat studies with feed supplements with short-chain oligofructose, long-chain inulin, or with diets including inulin or arabinoxylan had a variable bifidogenic effect, and, lower total SCFA concentrations with caecal pH also significantly decreased compared to the control (Simpson and Campbell, 2015).

Studies with resistant starch that escapes digestion in the small intestine revealed that *R. bromii* and *E. rectale* increased (Martínez et al., 2010; Walker et al., 2011). However, the specific effects depend largely on the type of resistant starch both in animal and in human studies (Simpson and Campbell, 2015). Inulin, another dietary fibre induced an increase in the numbers of *Bifidobacterium*, *Lactobacillus/Enterococcus* and the *Atopobium* group in one study but in another study no effect was recorded, probably due to a different inulin source and the mixing with other fibres (Costabile et al., 2010; Linetzky Waitzberg et al., 2012). *Bifidobacterium* enrichment was confirmed in yet other studies using inulin as a prebiotic together with an increase in *Faecalibacterium prausnitzii* or a reduction of *Prevotella* (Simpson and Campbell, 2015). Oligosaccharides increase the number of faecal bifidobacteria (Benus et al., 2010; Cloetens et al., 2010; Vulevic et al., 2013; Walton et al., 2012). The levels of the *Faecalibacterium prausnitzii* group and the *Roseburia intestinalis* group were reduced using (FOS) (Benus et al., 2010). GOS diminish the number of *Bacteroides* spp. and *Clostridium histolyticum* group of bacteria (Vulevic et al., 2013). Intake of polydextrose or soluble corn fibre resulted in a higher concentration of *Clostridiaceae* and *Veillonellaceae* and lower quantity of *Eubacteriaceae* compared with the control (Hooda et al., 2012). The number of *Faecalibacterium prausnitzii*, a butyrate producer known for its anti-inflammatory properties, was also elevated after fibre consumption. Another study with polydextrose reported an increase of *Ruminococcus intestinalis*, also a butyrate producer, and *Clostridium* clusters I, II, and IV, while there was a decrease of *Lactobacillus/Enterococcus* (Costabile et al., 2012). The impact of resistant maltodextrin was not consistent (Baer et al., 2014). Consumption of arabinoxylan-oligosaccharides-enriched breads led to increased faecal butyrate (Walton et al., 2012) and elevated *Lactobacilli* levels (Cloetens et al., 2010; Walton et al., 2012).

Non-starch polysaccharides can also inhibit the adherence of a range of different enteric gut pathogens including *Salmonella* spp., *Shigella* spp., enterotoxigenic *E. coli* and *C. difficile* (Simpson and Campbell, 2015).

6.6 Probiotics

Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit to the host (Guarner and Schaafsma, 1998). Often used probiotic microorganisms are *Lactobacillus rhamnosus*, *Lactobacillus reuteri*, *Bifidobacteria* and certain strains of *Lactobacillus casei*, *Lactobacillus acidophilus*-group, *Bacillus coagulans*, *Escherichia coli* strain Nissle 1917, certain enterococci, especially *Enterococcus faecium* SF68, and the yeast *Saccharomyces boulardii* (Pandey et al., 2015). Probiotic effects are very strain specific and cannot be generalised.

Live microorganisms which when administered in adequate amounts confer a health benefit to the host.

Fermented foods such as fermented milk or yoghurt contain lactic acid bacteria. Several groups have reported increased total bacterial load after regular consumption of fermented milk or yoghurt (Singh et al., 2017). Especially *Bifidobacteria* and/or

Lactobacilli have been seen to increase. The effect of probiotic VSL#3 consisting of three strains of *Bifidobacterium*, four strains of *Lactobacillus*, and one strain of *Streptococcus* in a trial with overweight healthy adults, was an increase in total aerobes; anaerobes *Lactobacillus*, *Bifidobacteria*, and *Streptococcus* compared to placebo. These subjects also had fewer total coliforms and *Escherichia coli*, as well as a reduced triglycerides, total cholesterol, LDL-cholesterol, very low-density lipoprotein (VLDL)-cholesterol, and high-sensitivity C-reactive protein (Rajkumar et al., 2014). High-density lipoprotein (HDL)-cholesterol and insulin sensitivity improved. In another study enteropathogens *E. coli* and *Helicobacter pylori* were reduced after *Helicobacter*-infected children consumed probiotic-containing yoghurt (Yang and Sheu, 2012).

Most probiotics do not colonise the host's gut. Therefore, continuous consumption often is necessary to achieve lasting effects.

6.7 Synbiotics

Synergistic combinations of pro- and prebiotics are called synbiotics (de Vrese and Schrezenmeir, 2008). The term is especially reserved for products in which the prebiotic compound(s) selectively favours the probiotic organism(s).

6.8 Microbiota influencing host appetite

The gut microbiota not only is influenced by the food, they themselves affect the host in his eating behaviour. The bidirectional communication pathway between the gastrointestinal tract microorganisms and the brain is called the microbiota-gut-brain axis.

The microbiota-gut-brain axis:

gut microbiota is influenced by the food and affects the host in his eating behaviour.

Signalling pathways may be neural, endocrine and/or immune pathways (Temko et al., 2017). Microbial-derived metabolites can activate these pathways. They signal from the gut to the brain and may impact the brain. Neural signalling from the brain to the gut can influence gut function and change the composition and function of the gut microbiota.

The gut microbiota has a key regulatory role in appetite (van de Wouw et al., 2017). Bacterial components and metabolites are able to influence intestinal satiety pathways, thus controlling host appetite and satiety. The main actors are the SCFAs acetate, propionate and butyrate. The signalling goes via the vagus nerve that connects the digestive tract directly with the brain. However, much is still not clear: obesity is associated with high levels of SCFAs, while supplementation with SCFAs tends to decrease acute food intake. Also, some gut microbes may produce short protein sequences that share a sequence that is identical to various appetite-regulating peptides (molecular mimicry).

The gut microbiota can alter host nutrient and taste receptors and therefore taste signalling, thereby influencing the host to eat specific nutrients (Alcock et al., 2014). As a result, the microbiota's preferred food substrates increase and thereby survival. It is hypothesised that this host-bacteria relation has evolved so as to enhance the individual bacteria's own survival or hinder that of competitive gut bacteria. Another pathway is through microbes releasing toxins due to low concentration of growth-limiting nutrients. These toxins induce dysphoria leading to increased eating.

Temko et al. performed a systematic review on the influence of gut microbiota in eating disorders and alcohol and substance use disorders (Temko et al., 2017). Eight of the reviewed studies dealt with eating disorders. The authors concluded that the studies support preliminary evidence for the role of the gut microbiota in these disorders, but more is needed to determine causativeness.

7 Effects of/on health and well-being

Dysbiosis -imbalances or alterations in microbial composition or activity- can influence health and is implicated in various diseases, such as obesity, type 2 diabetes, asthma, allergies and inflammatory bowel disease.

The microbiota produces signalling molecules and metabolites that influence several intestinal functions and various organs.

Inflammatory bowel disease is clearly associated with intestinal dysbiosis, with reduction in biodiversity as well as decreased representation of several specific taxa.

Data suggest that for type 1 diabetes mellitus, intestinal microbiota might be involved in the progression to clinical disease, not initiating the disease process. Several models, e.g. the Leaky Gut Hypothesis, the Old Friends Hypothesis, the Perfect Storm Hypothesis and the Hygiene Hypothesis link the gut microbiome with the development of type 1 diabetes.

The gut microbiota has a key role in the regulation of different metabolic pathways that are important in glucose homeostasis and type 2 diabetes pathogenesis.

Studies support the link between the microbiota and the onset of Coeliac disease, a complex multifactorial chronic immune-mediated enteropathy, triggered by the ingestion of gluten in genetically susceptible individuals.

Intestinal microbiota takes part in the development of obesity and subsequent insulin resistance.

Gut microbiota seems to be one of the factors involved in fatty liver diseases associated with alcohol, obesity, and the metabolic syndrome.

While the microbiome can influence cardiovascular diseases indirectly via its effect on type 2 diabetes and obesity, speculations about a more direct involvement via the metabolism of choline is still under debate.

Certain bacteria promote carcinogenesis directly by secreting substances that lead to DNA damage, whereas others promote carcinogenesis indirectly by maintaining a persistent pro-inflammatory microenvironment.

The most relevant function of the gut microbiome to autoimmunity is maintenance of the immune system involving SCFAs, secondary bile salts, and trimethylamines.

The development of allergies later in life is related to the development of the immune system in early life. The factors involved determine the composition of the intestinal microbiota that in turn modulates the immune system response.

Studies in animals suggest a role for gut microbiota in Alzheimer's disease-related pathogenesis. In general, the gut-microbiota-brain axis is instrumental for human and animal well-being.

Exercise leads to an increase in microbiota diversity. Exercise early in life, when the composition of the microbiota is still evolving, may positively influence this evolution and may create lasting adaptations in lean mass and psychological well-being.

Imbalances or alterations in microbial composition or activity – dysbiosis – can influence health and is implicated in various diseases. The factors that can disturb the balance of intestinal microbiota include: lifestyle, antibiotic treatments and pathogens. Diseases such as obesity, type 2 diabetes, asthma, allergies and inflammatory bowel disease (IBD), the so-called “diseases of civilisation”, have been associated with dysbiosis of the gut microbial ecosystem (Rampelli et al., 2016). There are also associations with

inflammatory skin diseases such as psoriasis and atopic dermatitis, autoimmune arthritis, and atherosclerosis.

The microbiota produce signalling molecules and metabolites that influence several intestinal functions: visceral-sensing, motility, digestion, permeability secretion, energy harvest, mucosal immunity, and barrier effect (Iebba et al., 2016). These products are also transported to various organs affecting their functionality: brain (cognitive functions), liver (lipid and drug metabolism), and pancreas (glucose metabolism). A gut microbiota in an eubiotic status is characterised by a preponderance of potentially beneficial species, belonging mainly to the two bacterial phylum Firmicutes and *Bacteroides*, while potentially pathogenic species, such as those belonging to the phylum Proteobacteria (*Enterobacteriaceae*) are present, but in very low relative abundance. In the case of dysbiosis this balance is disturbed. Dysbiosis induces an immune reaction from the host thereby promoting the dysbiosis status. Inflammation releases components in the gut that represent a growth advantage for potentially pathogenic species, such as the members of the *Enterobacteriaceae* family, in particular *E. coli*. The relative abundance of the obligate anaerobe *Faecalibacterium prausnitzii*, a butyrate producer defined as an anti-inflammatory bacterium, is reported to be significantly reduced. The ratio of the relative abundances of *F. prausnitzii* / *E. coli* is currently used to evaluate the dysbiosis status.

Besides being an energy source, SCFAs can act as signalling molecules (Dolan and Chang, 2017; Koh et al., 2016). Butyrate and, to a lesser extent, propionate are known to act as inhibitors of histone deacetylases that interfere with chromatin structures and gene expression. Butyrate protects against colorectal cancer and inflammation, at least partly, by inhibiting histone deacetylases. This inhibiting effect also works anti-inflammatory making the immune system hypo-responsive to beneficial commensals. SCFAs also regulate cytokine expression in T cells (e.g. IL-10) and generation of Tregs through histone deacetylase inhibition.

Acetate and propionate are activators of free fatty acid receptors promoting secretion of glucagon-like peptide 1 (GLP-1) and peptide YY (PYY), affecting satiety and intestinal transit.

Butyrate can also modulate the activity of the enteric nervous system modulating gut motility. Moreover, SCFAs affect the gut-brain neural axis and regulate the permeability of the blood-brain barrier.

The intestinal mucosa and its immune system maintain a status of tolerance to the antigenic stimuli of normal bacterial flora, but intolerance to pathogenic microorganisms (Lopetuso et al., 2016). Antigens are continuously presented to the mucosal effector cells that react through specific receptors, the pattern recognition receptors. Mucosal injury leads to inflammation. Intestinal epithelial cells react to repair the damage, a process regulated by cytokines. Several factors among which SCFAs and also gut microbiota, through the activation of TLRs, regulate intestinal epithelial cells' proliferation.

There is also an indication that gut microbiota may promote metabolic inflammation through TLR signalling upon challenge with a diet rich in saturated lipids (Caesar et al., 2015).

Communication between the liver and the intestine is facilitated by bile acids (Betrapally et al., 2016; Dolan and Chang, 2017). Bile acids are formed in the liver from cholesterol to facilitate digestion of fats. Bile acids are further transformed in the intestine by bacteria. They furthermore act as ligands for receptors that include nuclear receptor Farnesoid X receptor (FXR) and G protein-coupled bile acid receptor 1 (GPBAR1). FXR functions in a negative feedback pathway in which synthesis of bile acids is inhibited when cellular levels are already high. GPBAR1 regulates bile acid homeostasis, glucose homeostasis, energy metabolism as well as inflammation. The bile acid composition in the intestine determines the microbiota composition.

7.1 Inflammatory bowel disease

Inflammatory bowel disease (IBD) represents a heterogeneous group of chronic immune-mediated inflammatory diseases affecting the gastrointestinal tract (Lane et al., 2017). There are two major types: ulcerative colitis (UC) and Crohn's disease (CD).

IBD is a disease arising from both genetic and environmental factors (diet, smoking, stress, sleep patterns, hygiene, and antibiotic use) with the host genome potentially having a pivotal role in shaping the gut microbiota (Parekh et al., 2015). Research more and more demonstrates that the interaction between diet and microbes in a susceptible person contributes significantly to the onset of the disease (Dolan and Chang, 2017). IBD patients are thought to have a compromised mucus layer in the intestine, thus allowing luminal microflora to penetrate intraepithelial cells and drive inflammatory and proliferative processes. IBD is clearly associated with intestinal dysbiosis, with reduction in biodiversity as well as decreased representation of several specific taxa, including Firmicutes and Bacteroidetes (Lane et al., 2017; Lopetuso et al., 2016). A relative increase in the abundance of *Enterobacteriaceae*, including *E. coli* and *Fusobacterium* is noted. The presence in the mucus layer of *Pasteurellaceae* (*Haemophilus* sp.), *Veillonella parvula*, *Neisseriaceae corrodens*, and *Fusobacteriaceae nucleatum* positively correlates with the diagnosis of CD (Parekh et al., 2015). Also, fungal and yeast communities have increased diversity in CD including *Saccharomyces cerevisiae*, *Calvispora lusitaniae*, *Cyberlindnera jadinii*, *Candida albicans*, and *Kluyveromyces marxianus* (Lane et al., 2017; Lopetuso et al., 2016). In contrast, fungal biodiversity is reduced UC (Gilca et al., 2017).

In a meta-analysis, exposure to antibiotics during childhood reorganising the microbiota composition, was shown to be associated with increased risk of CD but not UC (Ungaro et al., 2014).

Microbiota is able to sustain mucosal healing and regeneration through various mechanisms (Lopetuso et al., 2016). An alteration in microflora composition, as in IBD, can sustain intestinal damage. In new-onset CD, the degree of dysbiosis is greater in ileal or rectal mucosal biopsies than in stool (Dolan and Chang, 2017; DuPont, 2014). The contact of mucosal bacteria with host tissues allows to regulate local immunity. If this balance is disrupted also the immune response is changed. Also eukaryotes like *Saccharomyces* spp. have a regulatory effect on dendritic cells, modulating various anti-inflammatory cytokine production in this way influencing IBD (Gilca et al., 2017).

The SCFAs acetate, butyrate and propionate are pivotal in several host physiological aspects such as nutrient acquisition, immune function, cell signalling, proliferation control and pathogen protection. SCFA levels are considered anti-inflammatory and important for maintenance of the mucosal barrier. Butyrate has a positive effect on cell proliferation, differentiation and maturation after epithelial injury (Lopetuso et al., 2016). Several studies have demonstrated that IBD patients possess lower faecal counts of *Roseburia* and other butyrate-producing bacteria than healthy subjects (Dolan and Chang, 2017; Lopetuso et al., 2016). Another butyrate producer *Faecalibacterium prausnitzii* is dramatically less abundant in CD patients (Dolan and Chang, 2017; DuPont, 2014). Healthy subjects, on the other hand, have 10-fold more abundant *Eubacterium rectale* in their intestines (Singh et al., 2017). A diet high in fruits and vegetables resulting in more SCFAs, reduces the risk of developing CD (Dolan and Chang, 2017; Lane et al., 2017).

Bacterial bile acid metabolism allows for signalling via bile acid receptors, promoting anti-inflammatory signalling and barrier function (Dolan and Chang, 2017). Normally bile acids are first deconjugated by bile salt hydrolase of the microbiota prior to further metabolism. In IBD patients higher concentrations of sulphated and conjugated bile acids are found in their stool than in healthy controls due to a decrease of Firmicutes-associated bile salt hydrolase genes. This results in a loss of anti-inflammatory properties.

Also in irritable bowel syndrome (IBS) the gut microbiota, the bidirectional gut–brain axis and inflammation play a role (DuPont, 2014). Several studies have supported that intestinal infection was strongly associated with a subsequent emergence of symptoms.

7.2 Diabetes

In type 1 diabetes there is a shortage of pancreatic β -cells, that produce insulin, due to autoimmune destruction. Type 2 diabetes is characterised by a low level of insulin receptors and/or insulin resistance due to a defect in the insulin cascade.

The first stages of type 1 diabetes typically develop early in life. The gut microbial community is then shaped influenced by factors such as host genetics, mode of delivery, diet and external factors such as treatment with antibiotics. The gut microbiota on its turn has a role in shaping the immune system early in life. The gut microbiota in individuals with preclinical type 1 diabetes mellitus is characterised by a high level of Bacteroidetes, a lack of butyrate and lactate-producing bacteria, reduced bacterial and functional diversity and low community stability (Knip and Siljander, 2016). Though, it seems that autoantibodies that are predictive of type 1 diabetes mellitus come first. The changes appear afterwards. This suggests that the intestinal microbiota might be involved in the progression to clinical disease, not initiating the disease process itself. The process leading to type 1 diabetes mellitus is often initiated during the first few years of life, when the intestinal microbiota undergoes dynamic development.

Most of the studies that are available only point to a correlation without determining causal relationships between the gut microbiota and preclinical or clinical Type 1 diabetes mellitus (Endesfelder et al., 2016; Knip and Siljander, 2016). Many studies rely on mice and rats that develop autoimmune diabetes mellitus after exposure to certain chemicals or viruses (Knip and Siljander, 2016). In humans, a Finnish study with children reported a shortage of the two most abundant *Bifidobacterium* species (*B. adolescentis* in the elder children and *B. pseudocatenalatum* in the younger children) and an increased abundance of *Bacteroides* compared with the controls. However, a German study with children between 3 months and 3 years did not see any differences, whereas a Finnish study examining the stools of children in the same age group did see an increase in *Bacteroides* levels. Other studies point to a similar evolution in bacterial presence. Also, a lower number in butyrate-producing bacteria and mucin-degrading species next to a drop in diversity are reported (Endesfelder et al., 2016).

Several models have been proposed linking the gut microbiome with the development of type 1 diabetes (Endesfelder et al., 2016). According to the Leaky Gut Hypothesis, increased permeability of the gut epithelium results in diet-derived macro-molecules and microbial antigens passing the epithelial barrier and consequently triggering intestinal inflammation possibly leading to pancreatic β -cell attack. A decreased number of butyrate-producing bacteria may be the cause. The Old Friends Hypothesis builds on the co-evolution of host and commensals. A lack of encounter with co-evolved commensal bacteria might substantially influence self/non-self-recognition patterns in the immune system. The Perfect Storm Hypothesis combines both models. The Hygiene Hypotheses claims that increasing type 1 diabetes incidences being observed in Western societies result from a lack of contact with infectious agents due to increased hygienic conditions. Lack of pathogenic encounter in early childhood, disrupts proper priming of the immune system, possibly resulting in over-reaction leading to autoimmunity. A shift in the butyrate production may be the key driver induced by diet, drug treatment, mode of delivery, etc.

The gut microbiota has a key role in the regulation of different metabolic pathways that are important in glucose homeostasis and type 2 diabetes pathogenesis (Muscogiuri et al., 2016). Several studies in mice have shown that diabetic obese mice showed a higher abundance of Firmicutes, Proteobacteria, and Fibrobacteres phyla compared to lean mice. Some probiotic strains (*Lactobacillus* or *Bifidobacterium*) are able to modulate the glucose homeostasis. This is also seen in humans. Also, a low percentage of bacterial

Clostridia species that are butyrate producing bacteria, was noticed in type 2 diabetes humans. Butyrate produced by certain bacteria prevents translocation of endotoxic compounds derived from the gut microbiota, which have been shown to drive insulin resistance.

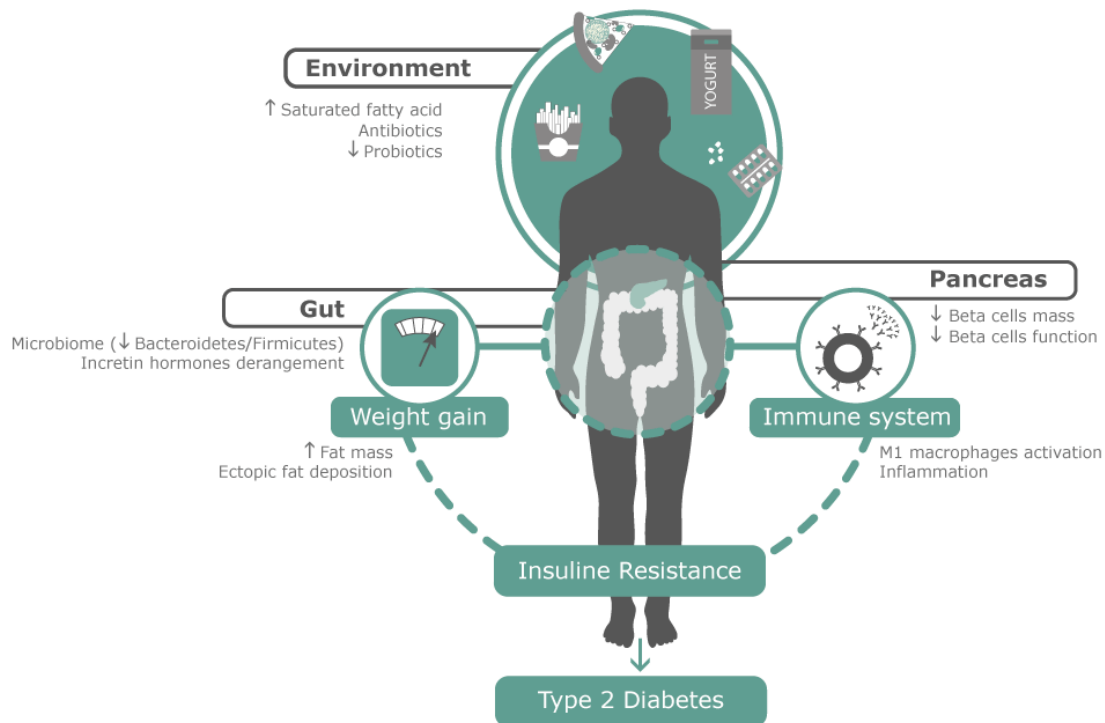


Figure 5. The gut microbiota plays an important role in the onset of type 2 diabetes (© Muscogiuri / Taylor & Francis, source: Muscogiuri et al., 2016)

The incretin hormones GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) stimulate insulin secretion and regulate postprandial glucose excursions, whereas GLP-1, cholecystikinin (CCK), and PYY inhibit appetite and food intake (Muscogiuri et al., 2016). The incretin hormones are required to maintain an adequate β -cell mass in adulthood and to maintain normal β -cell responses to glucose. In patients with type 2 diabetes this hormone action is reduced. Gut bacteria normally stimulate the production of these hormones.

7.3 Coeliac disease

Coeliac disease is a complex multifactorial chronic immune-mediated enteropathy, triggered by the ingestion of gluten in genetically susceptible individuals (Cenit et al., 2016). The majority of genetically susceptible individuals does not develop disease upon gluten exposure indicating that other factors play a role too. Studies support the link between the microbiota and the disease onset. Environmental factors may shape the composition of the microbiota, especially in early life: gestation mode, feeding pattern, infections, antibiotics and others. Dysbiosis in coeliac disease means an increase in gram-negative and Bacteroidetes species, and a decrease in *Bifidobacteria* and *Lactobacilli* (Losurdo et al., 2016). An unfavourable microbiota could amplify the immune response to gliadin. The probiotic *Bifidobacterium longum* was able to decrease this effect. *Bifidobacterium* strains are able to reduce the mucosal production of pro-inflammatory cytokines, notably tumour necrosis factor- α and IL-10.

However, the precise gut microbiota alterations that may precede disease onset are not known.

7.4 Obesity

Obesity results from the accumulation of excess adipose tissue. Causes include behavioural and environmental factors, such as excessive consumption of energy-dense foods and a sedentary lifestyle. But also intestinal microbiota turned out to take part in the development of obesity and subsequent insulin resistance (Villanueva-Millan et al., 2015).

Gut microbes ferment dietary polysaccharides resulting in the production of monosaccharides and SCFAs, that are absorbed and act as an energy source for the host. Microbiota from obese individuals has an increased capacity to harvest energy from the diet. In the obese population an increase in fermentation by the gut microbiota is seen. A high ratio of *Bacteroides* to *Prevotella* shows a decrease in SCFAs. A high ratio of Firmicutes to *Bacteroides/Prevotella* as in obese individuals enriches the microbial genes involved in polysaccharide degradation and increases the SCFA levels (Cardinelli et al., 2015; Parekh et al., 2015). The increase in Firmicutes is mainly the result of an increased abundance of *Clostridium* cluster XIVa, which contains many butyrate-producing species such as *Faecalibacterium prausnitzii* (Villanueva-Millan et al., 2015). Other studies found no alteration or even an increase in Bacteroidetes compared to lean persons.

While these are contradicting results, the lower species diversity and the presence of more aerotolerant bacteria in obese persons have been clearly demonstrated (Villanueva-Millan et al., 2015). Aerotolerant bacteria generate products that are easily converted to SCFAs. While there is no consensus on the specific pattern, alterations in gut bacteria are definitely involved in obesity.

Microbiota also influences the host's lipid metabolism through various mechanisms (Cardinelli et al., 2015). The microbiota can induce lipogenesis. Also, microbiota decreases expenditure of energy by decreasing fatty acid oxidation which, in turn, favours lipid deposition and storage in adipose tissue, liver, and/or muscle.

Butyrate, propionate, and acetate also regulate gut hormones. Through their specific free fatty acid receptors SCFAs regulate satiety and intestinal motility. Gut microbiota after each meal stimulate intestinal L-cells to excrete GLP and PYY that regulate satiety. PYY levels are negatively correlated with the tendency to obesity. GLP-1 stimulates insulin secretion from β -cells of the islets of Langerhans.

Obesity is accompanied by a low-grade inflammatory response. TLRs are a type of pattern recognition receptor for microbe-associated molecular patterns seen on bacteria, viruses, and fungi (Parekh et al., 2015). They recognise microorganisms as pathogenic or non-pathogenic. Normally gut microbiota induces anti-inflammatory effects that protect epithelial cells against pathogens via TLRs. TLR4 interacts with lipopolysaccharides (LPS, a determinant of Gram-negative bacteria cells, that normally circulate at low concentrations in the blood). This interaction plays a role in inflammation following a high fat diet by disrupting the intestinal epithelium and barrier (Villanueva-Millan et al., 2015). Once LPS is in the blood it can induce cellular inflammatory responses in several tissues/organs. The interaction between gut microbiota and TLR-5 results in the induction of inflammatory cascade and downstream transcription of various cytokines and inflammatory mediators resulting in a low-grade inflammatory state associated with obesity (Parekh et al., 2015). Other molecules (TLR2, myeloid differentiation primary response gene 88, nuclear oligodimerisation receptor) are also being studied (Villanueva-Millan et al., 2015).

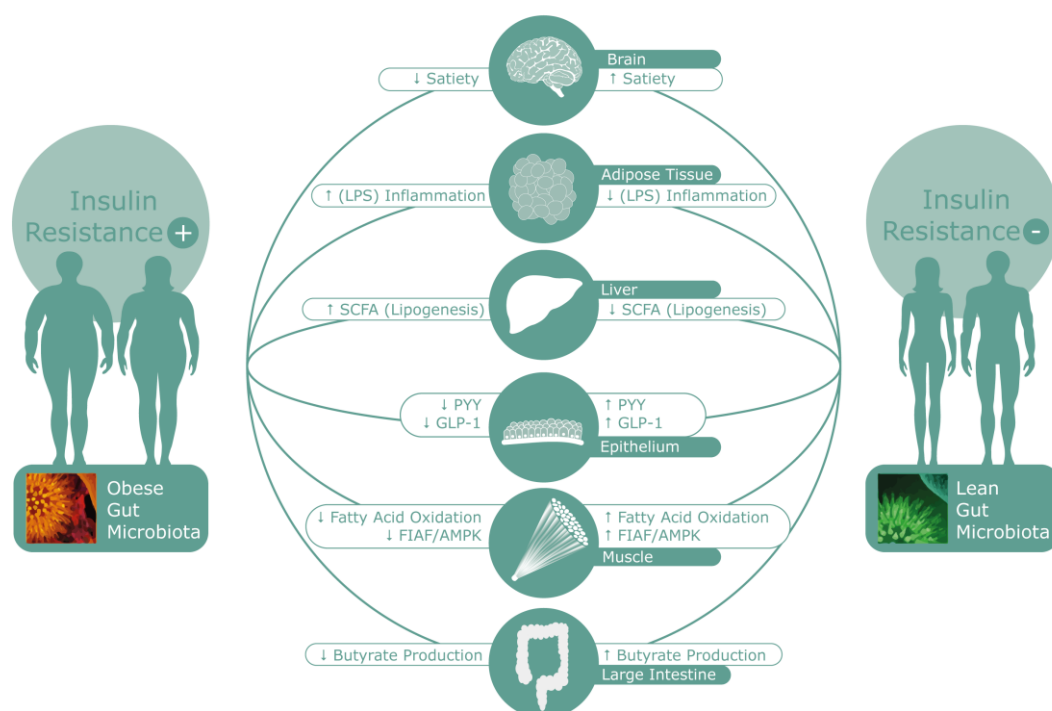


Figure 6. Gut microbiota and its influence on obesity
 (© Parekh / Springer Nature, source: Parekh et al., 2015)

7.5 Liver disease

Fatty liver diseases are associated with alcohol, obesity, and the metabolic syndrome. Diet and lifestyle together with the gut microbiota are involved, but the mechanisms of pathogenesis are not yet elucidated. Most knowledge is derived from animal experiments.

Non-alcoholic fatty liver disease (NAFLD) is characterised by fat accumulation, mainly as triglycerides, in the hepatocytes. It can progress to non-alcoholic steatohepatitis (NASH), liver cirrhosis, and hepatocellular carcinoma. The underlying pathogenesis of NAFLD and NASH is not clear, but alterations in gut microbiota are thought to be a major contributor to its development (Betrapally et al., 2016; Leung et al., 2016; Parekh et al., 2015). Studies show there is a significant increase in faecal volatile organic compounds, that affect the liver. Some studies report disproportionately low levels of bacteria from the *Ruminococcaceae* family (Firmicutes) and high levels of *Escherichia*, while others report lower levels of *Bacteroides* and high levels of Firmicutes (e.g. *Clostridium coccoides*).

Several mechanisms may lead to NAFLD/NASH (Figure 7). A higher amount of SCFA, as in obese, due to an increase in the Firmicutes to Bacteroidetes ratio, leads to a higher energy harvest inducing lipogenesis and gluconeogenesis in the liver. Dysbiosis also reduces butyrate production in favour of other SCFAs. Less butyrate decreases fasting-induced adipocyte factor (FIAF) secretion from intestinal cells, leading to activation of lipoprotein lipase (LPL) and subsequent triglyceride accumulation in both adipose tissue and the liver. LPS, an endotoxin found on the cell membrane of Gram-negative bacteria, binds to LPS-binding protein and CD14 and then activates TLR-4. TLR-4 in turn initiates a pro-inflammatory cascade. Bile acids suppress overgrowth of bacteria in the gut. A low level may result in small intestinal bacterial overgrowth (SIBO) that induces alterations of gut permeability and is also associated with NAFLD/NASH. Patients with NASH have an increased abundance of ethanol-producing bacteria such as *Escherichia coli* in their gut. Ethanol might contribute to liver injury by increasing intestinal permeability and portal LPS levels. Another mechanism is the catalysis of choline, a phospholipid component of

the cell membrane, by the gut microbiota into toxic methylamines. Hepatic uptake of these toxic metabolites results in the induction of the inflammatory cascade.

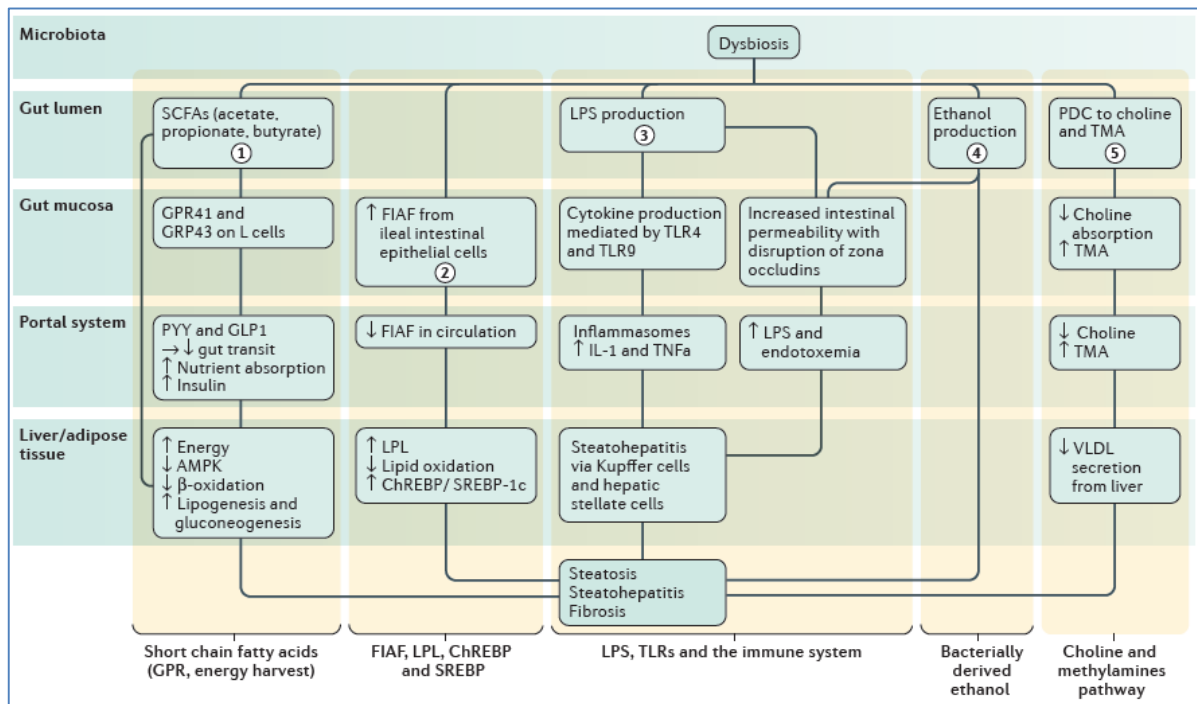


Figure 7. Gut microbiota and its influence on non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) proposed mechanisms. (© Leung / Springer Nature, source: Leung et al., 2016).

Alcohol-induced dysbiosis could lead to alcoholic liver disease (ALD) (Pevsner-Fischer et al., 2016). Excessive alcohol intake leads to an overgrowth of Gram-negative bacteria, that cause increased gut permeability, in turn leading to increased availability of bacterial metabolites to the liver, as well as pro-inflammatory molecules such as bacterial toxins, LPS and even living microbes. Alcohol also affects the composition of bile acids (Betrapally et al., 2016).

7.6 Cardiovascular disease

Traditional risk factors leading towards the development of cardiovascular disease (CVD), are mainly type 2 diabetes and obesity (Garcia-Rios et al., 2017). The role of microbiota in these conditions has been described above.

The metabolism of choline into trimethylamine (TMA) and TMAO by the gut microbiota also plays a role here (Griffin et al., 2015; Tuohy et al., 2014). L-carnitine derived from red meat may also be transformed into TMA and TMAO by microbes. TMAO also impacts on bile acid metabolism in the liver at multiple levels including cholesterol transporters and suppression of bile acid synthetic enzymes. TMAO seems to be correlated with subsequent CVD occurrence, though a causative relation is still under debate.

7.7 Cancer

Certain bacteria promote carcinogenesis directly by secreting substances that lead to DNA damage (Hold, 2016; Lv et al., 2017). Examples are *Helicobacter hepaticus*, *Enterococcus faecalis*, and *Bacteroides fragilis*. Other bacteria promote carcinogenesis indirectly by maintaining a persistent pro-inflammatory microenvironment. An example is *Fusobacterium nucleatum* that increases the permeability of colonic epithelial cells.

When compared with healthy people, patients with colorectal cancer (CRC) have higher amounts of *Enterococcus*, *Escherichia*, *Klebsiella*, and *Streptococcus*, and lower amounts

of *Rothia* and butyrate-producing bacteria (Lv et al., 2017). People susceptible to CRC have more species that generate secondary bile acids, but fewer that produce butyrate. Especially the bile acids lithocholic and deoxycholic acid can be proinflammatory. Chronic inflammation is a well-established risk factor for CRC. As such, the presence of IBD increases the risk of CRC. SCFAs have a protective role in colonic inflammation through signalling via GPCRs (Hold, 2016; Koh et al., 2016). Butyrate especially functions as a tumour suppressor in colon. Nonetheless, butyrate might have a pro-carcinogenic effect on CRC as demonstrated in different animal models (Hold, 2016).

In the development of CRC genetic alterations drive the progression of normal mucosa to pre-malignant lesions (adenomatous polyps) (Hold, 2016). However, not all adenomatous polyps become cancerous (adenoma-carcinoma sequence). In CRC progression, the involvement of the gut microbiota has been clearly demonstrated in numerous animal studies. Cancer progression is not attributable to specific species but rather to the metabolic functions and/or pathways of the microbiota as a whole.

Also, the liver is influenced by the nutrients, metabolites and also toxins and pathogens derived from the gut via the portal vein. Often chronic infections by hepatitis B virus (HBV) and hepatitis C virus (HCV) and diseases such as ALD and NAFLD lead to hepatocellular carcinoma (HCC). Gut microbiota can both influence the development of these diseases and the transition from these diseases into HCC (Pevsner-Fischer et al., 2016). TLR4 signalling activated by the LPS on Gram-negative bacteria, as in ALD, is crucial in the dedifferentiation of hepatocytes. In obese persons, a high-fat diet induces overgrowth of Gram-positive bacteria that can produce the secondary bile acid deoxycholic acid (DCA). DCA is known to cause DNA damage through the production of reactive oxygen species, as well as to promote liver carcinogenesis.

Dysbiosis and intestine mucosal injuries enhance HCC progression (Lv et al., 2017). Adjusting the gut microbiota may alleviate the symptoms of liver cancer. Also, the oral administration of probiotics protects the mucosa and microbiota homeostasis. They help to prevent inflammatory responses and support the differentiation of immune cells thereby changing the tumour microenvironment and inhibiting the growth of cancer cells. However, the authors ask for more clinical trials to study the exact role of microbiota and probiotics in HCC.

The interaction between the mucosal immune system and gut microbiota is important. In remedying dysbiosis the focus shifts from studying individual enterobacterial roles to considering gut microbiota as "a microbial community effect" (Yamamoto and Matsumoto, 2016).

7.8 Autoimmune disease

The most relevant function of the gut microbiome to autoimmunity is maintenance of the immune system involving SCFAs, secondary bile salts, and trimethylamines (Coit and Sawalha, 2016). SCFAs have an atheroprotective role by signalling through GPCRs (e.g., GPR43, GPR41 and GPR109A), and by inhibiting histone deacetylases (HDACs) and thus permitting gene transcription (Abdollahi-Roodsaz et al., 2016). The level of medium-chain fatty acids (MCFAs) is decreased in the intestinal lumen of patients with psoriasis. Their role is still unknown. Bile acids act via the FXR and the transmembrane GPCR TGR5. Disturbed bile acid metabolism has effects on adiposity, obesity and the metabolic syndrome that are all risk factors for some rheumatic diseases. Lastly, choline metabolites generated by the microbiota potentially have a causative relationship with the pathogenesis of atherosclerosis and cardiovascular disease.

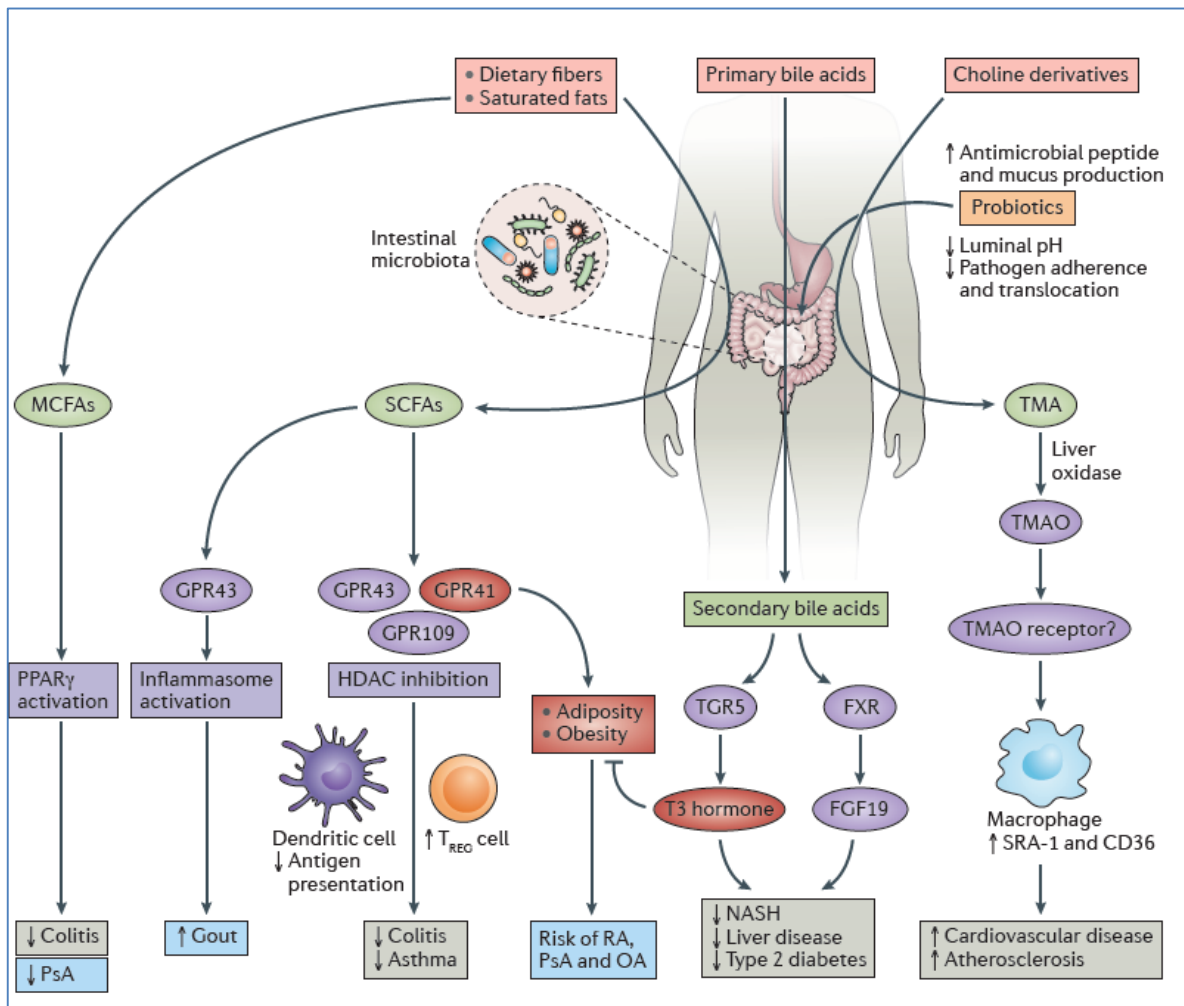


Figure 8. Immune and disease-modulating capabilities of intestinal microbial metabolites and probiotics. (NASH, nonalcoholic steatohepatitis; PPAR γ , peroxisome proliferator-activated receptor γ ; SRA-1, scavenger receptor A-1; TMAO, TMA N-oxide)
 (© Abdollahi-Roodsaz / Springer Nature, source: Abdollahi-Roodsaz et al., 2016)

Abdollahi-Roodsaz and colleagues (2016) provide a view on immune and disease-modulating capabilities of intestinal microbial metabolites and probiotics (Figure 8). The intestinal microbiota converts dietary fibres into SCFAs and MCFAs, primary bile acids into secondary bile acids, and choline derivatives into TMA. SCFAs act through various GPCRs to inhibit HDACs and alter the biology of Treg cells and dendritic cells or to activate the inflammasome. Secondary bile acids activate the transmembrane GPCR TGR5 and the FXR, inducing the T3 thyroid hormone and fibroblast growth factor 19 (FGF19), respectively. These pathways and their end products modulate a variety of inflammatory, metabolic and autoimmune diseases. Probiotics support the host's immune system, enhance intestinal barrier function and limit enteric pathogens. The dark blue colour boxes highlight rheumatic diseases that might be affected by the intestinal microbiota. These diseases include gout, psoriatic arthritis (PsA), rheumatoid arthritis (RA) and osteoarthritis (OA).

7.9 Allergy

Both genetic and environmental factors determine the occurrence of allergic disorders, including asthma, hay fever, and other types of allergic rhinitis, atopic dermatitis, urticaria, and food allergy. The development of allergies later in life is related to the development of the immune system in early life (Rachid and Chatila, 2016; Vuitton and Dalphin, 2017). Factors that are involved include the mode of delivery, number of

siblings, history of infection in mother and child, antibiotic treatments, exposure to pets and indoor allergens, and dietary components such as breast feeding, early food diversification, and regular consumption of fermented foods. These factors determine the composition of the intestinal microbiota that in turn modulate the immune response. *Acinetobacter lwoffii* F78, *Lactococcus lactis* G121, and *Bacillus licheniformis* might be candidates for use in allergy prevention.

In asthma an inadequate immune regulation and/or compromised airway epithelium result in an allergic airway disease. SCFA's modulation of HDACs and GPCR-induced signalling can be important for shaping the immune niche in the lungs (Koh et al., 2016). In the concept of a "common mucosal response" antigen presentation at a single mucosal site stimulates lymphoid cell migration to other mucosal sites, thus influencing the immune responses of remote sites (systemic immunity) (Ipci et al., 2017). A reduced density and diversity of Bacteroidetes, producers of butyrate that help in establishing the immune system in early infancy precedes the development of allergies. At the onset of allergic symptoms, the microbiota of allergic children shows lower counts of *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, and *Clostridium spp.*, a higher prevalence of *Bifidobacterium adolescentis*, lower levels of *Bifidobacterium catenulatum* and *Staphylococcus aureus*; and decreased bacterial diversity overall.

Food allergies coincide with low species diversity, reduced Clostridiales, and increased Bacteroidales (Hirata and Kunisawa, 2017). Not only SCFAs but also long-chain fatty acids (LCFAs) are acting as energy sources as well as in the regulation of immune responses. Omega-3 and omega-6 fatty acids have anti-allergic and anti-inflammatory properties. Commensal bacteria participate in LCFA metabolism. e.g. epithelial barrier function is enhanced by *Lactobacillus*-derived 10-hydroxy-cis-12-octadecenoic acid preventing food allergy. Microbiota also act through the production of essential vitamins. Besides nutritional functions they exercise immunologic functions, especially folate (vitamin B9) and riboflavin (vitamin B2).

Members of the *Clostridium* clusters XIVa, XIVb and IV may be protecting against some food sensitisation (Rachid and Chatila, 2016). The mechanism involves modulating the innate lymphoid cells. A second mechanism targets the adaptive immune response to promote tolerance. The commensal microbiota acts directly on Treg cells through their toll-like receptors and on the β cells.

It is not clear whether *Staphylococcus aureus*, commonly found on the skin of eczema sufferers, is cause or effect of the development of eczema (Marrs and Flohr, 2016). However, here too a diminished diversity of gut microorganisms in early life precedes the onset of eczema, together with greater prevalence of *Clostridium* species.

7.10 Alzheimer's disease

Studies in animals suggest a role for gut microbiota in Alzheimer's disease-related pathogenesis (Jiang et al., 2017). The bidirectional communication system, the microbiota-gut-brain axis, includes neural, immune, endocrine, and metabolic pathways. Dysbiosis increases the permeability of the gut and blood-brain barrier. Bacteria can also secrete large amounts of amyloids and LPS, interfering with signalling pathways and the production of pro-inflammatory cytokines associated with Alzheimer's disease.

7.11 Mental health

Again, the gut-microbiota-brain axis is instrumental for human and animal well-being (Sherwin et al., 2016). Dysfunction of the microbiome-brain-gut axis has been implicated in stress-related disorders such as depression and anxiety and in neurodevelopmental disorders such as autism (Borre et al., 2014). Inflammatory mediators (various cytokines and chemokines) produced by microbes may affect the gut epithelium integrity, infiltrate and induce an immune response (Figure 9). Also, neurotransmitters and SCFAs that have neuroactive properties, are produced by the gut microorganisms. Tryptophan is an essential amino acid which is the precursor of serotonin, kynurenine, and metabolites of

the kynurenine that are neuroactive as well. The gut microbiota may affect the rate of the tryptophan metabolic pathway (Kennedy et al., 2016). Specific bacterial species, can regulate central neurotransmitter levels and receptor expression.

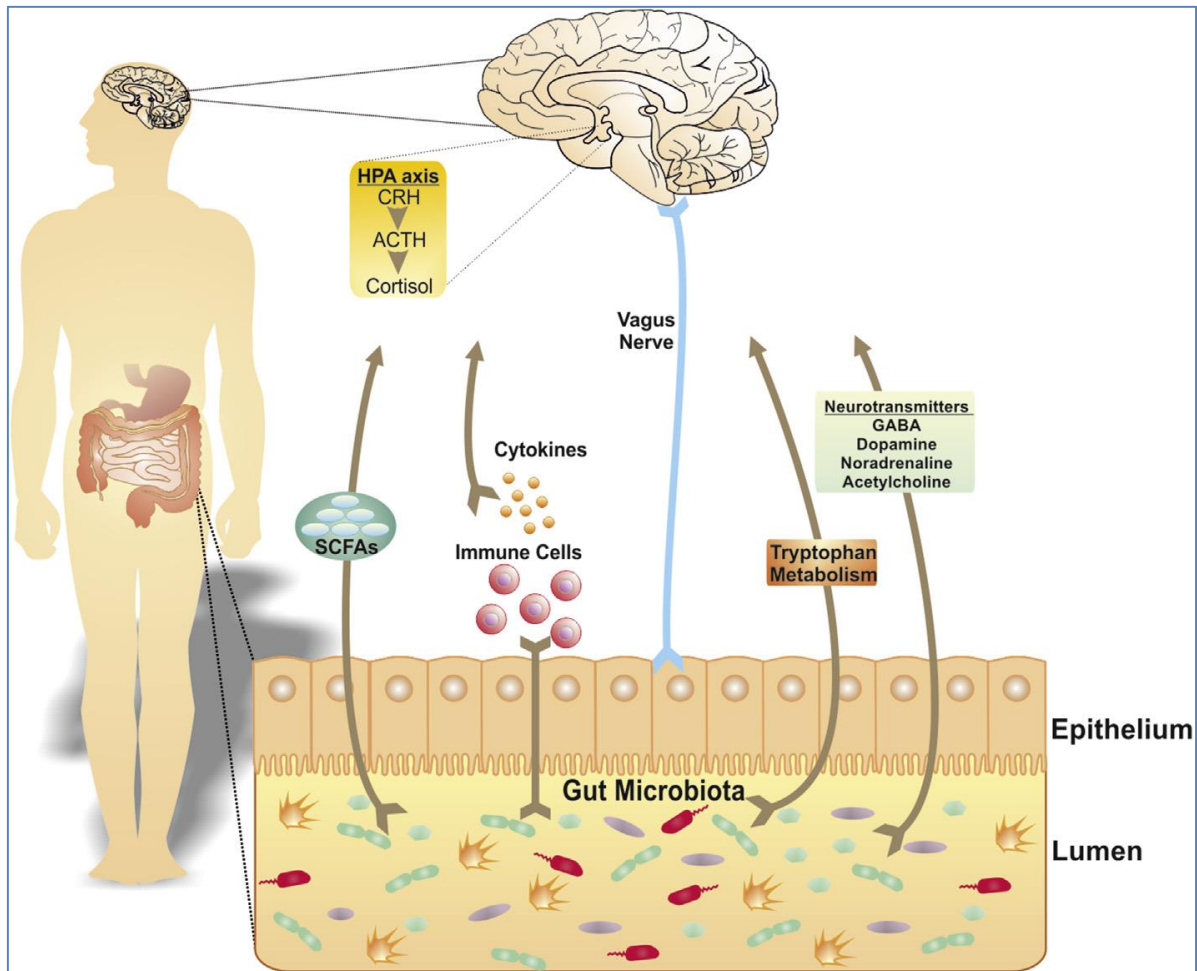


Figure 9. The microbiome-brain-gut axis and its variety of pathways. (ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; GABA, gamma-aminobutyric acid; HPA, hypothalamic-pituitary-adrenal; SCFAs, short chain fatty acids).
 (© Kennedy / Macmillan Publishers Limited, Source: Kennedy et al., 2016)

Psychiatric conditions such as depression and anxiety can be traced back to deficits in serotonergic neurotransmission, alterations in the brain derived neurotrophic factor, immune activation, and dysregulation of the hypothalamic-pituitary-adrenal axis (Sherwin et al., 2016). The gut microbiota regulates all of these biological parameters. In depressed individuals increase in bacterial diversity was shown, with a decrease in the level of Firmicutes and an increase in Proteobacteria, Actinobacteria, and Bacteroidetes. An increased microbial diversity in depression may suggest the presence of harmful bacteria. There are indications that prebiotics and probiotics may be used as antidepressants.

Also in the pathophysiology of autism spectrum disorders gut microbiota may have a role by influencing neurodevelopment, as preclinical and clinical evidence suggests (Kennedy et al., 2016). Cognitive decline during ageing is associated with heightened immune activity and hypothalamic-pituitary-adrenal axis dysfunction, that in turn is related to the changing composition of gut microbiota, as seen above. But cognitive function in general encompassing the life-long process of learning, both long- and short-term processes, is influenced by the intestinal microorganisms (Gareau, 2016).

As consumed food has a central role in programming gut microbiota composition, diversity, and functionality throughout life, a diet rich in polyphenols, omega-3 polyunsaturated fatty acids, prebiotics and probiotics, may help maintain normal brain function and mental health (Kennedy et al., 2016).

7.12 Exercise

Exercise leads to an increase in microbiota diversity. Athletes show lower levels of Bacteroidetes and greater amounts of Firmicutes than non-athletes. However, this effect may also be induced by differences in diet. Studies show that several immune responses are suppressed during prolonged periods of intense exercise training, causing an acute-phase inflammatory response. Also, the permeability of the gastrointestinal epithelial wall increases and the gut mucous thickness decreases leading to pathogens or endotoxins (e.g. LPS) crossing the intestinal barrier into the bloodstream (endotoxemia) triggering immune and inflammatory responses. An adequate gut microbiota composition in athletes and their resulting SCFA metabolites could neutralise these phenomena.

Endurance exercise has a profound impact on oxidative stress, intestinal permeability, muscle damage, systemic inflammation, and immune responses (Mach and Fuster-Botella, 2017) leading to gastrointestinal disturbances, anxiety, depression, and underperformance (Clark and Mach, 2016).

Microbiota has a role in oxidative stress modifying the activity of the antioxidant enzymes thereby reducing exercise-induced fatigue. Gut microorganisms can regulate the hypothalamus-pituitary-adrenal axis that affects the stress response, through the synthesis of hormones and neurotransmitters (Clark and Mach, 2016). They also maintain a proper hydration state during exercise influencing the cellular transport of solutes through the gut mucosa (Mach and Fuster-Botella, 2017). Athletes usually consume high amounts of simple carbohydrates and proteins and low amounts of fat and fibre in order to provide a quick source of energy (Clark and Mach, 2016). Nevertheless, these diets do not promote a healthy gut microbiota composition nor do they produce beneficial SCFA. High protein diets can also affect the microbiota composition. They then ferment amino acids in the colon producing undesirable metabolites (e.g. phenol, hydrogen sulfide and amines) and urea. Athletes in general do not consume sufficient fibre and resistant starch for commensal bacteria to produce beneficial SCFAs and active neurotransmitters, and at the same time to inhibit the bacteria from producing harmful metabolites from proteins. Taking probiotics regularly may shift the microbial composition in a positive direction, but it is not clear yet which strains would be beneficial for athletes. They also may counteract anxiety and depression.

Exercise early in life when the composition of the microbiota is still evolving may positively influence this evolution and may create lasting adaptations in lean mass and psychological well-being. (Mika and Fleshner, 2016). Early-life exercise increases Bacteroidetes and decreases Firmicutes. As neural circuits are in full development in young children, exercise through the impact of commensals can protect the brain against stress-induced psychiatric disorders, such as depression and anxiety later in life.

8 Effects of/on infections

When a pathogen infects a host, both the host-pathogen interaction and the microbiota are essential.

When the normal intestinal microbiota is disrupted (e.g. by an antibiotic treatment), naturally residing bacteria may become harmful.

Microbiota influence infection directly by inhibiting or promoting colonisation, and indirectly via the immune system.

When a pathogen is infecting a host, it is not only the host-pathogen interaction that is at play, but also the microbiota which plays an essential role. Host and microbiota depend on each other for their metabolism. Disruption of the microbiota community disrupts this relationship and this may lead to infection (Leslie and Young, 2015).

Clostridium difficile is a natural resident of the intestinal microbiota; however, it becomes harmful when the normal intestinal microbiota is disrupted, and overgrowth and toxin production occur. Theriot and Young found that antibiotic induced changes in the microbiota shift the caecal metabolome to one that supports *Clostridium difficile* colonisation, including bile acids, carbohydrates and amino acids (Theriot and Young, 2014). Bile acids produced by the host are normally converted by the microbiota into secondary bile acids. Disruption of the microbiota may lead to increased levels of primary bile acids in the large intestine, giving an advantage for germination of *C. difficile* spores (Leslie and Young, 2015).

Carbohydrate fermentation lowers colonic pH (5.5–6.5 in proximal colon where fermentation is highest, compared to pH 6.5–7.0 in the distal colon) and inhibits growth of Gram-negative *Enterobacteriaceae* including familiar pathogens *Salmonella* spp. and *E. coli* (Simpson and Campbell, 2015).

Also, bacteria-bacteria interaction is key. One way that bacteria gain a competitive advantage is via production of microbial products such as bacteriocins. Lactic acid bacteria and others produce bacteriocins in order to combat other bacteria. Also, pathogens must compete with resident microbes for the nutrients. *E. coli* strain Nissle 1917 provides colonisation resistance to infection by *Salmonella enterica* serovar *typhimurium* by competing for iron (Leslie and Young, 2015).

Besides this direct effect inhibiting or promoting colonisation, the microbiota also indirectly influences infection via the immune system. A diversity of bacterial signals modulate host immunity (Leslie and Young, 2015). Butyrate produced by the microbiota aids in the development of peripheral anti-inflammatory T regulatory cells. Some bacterial taxa drive intestinal Treg development, whereas others induce Th17 T cell development (Caballero and Pamer, 2015).

Studies with germ-free mice underscore the importance of the microbiota in the defence against pathogens (Costa et al., 2016). Germ-free mice were more susceptible to *Cryptococcus gattii* infection and showed reduced levels of IFN-gamma, IL-1 beta and IL-17, and lower NF kappa B p65 phosphorylation compared to conventional mice.

Th17 immunity is regulated by the intestinal microbiota composition, especially by segmented filamentous bacteria, not only in the gastrointestinal tract, but also in the lungs (Gauguet et al., 2015). The authors challenged mice with methicillin-resistant *Staphylococcus aureus*. Higher cytokine IL-22 levels and type 17 immune effector levels in the lung were reported in the presence of segmented filamentous bacteria.

In another study with germ-free mice it was shown that the lack of the microbiota influences *Salmonella* colonisation of the mesenteric lymph nodes (Fernandez-Santoscoy et al., 2015). IFN-gamma in the mesenteric lymph nodes of infected germ-free mice increased due to the absence of commensals at the time of infection but also due to the lack of immune signals provided by the microbiota from birth.

A study examining the influence of acidic oligosaccharides derived from pectin on *Pseudomonas aeruginosa* infection demonstrated again the involvement of the microbiota (Bernard et al., 2015). Next to other effects, pectin derived acidic oligosaccharides modified the intestinal microbiota by stimulating the growth of species involved in immunity development, such as *Bifidobacterium* spp., *Sutterella wadsworthia*, and *Clostridium* cluster XIVa organisms, and at the same time increased the production of butyrate and propionate.

The human gut microbiota composition was investigated before, during, and after natural *Campylobacter* infection comparing individuals who became culture positive for *Campylobacter* and those who remained negative (Dicksved et al., 2014). Individuals who became *Campylobacter* positive had a significantly higher abundance of Bacteroides, *Escherichia*, *Phascolarctobacterium* and *Streptococcus* species. The *Campylobacter*-negative group, had more Clostridiales, unclassified *Lachnospiraceae* and *Anaerovorax*. For the *Campylobacter*-positive group this resulted in long-term changes in the composition.

The use of antibiotics may have a strong or mild effect on the composition of the microbiome depending on the type of antibiotic and the time of treatment in life. In young children, the microbiome is still developing. Antibiotics therefore may have a lifelong negative effect. In adults, the microbiome usually recovers very well, but even then, some bacterial groups may not recover with permanent effects on health. Khanna and Pardi studied the effect of antibiotics on *Clostridium difficile* infection and recurrence (Khanna and Pardi, 2016). They plead for antibiotic stewardship to protect native microbiota and to prevent infection recurrence.

9 Effects of/on therapeutic products

Gut microbiota may inactivate therapeutic drugs rendering them less effective. Alternatively, drugs may be biotransformed into active or even toxic derivatives.

Composition of microbiome may also affect vaccine efficacy.

Gut microbiota may inactivate therapeutic drugs rendering them less effective. Alternatively, drugs may be biotransformed into active or even toxic derivatives (Daliri et al., 2017). Xenobiotics¹ are detoxified through the host and microbiota metabolism (Li et al., 2016). In a first step, oxidation, reduction, hydroxylation reactions are to facilitate the excretion of foreign compounds in urine by increasing the polarity. The next step is the conjugation reaction (glucuronidation and sulfonation), where they are conjugated with endogenous metabolites, again to increase their urinary excretion.

Microbiota interferes either directly producing enzymes or by altering the capacity of drug-metabolizing enzymes or expression of genes. Besides modulating the oral drug bioavailability, gut microbiota may also increase drug efficacy (e.g. antitumor chemotherapy) or inactivate them (Jourova et al., 2016; Li et al., 2016). The personal composition or function of gut microbiota may explain the individually different responses towards drug therapy.

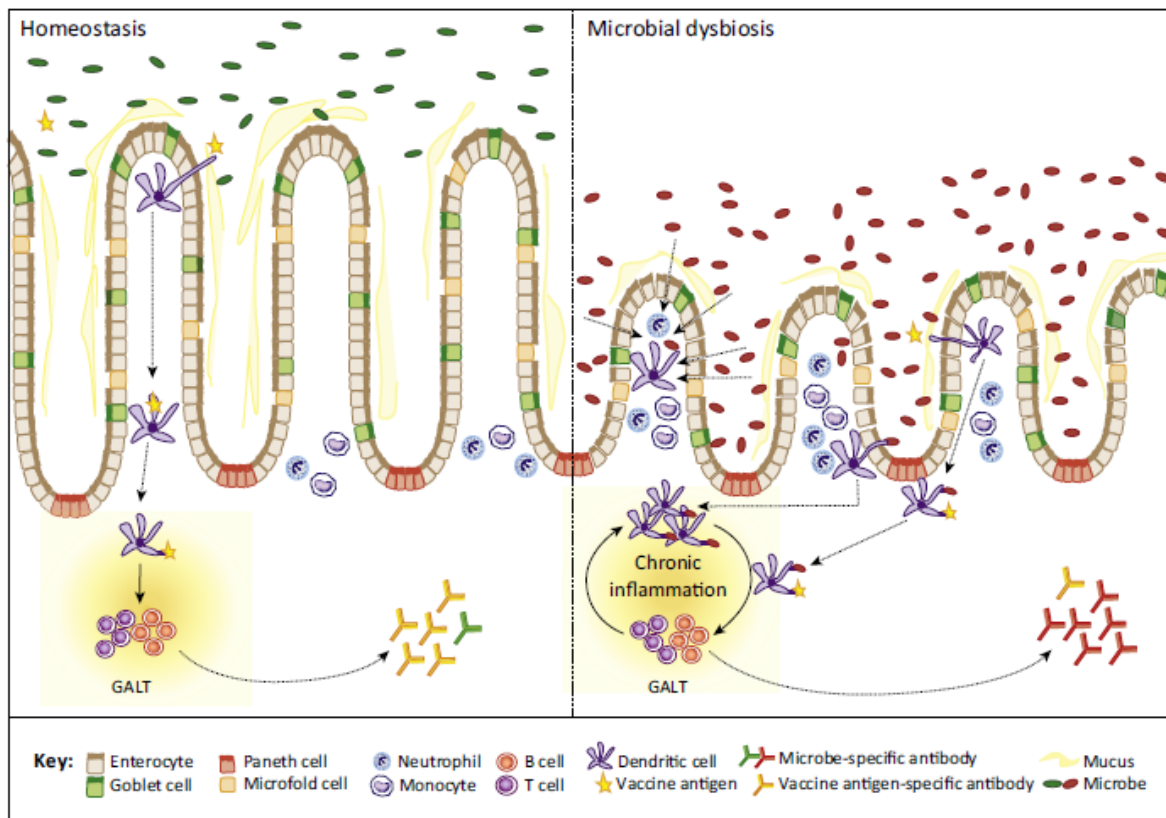


Figure 10. Intestinal homeostasis, dysbiosis and oral vaccine effectiveness (GALT: gut-associated lymphoid tissue)

(©Valdez et al./CellPress, Source: Valdez et al., 2014)

The composition of microbiome may also affect vaccine efficacy (Valdez et al., 2014). Dysbiosis results in villous blunting, increased intestinal permeability, and chronic

¹ a chemical compound foreign to a given biological system. With respect to animals and humans, xenobiotics include drugs, drug metabolites, dietary and environmental compounds such as pollutants that are not produced by the body.

inflammation (Figure 10). Immune cells in the gut-associated lymphoid tissue (GALT) respond by creating dendritic cell-mediated T cells and antibody responses to invading microbiota. Because the immune system is preoccupied with preventing a systemic microbial breach of the intestine, the immune response to a vaccine decreases.

Dysbiosis may result from e.g. poor hygienic living conditions and poor nutritional status. The effect of administering probiotics is varying, probably depending on probiotic strain, dose and type of vaccine. Nevertheless, probiotics may have adjuvant effects and act in modulating tissue homeostasis. Prebiotics fail to show a positive effect on vaccine effectiveness in humans. Studies also suggest that a more diverse intestinal microbiota fosters a more protective immune response to oral vaccines against intestinal pathogens (Valdez et al., 2014).

10 Effects of host genome and life stages

The effect of the host genome on and heritability of the microbiome diversity is rather limited.

The gut microbiota is established as of early life (maternal effect before birth). During the first 2-3 years of life there are significant changes as a result of nutrition and overall environment.

With aging, both the physiological modification of human organs and systems as well as changes in lifestyle have an effect on the gut microbiota and its interaction with the host.

10.1 Host genome

Comparing groups of monozygotic and dizygotic twins the influence of the shared environment can be distinguished from the effects of shared genetics (Abdul-Aziz et al., 2016; Kurilshikov et al., 2017). The phyla Firmicutes, Actinobacteria, Tenericutes, and Euryarchaeota were shown to be more heritable, while the highly abundant Bacteroidetes phylum shows very little heritability.

Heritability is also found in microbial gene type groups, including branched-chain amino acid biosynthesis and sulphur reduction pathways. But microbial quantitative trait mapping in genome-wide association studies reveals that the effect size of host genetics on the microbiome is rather modest: it may explain about 10% of microbiome variance. Several associations are found between the microbiome and genes associated with the host's innate immunity: pattern recognition receptors sense microorganisms in the intestines and therefore modulate microbiome composition and microbiome-associated disease. The strongest association is with the C-type lectin receptors with diet, vitamin D receptors, and metabolism. Host genetic background through bacteria attachment sites exert an important role for the first colonizing bacteria (pioneer flora) (Iebba et al., 2016). Pioneer flora in turn modulates host genes expression, influencing the successive microbial flora.

10.2 Early life

Already before birth, the unborn child comes into contact with microorganisms and the gut microbiota is established. Also, the mode of birth, vaginal *versus* caesarean section, has an effect on the baby's initial microbiota. During the first 2-3 years of life there are significant changes as a result of nutrition and overall environment. After that, the composition of the microbiota stabilises. Microbial colonisation runs in parallel with immune system maturation. Disruptions during this complex process of microbial colonisation have been shown to predispose to diseases later in life.

With pregnancy endocrine, metabolic, and immune changes occur that have an effect on the microbiota at different body sites of the mother (Nuriel-Ohayon et al., 2016). Even though the effects are probably bidirectional as seen above. The abundance of species of the Actinobacteria and Proteobacteria phyla in the gut gradually increases, while the level of *Faecalibacterium*, a butyrate-producing bacterium with anti-inflammatory activities decreases, as well as individual species richness. This coincides with weight gain, insulin insensitivity, and higher levels of faecal cytokines, reflecting inflammation, especially in the third trimester of pregnancy.

Through microbial exposure (probiotics and/or external) there is an early maternal effect on the offspring's microbiota. In a mouse study, maternal microbiota was shown to shape the offspring's immune system in order to respond appropriately to pathogens and commensals after birth. After delivery, it is not clear how long it takes for the mother to return to baseline microbial populations, if ever.

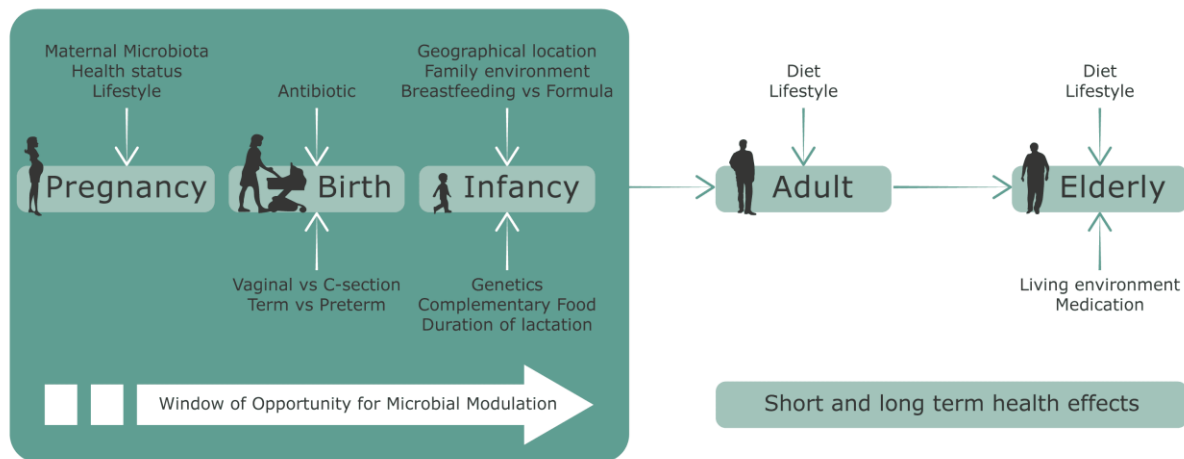


Figure 11. Factors influencing the microbiota of the intestines in humans throughout life.
 (© Rodriguez/ *Microb Ecol Health Dis*, Source: Rodriguez et al., 2015)

The human vaginal microbiota is a key component in the defence system against microbial and viral infections. Especially the *Lactobacillus* genus bacteria can create a barrier against invaders by maintaining a low pH (< 4.5) and secreting inhibiting metabolites. During pregnancy, the level of *Lactobacillus* increases.

A normal healthy placenta contains bacteria, although at low levels, with a composition more resembling the oral microbiome (Kashtanova et al., 2016; Nuriel-Ohayon et al., 2016; Rodriguez et al., 2015). The major phylum is Proteobacteria. Also, in the amniotic fluid and umbilical cord blood microbes are present. Colonisation of the foetus' gut begins prior its birth as shown from the infant meconium. Meconium bacterial populations are dominated by Firmicutes including *Enterococcus*, *Escherichia*, *Leuconostoc*, *Lactococcus*, *Lactobacillus* and *Streptococcus*. The potential mechanisms by which bacteria pass from the mother to the foetus are still unknown.

The mode of delivery is determinant (Castanys-Munoz et al., 2016; Kashtanova et al., 2016; Nuriel-Ohayon et al., 2016; Rodriguez et al., 2015; Rutayisire et al., 2016). Infants delivered by caesarean section have lower total gut microbiota diversity in the first weeks of life compared with vaginally delivered. The gut of vaginally born infants is characterised by bacteria from the maternal vagina, i.e. enriched in the *Prevotella*, *Sneathia*, *Bifidobacterium* and *Lactobacillus* genera, and also includes bacteria present in the maternal gut (e.g. Enterobacteriaceae). Children born via caesarean section carry a gut microbiota resembling the maternal skin and oral microbiota dominated by *Propionibacterium*, *Corynebacterium*, and *Streptococcus*. They have lower counts of *Bifidobacterium* spp. and *Bacteroides fragilis* but increased numbers of *Clostridium difficile*. Colonisation by the phylum Bacteroidetes is delayed. The mode of delivery may also have an effect on the maturation of the immune system, with caesarean section potentially leading to immune disorders. Studies have found a higher risk for developing asthma, obesity, celiac disease, and type 1 diabetes in children born via caesarean section compared with vaginally delivered (Rutayisire et al., 2016). At the age of 6 months the differences in microbial composition start disappearing.

Gestational age also influences the establishment. Preterm infants show higher amounts of facultative anaerobes belonging to *Enterobacteriaceae*, and potentially pathogenic species such as *Clostridium difficile* or *Klebsiella pneumoniae*, and low levels of *Bifidobacterium* and *Bacteroides*. Term babies had higher genus diversity with genera such as *Bifidobacterium*, *Lactobacillus* and *Streptococcus*.

Breastmilk vs. formula feeding and later solid foods all have their impact on microbiota composition next to exposure to several microorganisms from the environment and family members. The microbiome matures during the first year of life. Individual species

diversity increases and diversity between individuals decreases with age. Human milk not only contains bacteria, mainly including streptococci and staphylococci, but is the predominant source for establishing a “healthy microbiome” in the new-born. The most dominant bacteria in the colostrum included *Weisella*, *Leuconostoc*, *Staphylococcus*, *Streptococcus*, and *Lactococcus*. The composition later on changes over the course of the lactation period. In breast-fed infant intestines *Bifidobacteria* are the most abundant species. The gut microbiota of formula-fed infants is dominated by *Enterococci* and *Clostridia*. *Bifidobacterium* can digest the complex oligosaccharides in breast milk, that cannot be digested by the infant itself. These oligosaccharides (natural prebiotics) selectively promote the growth of beneficial bacteria while inhibiting the growth of pathogens. Nowadays, the addition of prebiotics such as GOSs and FOSs to formulas has contributed to bringing the microbiota of formula-fed infants closer to that of breast-fed infants. The more structurally complex human milk oligosaccharides are not yet present in formula. After weaning the gut microbial composition changes to species adapted to digestion of solid foods such as butyrate producers, including *Bacteroides* and certain *Clostridium* species. The number of *Bifidobacterium*, *Lactobacillus*, and *Enterobacteriaceae* decreases.

In new-borns the composition of human microbiota progresses from microbes that can metabolise the components of breast milk during the lactation period, to microorganisms that can utilise components of a solid diet (Rampelli et al., 2016). Next the composition becomes gradually more diverse reaching a maximum between 3 and 5 years of age. During this maturation stage, there are shared functional stages over time regardless of the population or geography. From then on, the composition of the human gut microbiota is rather stable throughout life at the phylum level and in overall function (Yatsunenکو et al., 2012), although differences in composition between individuals may be large. Short-term dietary interventions do not strongly change the microbiota composition. Nevertheless, gene expression and therefore the functional profiles seem to adapt to changes in diet rapidly (Graf et al., 2015).

Coyte et al. tried to understand the mechanisms for maintaining stability (Coyte et al., 2015). They applied concepts and tools from community ecology to gut microbiome assembly. The conclusion was that a high diversity of species is likely to coexist stably when the system is dominated by competitive, rather than cooperative, interactions.

10.3 Aging

In studying the microbiota of elderly people, one has to distinguish the effects of the aging process itself, i.e. the physiological modification of human organs and systems, from those of changes in lifestyle. Both have an effect on the gut microbiota and its interaction with the host (Mello et al., 2016; Salazar et al., 2017). Next to alterations in diet (less vegetables and fruit), lifestyle (decreased mobility), digestive physiology and immune function, also frequent multi-drug therapy (including antibiotics) has influence. In elderly people a great proportion of phylum Bacteroidetes and a lower proportion of phylum Firmicutes with respect to that of younger adults are found. The metabolic consequences are that less SCFAs are produced while proteolytic functions are enhanced. In turn, they increase the inflammation status of aging people as well as age-associated diseases and mental disorders (via communication by the vagus nerve and other pathways).

Some probiotic strains have positive effects by reducing the inflammatory status and reduction of influenza infections. They may also reduce the incidence of antibiotic-associated diarrhoea and *Clostridium difficile* -associated diarrhoea.

Experiments with African turquoise killifish (*Nothobranchius furzeri*) showed that replacing the gut microbiota of middle-aged fish by the microbiota of young fish, extended the life span and delayed behavioural decline (Smith et al., 2017). In this way the aging-related decrease in species diversity was prevented. Young fish receiving middle-aged fish microbiota had no effect on life span.

Claesson et al. studied the influence of residence location in the community, day-hospital, rehabilitation or in long-term residential care and therefore diet on gut microbiota (Claesson et al., 2012). Persons in long-stay care had a significantly less diverse microbiota than community dwellers.

11 Effects of environmental factors

The influence of geographic location can be linked with differences in dietary patterns and lifestyle in a specific area.

Co-habitation creates microbial homogeneity.

The microbiome may interact with environmental chemicals and pollutants in different ways.

As exposures to environmental chemicals induce microbiota alterations that modulate adverse health effects, screening environmental chemicals should include toxicity end-points for the microbiome.

11.1 Geography

The influence of geographic location can be linked with differences in dietary patterns and lifestyle in a specific area (city, countryside, country, religion, etc.).

Comparing microbiota composition of volunteers (0-70 years of age) from Venezuela, Malawi, and the United States revealed that irrespective of age, the microbiota composition clustered according to country (Yatsunenکو et al., 2012). The least microbial diversity in this study was observed for adult Americans with the genus *Prevotella* underrepresented. When comparing African with European children, De Filippo et al. observed increased amounts of *Prevotella* in African children (De Filippo et al., 2010). Faecal microbiota of the African children was rich in Actinobacteria and Bacteroidetes but had lower levels of Firmicutes. Conversely, European children were rich in Proteobacteria and had over twice the relative abundance of Firmicutes to Bacteroidetes. Likewise, Ou and colleagues saw enrichment in *Prevotella* in Africans compared with African Americans (Ou et al., 2013). Similar observations were done in the Tanzanian Hadza hunter-gatherers compared with Italians (Schnorr et al., 2014). In several African populations in these studies an enrichment has been reported in *Succinivibrio* and *Treponema*, bacteria that have a high-fibre-degrading potential. These characteristic features are consistent with a heavily plant-based diet. Also, the Hadza gut microbial ecosystem is depleted in *Bifidobacterium*. This is assumed to be the result of the lack of dairy consumption and contact with livestock.

11.2 Industrialised environment and cities

The Western lifestyle not only includes a typical diet and lack of exercise that influences the gut microbiota, also the physical environment is important (Broussard and Devkota, 2016). Because humans in the industrialised world spent most of their time indoors, microorganisms are exchanged between them and other individuals and the microbial environment. Co-habitation creates microbial homogeneity.

People engaged in shift-work or having a jetlag experience circadian misalignment (Broussard and Devkota, 2016). Gut microbiota shows diurnal oscillations, driven primarily by the rhythms of food intake, leading to rhythmic composition and functional profiles of intestinal bacteria. Circadian misalignment disturbs that rhythm altering the gut microbiome in a way that promotes increased energy absorption and positive energy balance.

11.3 Pollution

Environmental chemicals and intestinal microorganisms might interact in different ways (Claus et al., 2016; National Academies of Sciences Engineering and Medicine, 2017):

- Gut microorganisms themselves can metabolise a variety of environmental chemicals;

- Microbiota can metabolise environmental chemicals after their conjugation by the liver;
- Environmental chemicals can interfere with the composition of the intestinal microbiota;
- Environmental chemicals can interfere with metabolic activity of the microbiota, with potentially deleterious consequences for the host;
- Microbiota can regulate host genes involved in chemical metabolism.

Ingested chemicals may pass the gastrointestinal tract until the distal small intestine and caecum where they may be neutralised by the microbiota or alternatively converted to harmful molecules. An example of the first is 2-nitrofluorene, of the latter, some other polycyclic aromatic hydrocarbons that can be transformed into substances with oestrogenic properties. Another example: the herbicide propachlor is first absorbed and converted by the liver in glutathione, and cysteine conjugates and then deconjugated by the intestinal microbes into toxic compounds. Pollutants such as heavy metals and some pesticides may be toxic to some microorganisms and in this way dysbiosis may be caused. The altered composition and activity of the gut microbiota interfere with the intestinal epithelial-barrier function.

Nevertheless, there is also a reason to look cautiously at these results. e.g. some studies suggest that artificial sweeteners such as aspartame, sucralose and saccharin induce dysbiosis in animals and humans and that this dysbiosis is responsible for deleterious metabolic effects in the host. These studies have been criticised for conclusions not supported by data, small sample sizes, non-representative sample, lack of control group, lack of baseline data, limited testing episodes and recall bias.

Mycotoxins produced by filamentous fungi can damage intestinal tight junction proteins, cytokine synthesis and viability of epithelial cells leading to increased intestinal permeability and degradation of the intestinal mucosal barrier (Du et al., 2017). This influences digestion, absorption, metabolism and transport of the nutrients. Mycotoxins can exhibit antimicrobial properties modulating the composition of the gut microbiome. Also, the microbial activity can be disturbed via modulation of intestinal mucus. Beneficial *Candidatus savagella* and *Lactobacillus* levels are reduced. The segmented filamentous bacterium *Candidatus savagella* is involved in host gut-associated immune systems. Some strains of lactic acid bacteria *Lactobacillus* and *Propionibacterium* can effectively eliminate potent mycotoxins in the intestinal lumen.

The role of the human microbiome in modulating absorption, distribution, metabolism (activation or inactivation), and elimination (ADME) of environmental chemicals should be further studied. As exposures can induce microbiota alterations that modulate adverse health effects, screening environmental chemicals should also include toxicity end-points for the microbiome (National Academies of Sciences Engineering and Medicine, 2017). The research strategy should focus broadly on the three general topics: the effects of environmental chemicals on the human microbiome, the role of the human microbiome in modulating environmental-chemical exposure, and the importance of variation in the human microbiome in modulating chemical-microbiome interactions. This individual-specific microbiome composition will result in an individual-specific response to chemicals.

12 Manipulation of the gut microbiome

Modulation of either the composition or the immune-metabolic activity of the gut microbiota has been tested to restore health from a diseased microbiome.

Therapeutic options include a change of diet, addition of non-digestible prebiotics, probiotics, and synbiotics, antibiotics and/or faecal microbiota transplantation.

While some treatments seem effective, most authors ask for studies with larger sample sizes (adequate statistical power), homogeneous patient groups, standardised treatments, elimination of confounding factors, inclusion of measurements of biomarkers related to the immune system and intestinal health, etc. to be able to compare results and understand the underlying phenomena.

Faecal microbiota transplantation enhances microbial diversity, but strict criteria must be implemented to ensure quality and prevent risks (e.g. of transferring pathogens and disease phenotypes).

12.1 Diet

Eating habits, and therefore dietary components, are the main significant determinants of the microbial composition of the gut, influencing both microbial populations and their metabolic activities, as explained above. Dietary intervention trials to examine the effect on diseases share some limitations (Matijasic et al., 2016): the lack of a placebo control group, the lack of accuracy in information on dietary intake, complex interactions between the consumed food components, individual differences in food metabolism. Moreover, short-term interventions are not able to drastically change the microbiota. A long-term change in dietary habits might be needed.

12.2 Prebiotics

The use of prebiotics or probiotics have variable success in treating diseases. They certainly are not one-fits-all. Clinical trials show large differences in response to treatment, depending on the disease and the type and amount of prebiotics or probiotics.

Prebiotics have been shown to be beneficial in the treatment of colorectal cancer in some studies (Serban, 2014). Inulin and oligofructose reduced the severity of the disease in rats. The best results have been obtained with a combination of probiotic bacteria and inulin-oligofructose in both animal and human studies for reducing and preventing colorectal cancer. They act via the production of SCFAs and upregulating apoptosis, and enhancement of the host's immune response. However, studies are heterogenic and outcomes are varying.

Oligofructose was shown to be advantageous in the treatment of recurrent *Clostridium difficile* infection (Patel and DuPont, 2015). Prebiotic lactulose showed a trend toward clinical benefit in ulcerative colitis (Ghoury et al., 2014).

A systemic review on the use of dietary fibre (e.g., germinated barley, inulin, oligosaccharide/inulin, and psyllium, and high-fibre diet) revealed that only weak evidence for improvement is given for UC and pouchitis (Wedlake et al., 2014). For CD no positive result was reported. Positive effects of fibre are attributed to its fermentation products, SCFAs, in particular, butyrate.

12.3 Probiotics

Various inflammatory and metabolic disorders described above are characterised by dysbiosis, i.e. disruption of the interactions between microbes and the host. Probiotics are proposed to re-establish gut homeostasis and promote gut health. Specific bacterial species originally derived from fermented food (dairy products in particular such as

yoghurt and kefir, but also sauerkraut, cabbage kimchee and soy bean based miso and natto), have beneficial activities.

The probiotic bacteria act by producing SCFAs (lowering of intestinal pH), by metabolising carcinogenic substances, by synthesising vitamins such as B and K, by stimulating the immune response either directly increasing the activity of macrophages or natural killer cells and modulating the secretion of immunoglobulins or cytokines, or indirectly enforcing the gut epithelial barrier (modulating the expression of tight junction proteins) and altering the mucus secretion (increasing the expression of mucins), by competing with pathogenic and opportunistic microbes and suppressing their growth (producing bacteriocins) (La Fata et al., 2017; Raman et al., 2013).

Health benefits from probiotic supplementation are regarded as being strain specific. Strains may be beneficial on their own or in combination. The most common strains belong to the species *Lactobacillus* sp. and *Bifidobacterium* sp.. A successful combination in studies is VSL#3 (*Lactobacillus plantarum*, *L. delbrueckii* subsp. *bulgaricus*, *L. casei*, *L. acidophilus*, *Bifidobacterium breve*, *B. longum*, *B. infantis*, and *Streptococcus salivarius* subsp. *thermophilus* (Mimura et al., 2004)).

Current criteria to qualify for a probiotic are (Grant and Baker, 2016):

- ability to survive during processing, transport and storage,
- ability to survive gastric transport,
- ability to adhere to and colonise the gastrointestinal tract,
- ability to compete pathogenic bacteria,
- demonstration of clinical health outcomes.

Probiotics may be employed for prevention and treatment of colorectal cancer (Ambalam et al., 2016; Raman et al., 2013). A few human studies support their beneficial effect. Potential modes of action are: mutagen binding, degradation and mutagenesis inhibition, prevention of non-toxic pro-carcinogen conversion to carcinogens, lowering of intestinal pH by SCFA production, secretion of anti-inflammatory molecules enhancing the innate immune response.

In a systematic review on the role of probiotics in induction or maintenance of remission in CD, none of the studies provided conclusive evidence of a beneficial effect (Ghouri et al., 2014). Though, in the ulcerative colitis studies various agents showed a trend toward improved rates in both induction of remission and maintenance.

It has been suggested to prevent and combat infections with probiotics (Wolvers et al., 2010). For infectious diarrhoea in infants and traveller's diarrhoea, antibiotic-associated diarrhoea some evidence exists of positive effects in certain conditions with certain strains (e.g. *Saccharomyces boulardii*, *Lactobacillus rhamnosus* GG, *Lactobacillus casei* DN 114 001). Results for *Helicobacter pylori* infection are not conclusive.

A systematic review on clinical trials discussed the effect of probiotics on constipation (Miller et al., 2016). Short-term supplementation of probiotics, mostly yoghurt or other forms of fermented milk, in constipated subjects statistically decreased intestinal transit time in comparison to the placebo. This effect was not seen in healthy adults. Single-strain probiotics were more efficacious than multiple strain probiotics. Another systematic review selected clinical trials with children (Huang and Hu, 2017). Constipation was significantly reduced using probiotics from the *Lactobacillus* and *Bifidobacterium* genera, but also *Streptococcus thermophilus*.

In a study to verify prevention of gestational diabetes mellitus, different treatments during pregnancy were compared: a combination of administration of probiotics and dietary intervention, a placebo and dietary intervention, and, dietary intervention alone (Barrett et al., 2014). This study shows a lower rate of gestational diabetes mellitus in the probiotics group.

Villanueva-Millan et al. mentioned the use of probiotics in animal studies to treat obesity (Villanueva-Millan et al., 2015). Mimura et al. noted a positive effect in treating recurrent or refractory pouchitis (Mimura et al., 2004).

Petrof and colleagues reported on a systematic review on the effect of probiotics on critically ill patients (Petrof et al., 2012). Data on ventilator-associated pneumonia show a rate reduction with probiotics. The analysed clinical trials did not show a reduction in antibiotic-associated diarrhoea and *Clostridium difficile* infections. The heterogeneity of the trials reported outcomes that prevent clear conclusions.

Microbiota regulate gene expression in specific tissues. A systematic review on probiotic-mediated modulation of gene expression associated with the immune system and inflammation was performed trying to understand the underlying mechanisms (Plaza-Diaz et al., 2014). Certain strains of *Bifidobacterium*, *Lactobacillus*, *Escherichia coli*, *Propionibacterium*, *Bacillus* and *Saccharomyces* induce an anti-inflammatory response: downregulation of pro-inflammatory genes, e.g. producing certain chemokines and cytokines, and upregulation of anti-inflammatory genes, such as mucin genes and Toll-like receptors, in enterocytes, dendritic cells. These findings are from *in vitro* and animal studies. Studies in humans are scarce.

As the microbiota has an effect on the brain, probiotics are proposed as a therapeutic alternative to reduce mood disorders such as stress, anxiety and depression. A meta-analysis performed in 2016 showed that supplementation with probiotics, mostly including lactobacilli and bifidobacteria but also *Lactococcus* and *Streptococcus*, resulted in a statistically significant improvement in psychological symptoms of depression, anxiety, and perceived stress in otherwise healthy volunteers (McKean et al., 2017). The mode of action might be the competitive exclusion of harmful pathogens, the decrease in pro-inflammatory cytokines and the communication with the brain through the vagus nerve, leading to changes in neurotransmitter levels or function (Grant and Baker, 2016).

Most authors ask for studies with larger sample sizes (adequate statistical power), homogeneous patient groups, standardised treatments, elimination of confounding factors, inclusion of measurements of biomarkers related to the immune system and intestinal health, to be able to compare results and understand the underlying phenomena.

12.4 Antibiotics

Antibiotics are not only beneficial in treating infectious diseases but are also potentially harmful agents. Antibiotics are able to shift the gut microbiota (Ferrer et al., 2017; Ianiro et al., 2016; Langdon et al., 2016; Lange et al., 2016). This post-antibiotic dysbiosis is in general characterised by a loss of diversity both in luminal and mucosal bacteria species, a loss of certain important taxa, shifts in metabolic capacity, and by reduced colonisation resistance against invading pathogens. Especially in early life this has long lasting effects with impaired immune system maturation.

Dysbiosis might lead to metabolic, immunological, and developmental disorders, and the use of antibiotics may have an effect on the prevalence and course of disease. The main immediate consequence of antibiotic treatment is the disruption of the ecosystem balance, leading to antibiotic-associated diarrhoea. Both opportunistic and exogenous pathogens benefit from the dysbiosis status (Iebba et al., 2016). The rise of *Clostridium difficile* infections after antibiotic treatment especially in the elderly is a striking example (Ianiro et al., 2016). The impact of antibiotics is dictated by both the type of the antibiotic, pharmacokinetics, pharmacodynamics and range of action, dosage, duration and administration route as well as by host-related factors including age, lifestyle and microbiota composition (Ianiro et al., 2016).

Nevertheless, antibiotics may be used in treating non-infectious diseases. Again, results are varying depending on disease, type of antibiotic and individual patients. IBD (UC and CD) potentially can benefit from antibiotic treatment as these diseases are characterised

by high prevalence of harmful bacterial genera (Matijasic et al., 2016). Not enough trials have been performed and results are controversial. Thus, on one hand exposure to antibiotics appears to increase the likelihood of diagnosing CD (but not UC), and antibiotic treatment in IBD appears to be associated with more severe disease course, there might be on the other hand a beneficial effect of some antibiotics in certain treatment regimens (Ianiro et al., 2016; Lange et al., 2016). Likewise, antibiotics may be a risk factor in the development of IBS, but certain antibiotics (e.g. rifaximin) may be used in the treatment of this disorder (Ferrer et al., 2017; Ianiro et al., 2016). Rifaximin is also successful in treating hepatic encephalopathy.

12.5 Faecal microbiota transplantation

Faecal microbiota transplantation is becoming an accepted method for the restoration of a disrupted microbiota. A faecal suspension from a healthy donor is prepared and introduced in the gastrointestinal tract of a diseased person, either by oral capsules, or enemas, or duodenal infusions (nasointestinal tube) or colonoscopy. The treatment is successful in treating diseases of the gastrointestinal tract, e.g. due to *Clostridium difficile* infection, and to a lesser extent inflammatory bowel disease and ulcerative colitis (Gianotti and Moss, 2017). The mode of administration is of little impact on the efficacy of reducing *Clostridium difficile*.

A systematic review undertaken in 2013 investigated the efficacy and safety of faecal microbiota transplantation therapy (Sha et al., 2014). The authors included clinical trials with adults and children. The treatment was found successful in *Clostridium difficile* infection, it improves UC, but is disappointing in CD. Also in children the treatment was beneficial and safe. For chronic fatigue syndrome and metabolic syndrome in adults some effect was reported. Stool composition after faecal microbiota transplantation showed an increase in microbial diversity including anti-inflammatory and/or SCFA-producing bacteria. Adverse events were uncommon but transient, and may include flatulence, rectal discomfort, diarrhoea, nausea, abdominal cramping, etc. An earlier systematic review on the usefulness of faecal microbiota transplantation in patients with *Clostridium difficile* infection came to the same conclusion (Gough et al., 2011). Results depended on the type of donor, the preparation of the material, the dosage and patient pre-treatment.

The exact mechanism of disease remission is not known. It might be due to the change in bacterial communities, alterations in host metabolic profiles, or the introduction of peptides from the donor that modify host immune responses. (Gianotti and Moss, 2017). It is even not known whether *Clostridium difficile* is effectively eradicated or reverted to a sporulating state.

Faecal microbiota transplantation has also been suggested to remediate neuro-developmental disorders, autoimmune diseases and allergic diseases (Borody and Khoruts, 2011). Preliminary reports exist for Parkinson's disease, fibromyalgia, chronic fatigue syndrome, multiple sclerosis, myoclonus dystonia, obesity, insulin resistance and the metabolic syndrome and childhood regressive autism.(Sha et al., 2014).

Faecal microbiota transplantation enhances microbial diversity. Microbiota of treated patients has been shown to resemble that of the donor after therapy. The preparation should be standardised. It is not known what contact with oxygen might result in. The incomplete characterisation of the material delivered into the patient might be a drawback and hampers standardisation. Another disadvantage is the risk of transferring microbial pathogens, or undesired disease phenotypes, such as obesity, metabolic syndrome, and fatty liver, as shown in mouse studies (Hansen and Sartor, 2015). In selecting donors, the primary criterion should be the overall donor's health. Medical examination, screening test and medical history should not reveal gastrointestinal diseases or other diseases correlated with dysbiosis, or infections (e.g. HIV, hepatitis). The donor should not have used antibiotics recently (Borody and Khoruts, 2011; Sha et al., 2014).

Stool banks have been established in some countries, for example, OpenBiome and AdvancingBio in the United States, the Taymount Clinic in the United Kingdom, the Netherlands Donor Feces Bank (NDFB), and the Chinese FMT bank (Ma et al., 2017).

With more knowledge becoming available faecal microbiota transplantation may be replaced by defined preparations of their constituent therapeutic factors (Langdon et al., 2016).

13 Legal and ethical aspects

Probiotics are subject to a scattered legal framework for food, feed, and health claims.

Faecal microbiota transplantation is not regulated at the EU level.

Since the human metagenome (combination of the human genome and the microbiome) together encode a person's physiological and psychological traits, the microbiota may be considered to be part of a person's identity.

Commercial application raises concerns about property rights, accessibility of data, patentability of faecal microbiota profiles, financial benefits, etc. When performed outside of the regulated establishment, there are additional concerns on safety, follow-up, and exaggerated expectations.

The delay in appropriate governance hinders further clinical trials and applications and therefore prevents adequate therapies to be developed to replace the current, costly treatments.

13.1 Legal analysis

The World Health Organisation's definition of probiotics is: "live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host" (WHO, 2006). In EU legislation the notion probiotics does not exist.

In the EU probiotic microorganisms used as or in foods and food supplements are considered as food ingredients and are not subjected to a centralised pre-market safety assessment due to traditional and safe use in fermented foods. Food businesses have general obligations under the EU **Food Hygiene Regulation (EC) No 853/2004** (EU, 2004) essentially in relation to the compliance with microbiological criteria for foodstuffs. However, if new, they need to comply with the EU **Novel Food Regulation (EU) 2015/2283**, which lays down rules for novel foods that were not used before 1997 (EU, 2015).

Member State regulators, responsible for control of food businesses under the food hygiene regulation, often use requirements and guidance documents (e.g. from EFSA) developed for feed probiotics as reference. Since these are regulated as feed additives (see below), they require a substantial registration dossier prior to the EU authorisation.

Food Supplements Directive 2002/46/EC lays down specific rules for vitamins and minerals used as ingredients of food supplements (EU, 2002). Substances other than vitamins and minerals are not directly covered by this directive and rules regulating these substances are still governed by individual EU Member States.

Regulation 609/2013 on dietetic food covers infant formula and follow-on formula, processed cereal-based food and baby food, food for special medical purposes, and total diet replacement for weight control (EU, 2013a).

From a marketing point of view, probiotic manufacturers try to label their products with a health claim. Health claims are regulated by the **Health Claim Regulation (EC) 1924/2006** (EU, 2006) and its implementing legislation (EU, 2008). The regulation deals with beneficial nutritional properties (nutrition claim), the relationship between a food/constituent and health (health claim) and food/constituents significantly reducing a risk factor in the development of a human disease (reduction of disease risk claim). The risk assessment is based on evidence weighing of human studies (but not studies designed for the treatment of diseases), efficacy studies in animals and non-efficacy studies in humans, animals and/or *in vitro*. The only approved probiotic claim in the EU is generic for yoghurt bacteria: production of lactase and aid in digesting lactose in subjects with intolerance (EU, 2012).

Claims on probiotics have not been approved in the EU because of:

- Insufficient characterisation
- Non-defined claims
- Non-beneficial claims
- Not all measurable outcomes reflect a direct benefit for humans
- Lack of pertinent human studies
- The quality of studies

Nutrition and health claims will only be allowed on food labels if they are included in one of the EU positive lists (Register for claims: <http://ec.europa.eu/nuhclaims/>). The use of the term probiotic is not permitted under the Health Claim Regulation as of 14 December 2012 (EU, 2012). The term 'probiotic' is considered an implied health claim. This means that products cannot be sold in the EU claiming to be probiotics.

Food products carrying claims must also comply with the provisions of **Nutritional Labelling Regulation 1169/2011** on information to consumers (EU, 2011).

In 2013, the European Commission introduced the **Generic Descriptors Regulation No. 907/2013** (EU, 2013b), which sets out the rules for applications concerning the use of generic descriptors. Generic descriptors are words which have traditionally been used to indicate a characteristic of a class of foods or beverages which could imply an effect on health such as "digestive". In the past, these words have been exempt from the ban under the Health Claim Regulation. The Generic Descriptors Regulation foresees that generic descriptors for food and beverage products, which could be perceived as health claims, would only be allowed if they have been in use for the product for more than 20 years in a Member State. Where a company demonstrates use of these descriptors prior to the entry into force of the Generic Descriptors Regulation, then, it would be possible to apply for an exemption to the ban.

Microorganisms to be included in feed or animal drinking water are regulated by the **Feed Additives Regulation (EC) No 1831/2003** (EU, 2003), if they are intended to perform functions such as favourably affecting animal production, performance or welfare, particularly by affecting the gastro-intestinal flora or digestibility of feedingstuffs, and others.

When microorganisms are proposed for use in regulated products that require market authorisation, EFSA is required to assess their safety. Independently of any particular specific notification in the course of an authorisation process, EFSA's Qualified Presumption of Safety (QPS) (<https://www.efsa.europa.eu/en/topics/topic/qualified-presumption-safety-qps>) provides a generic safety pre-assessment approach of a defined taxonomic unit for use within EFSA that covers risks for human, animals and the environment. Several microorganisms that are present in the human gut have been included in the QPS list (Ricci et al., 2018).

In the absence of harmonisation—probiotics are subject to national provisions, resulting in a fragmented EU market place. At the moment, most EU countries consider the term "probiotic" a health claim.

Faecal microbiota transplantation is not regulated at the EU level, in Australia or China (Edelstein et al., 2015). However, the Dutch authorities consider stool samples to be drugs. The United States' FDA considers it as an investigational new drug (IND) meaning a long and arduous IND procedure as for medications (FDA, 2013). FDA has waived the IND requirement only for treatment of recurrent *Clostridium difficile* infection, under conditions (Ma et al., 2017). In Canada, it is regulated as a new "biologic drug" that can only be used in clinical trials.

13.2 Ethical considerations

Due to the close interaction with the human body and the fact that each individual's microbiota is unique, the microbiota may be considered to be part of a person's identity (Metselaar and Widdershoven, 2017; Rhodes, 2016). Both the human genome and the microbiome (together the human metagenome) encode a person's physiological and psychological trait. The question arises whether it is therefore also the person's property. For use in research, clinical trials and eventually commercial applications, microbiota samples may be compared with other biological samples. Participation in clinical trials follows the normal rules. With a person's informed consent the participant explicitly agrees with the study goals and potential risks. It is also worthwhile to think about participating in research on microbiota and providing material for biobanks for the interest of the general public health, as the gathered knowledge would be to the benefit of everybody.

Being part of identity the question rises to what extent faecal microbiota transplantation may alter essential characteristics of a person (Metselaar and Widdershoven, 2017). Changes to the psychology may alter family relations. Also, an altered microbiota can be transmitted through offspring; faecal microbiota transplantation therefore might have consequences to the next generation. The discussion has some similarities with altering germline genomics.

The human gut microbiota is sometimes presented as a "virtual organ" that should be treated as human tissue (Ma et al., 2017). Faecal microbiota transplantation is then a form of organ transplantation, but simpler to perform than other organ transplants, without the need for immunological matching of donor and recipient or the need for immunosuppression following the procedure (Borody and Khoruts, 2011). However, extensive screening of donors needs to be performed in order not to transfer pathogens or the risk for other diseases that might be associated with the gut microbiota, as discussed before. The influence of other factors such as gender, age, pregnancy, religious background (diet) is still uncertain (Ma et al., 2017).

For the donor the necessary guarantees must be in place to ensure privacy and confidentiality. At the same time, it must be clear for donor and medical staff how unsolicited and secondary findings should be handled. As a person's microbiota is unique, it may become possible to identify an individual analysing its faeces (its microbial fingerprint that also contains human DNA). Microbiota can reveal a person's lifestyle, travelling history, etc.

Potential patients are extra vulnerable as faecal microbiota transplantation is often the last resort when other treatments fail (Ma et al., 2017)., It is challenging to obtain an informed consent of the patient in an area where knowledge on the treatment is still limited especially regarding possible side effects on mood and behaviour. Faecal microbiota transplantation has until now only been proven effective in treating *Clostridium difficile* infection. Patients might be tempted to consent without fully understanding the risks of new or still to be verified therapies.

Commercial application raises concerns about property rights, accessibility of data, patentability of faecal microbiota profiles, and financial benefits (Ma et al., 2017). Commercial faecal microbiota transplantation circumventing the guidance of the hospital and health care professionals (DIY kits) may introduce issues as with the reproduction industry: safety, follow-up, exaggerated expectations, etc.

Faecal microbiota transplantation therapy may induce reactions of disgust toward the object of faeces, fear of transmission of potential pathogens and feelings of violation and degradation of human dignity (Ma et al., 2017).

The delay in appropriate governance hinders further clinical trials and applications and therefore prevent adequate therapies to be developed to replace the current, costly treatments.

14 Discussion/Conclusion

Research on human gut microbiota has made great progress in the last decade. Various techniques make it possible to identify its composition concerning organisms, genes, proteins and functions. Nevertheless, what really constitutes a “healthy” gut microbiota remains still unclear. The biodiversity between different healthy individuals is even greater than expected. There is consensus that the healthy adult gut is dominated by Firmicutes, Bacteroidetes, Actinobacteria, and Verrucomicrobia (Human Microbiome Project Consortium, 2012). Within these phyla, there is still large inter-individual (and intra-individual) variability, with each person harbouring a unique microbiota profile. Species are shared within families and communities. Despite the differences on the species level, the functions carried out by these species appear to be similar in every person’s gastrointestinal tract (Marchesi et al., 2016).

It must be stressed that to date research on gut microbiota is very bacteria-centric. Very few studies have looked at the viral component (or virome) and eukaryotes such as protozoa, yeast and fungi or even bacteriophages.

The microorganisms in the gut not only assist in the digestion of food, but they are also, perhaps more importantly, involved in establishing the immune system response, the defence against pathogens, the endocrine system and even mental health. Already before birth they are present and influence human health.

The composition is determined by and varies greatly according to the diet, but also lifestyle, age, genetics, disease, antibiotic use, etc. are important (Figure 12). Dietary changes can account for up to 57% of gut microbiota changes, whereas genes account for no more than 12% (Clark and Mach, 2016).

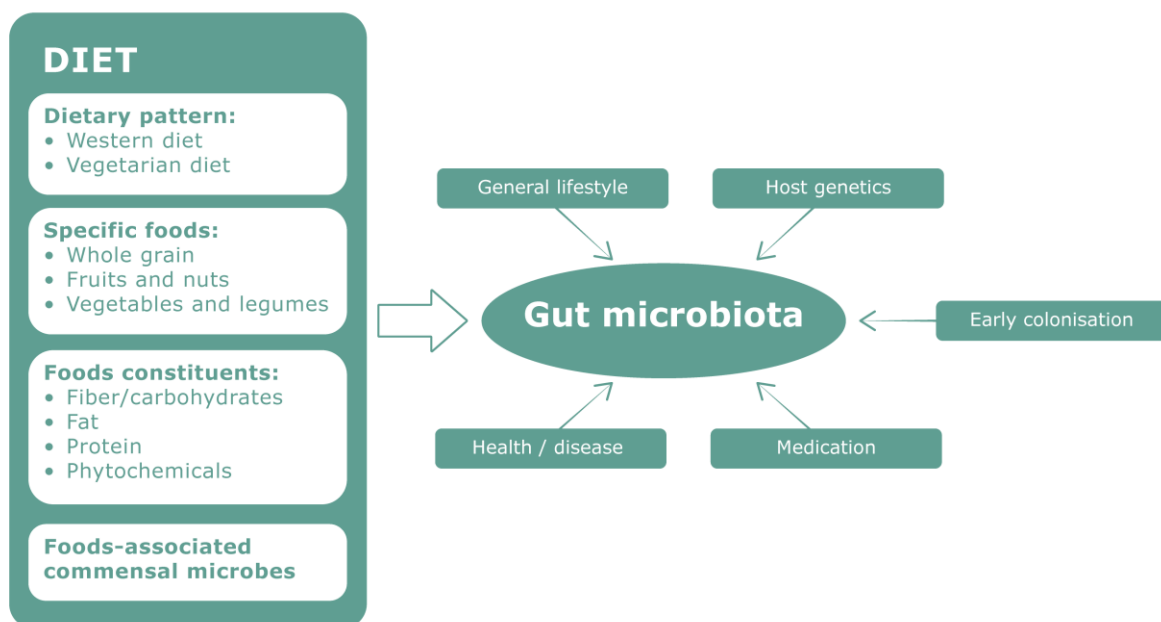


Figure 12. Factors, which influence the composition of the human gut microbiota, with special focus on diet.

(© Graf/ *Microb Ecol Health Dis*, Source: Graf et al., 2015)

Diet can modify the intestinal microbiome, which in turn has a profound impact on overall health. A load of evidence exists for the link between dysbiosis, the disturbed balance in the microbiota composition, and disease. However, not always a causal relation is established. We still lack much in terms of mechanistic insight into how microbes contribute to the onset of disease. Many animal studies have been conducted, while limited data is available concerning human studies. A major difficulty is the variation in

the normal functional human microbiome, but also the use of different techniques to assess dysbiosis in humans which might lead to the generation of different results (Leung et al., 2016).

Not only diseases related to the gastrointestinal tract (IBD, *Clostridium difficile* infection, coeliac disease, liver diseases) are correlated with the microbiota, but also diseases like cardiovascular diseases, autoimmune diseases and mental disorders like depression, Alzheimer's disease are affected.

Considering the relationship between microbiota and disease, and the fact that diet has a dramatic effect on its composition, many attempts have been made to cure diseases using prebiotics, probiotics or synbiotics. Trials have varying success.

Interfering by changing the diet or supplements may have effect, but the results in individual cases are highly influenced by the initial composition of an individual's microbiota (Flint et al., 2012). Clinical trials prebiotics, probiotics or synbiotics are hard to compare due to confounding factors. Treatment with antibiotics has also been suggested. Faecal microbiota transplantation therapy is clearly beneficial in curing *Clostridium difficile* infections, but more research needs to be performed for other diseases.

Existing legislation in relation to probiotics does not facilitate their use in preventing and curing diseases. Furthermore, the legal description of faecal microbiota transplantation is balancing between the notion "drug" and "biologic". Ethical issues are especially important concerning faecal microbiota transplantation therapy.

15 Examples of national and international initiatives

Initiative	Objective	Perio	Webpage
Human Microbiome Project NIH, USA	to characterise the diversity of the microbiota sampled at multiple body sites exclusively in healthy humans	2008 - 2013	hmpdacc.org
EU FP7 f project MetaHIT, Metagenomics of the Human Intestinal Tract	to sequence the microbial genomes of faecal samples derived from both diseased (IBD and obesity) and healthy individuals	2008 - 2012	www.metahit.eu
EU FP7 project MyNewGut"	research how the human gut microbiota and its genome (microbiome) influence obesity, behavioural- and lifestyle-related disorders and vice versa. It also aims to identify specific dietary strategies to improve the long-term health of the population	2013 - 2018	www.mynewgut.eu
European Network for Gastrointestinal Health Research (ENGTHR)	European Science Foundation Research Networking Programme (RNP) which promotes interactions between researchers interested in gut health research in Europe	2010 - 2014	www.engthr.eu
APC Microbiome Institute, University College Cork, Ireland	Trans-disciplinary environment with clinicians, clinician-scientists and basic scientists from diverse backgrounds focused upon the gastrointestinal bacterial community		apc.ucc.ie
American Gut Project, Citizen Science project	a crowd-sourced, open source citizen science project aiming to characterise the human microbiome		americangut.org
Vlaams Darmflora Project Professor Jeroen Raes VIB Metagenomics	To study the role of gut bacteria in health (5000 participants)		www.vib.be/nl/mens-gezondheid/darmflora-project

16 Methodology of this study

The study is focussed on the human gut microbiome. As a consequence information on microbiomes from other organisms (e.g. animals) or other human compartments (e.g. skin) are not covered.

This report is based on literature searches performed on the Web of Science and PubMed databases in Fall 2017. The search string that was used, is:

(Microbiome OR Microbiota)

AND

Influenca*

AND

(Nutrition* OR Nutrient* OR Probiotic* OR Environment* OR Pollution OR Infect* OR Health OR Well*being OR Disease OR Obesity OR Cancer OR Aging OR Therapeutic* OR ADME OR Pharma* OR Toxic*)

Only articles that focussed on the gut microbiota were retained. The search was limited to articles available in English. A total of 5810 articles were left. Manual searches through reference lists of the articles were also performed to identify additional studies. Review articles were also kept.

Based on this information, a selection was made in order to cover the range of findings and subjects, acknowledging that it was not the purpose of this study to provide a detailed analysis of a specific field. The outcome should only be regarded as a top-level view of the different directions in which research is conducted and in which areas information is still relatively weak and others for which the body of evidence is convincing

The information was classified and grouped according to the requested topics aiming at providing an overall picture. However, these classifications may not be as intended by the authors of the original research. Also, some wording may reflect the understanding of the authors of this study rather than the claims of the authors of the original publications. Nevertheless, the authors of this study have tried to remain unbiased and only presenting discussions and/or diverging views as they appear in publications.

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