Nutrition-mediated effects of the intestinal microbiota

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Summary
Recent studies show that the fetus already comes into contact with bacteria. However, the actual colonization of the infant intestine takes place during and following birth. A vast majority of the microorganisms in the human intestine are bacteria. Furthermore, lower concentrations of Archaea and eukaryotes (fungi) are also present. For the most part, commensal bacteria in the intestine use constituents of the host’s diet for their own growth. The diet therefore has a significant impact on the bacterial composition.

The microbiota plays an essential role in maturation of the immune system and maintenance of its functions. Intestinal bacteria interact closely with the mucosal immune system. However, the intestinal microbiota does not cause a systemic inflammatory response as long as the intestinal barrier is not disrupted and intestinal bacteria are contained in the gut lumen. An imbalance between immune tolerance and immune response toward intestinal bacteria can promote the development of various diseases.

The interrelationships between the microbiota and various diseases are currently under intense investigation. It is well known that the microbiotas differ between healthy humans and obese individuals, and in those with chronic inflammatory bowel disease. Conversely, the intestinal microbiota exerts an influence on these diseases. There are also indications for the involvement of the intestinal microbiota in the development of other diseases.

Keywords: nutrition, fiber, fermentable substrates, intestinal microbiota, mucosal immune system, inflammatory bowel disease, obesity

Introduction
The digestive tract of humans and animals is one of the most densely populated microbial habitats. It harbors microorganisms that obtain their energy primarily through fermentation, which is due to the lack of oxygen in the intestines. For growth, the microorganisms depend on substrates that serve as a source of cellular constituents and energy. These substrates mainly come from foods and the host organism. Only those bacteria capable of using the substrates available in the intestine and of tolerating the physicochemical conditions therein will be able to permanently colonize this ecosystem. Thus, the initial colonization and diet are the primary factors responsible for the composition of the microbial community in the intestine, also referred to as intestinal microbiota or intestinal microbiome, whereas the latter term emphasizes the microbial gene repertoire. The growing interest in the intestinal microbiome is due to its wide-ranging influences on the physiology of the host. The interactions between the host and his intestinal bacteria affect the entire metabolism and the immune system. It is therefore not surprising that many diseases are associated with an imbalance of the microbiome. However, whether disease development is caused by changes in the gut microbiome or whether changes in the microbiota are the result of the respective disease has not yet been fully clarified.

Colonization of the gastrointestinal tract
The microorganisms colonizing the digestive tract reach the intestine via the oral route. Contrary to earlier assumptions, recent studies show that the placenta is not sterile during pregnancy and that the fetus is already exposed to bacteria before birth [1]. Bacterial DNA has been found in umbilical cord blood [2] and in the placenta [3], and bacteria have been isolated from meconium [4]. Observations, that the oral intake of probiotic bacteria by pregnant women influences the expression of physiologically relevant genes in newborns, imply
occurs only after birth, despite earlier intrauterine contact of the fetus with bacteria. Colonization of the digestive tract in newborns proceeds in variable ways and apparently at random [6]. However, the authors of a recent study come to the conclusion that primarily representatives of the bacterial classes Bacilli, Clostridia, and Gammaproteobacteria colonize the gastrointestinal tract within the first two weeks after birth. This always occurs in a certain order, despite some intermittent abrupt changes. Therefore, the administration of antibiotics, type of birth (vaginal or Cesarean delivery) and type of diet only affect the speed of colonization [8]. The composition of the intestinal microbiota stabilizes within the first year of life and approaches that of adults [6].

Population of intestinal microbiota in adults

The intestinal microbiota of adults is mainly composed of prokaryotes, Bacteria for the most part. Methane-producing Archaea are found in

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<td>Coriobacteriales</td>
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<td>Ascomycota</td>
<td>Saccharomycetales</td>
<td><em>Candida</em></td>
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Tab. 1: Important groups of microorganisms in the human intestine
In principle, all dietary constituents are the most important substrates for the growth of intestinal bacteria. The quantitatively most important substrates are fermentable carbohydrates, primarily polysaccharides from plants, which are not or only partially degraded by the host’s digestive enzymes. These include resistant starches, cellulose, hemicellulose, pectin, and inulin. The starches in legumes and unprocessed grains as well as in raw potatoes or unripe bananas are present in forms that are not readily accessible to digestive enzymes. The heating then cooling of starchy foods can also convert starch into a resistant form (retrograded starch), which is resistant to digestion by enzymes of the host.

Indigestible polymeric carbohydrates and lignin are traditionally referred to as dietary fiber [11], suggesting that fiber is unnecessary, even though it is an important part of a healthy diet. The term fiber originally was associated with carbohydrates such as cellulose and hemicellulose, which lend a structural rigidity to plant cell walls and are largely resistant to digestion by intestinal bacteria during colon passage. In contrast, plant storage substances such as inulin are readily fermented by intestinal bacteria. The term “fiber” is less appropriate for these substances. A more appropriate term is “indigestible, fermentable carbohydrates”, but this is more lengthy and unwieldy than simply “fiber”. Indigestible carbohydrates are found in whole-grain products, legumes, nuts, vegetables and fruits. They differ in their physicochemical properties, including solubility, viscosity, and water-binding capacity. These properties are dictated by the compounds present (glucose, galactose, mannose, arabinose, xylose, uronic acid), how they are interlinked, and the degree of polymerization. Depending on their properties, indigestible carbohydrates are degraded to varying extents by the intestinal bacteria. Cellulose (up to 10,000 glucose units linked together with β-1,4 glycosidic bonds), due to its crystalline structure and low solubility, cannot be fermented by intestinal bacteria or only to a small degree. In contrast, water-soluble polysaccharides such as guar gum (galactomannan: β-1,4 glycosidic linked mannose chains with α-1,6 glycosidic linked galactose) or pectin (chains of α-1,4-linked D-galacturonic acid partially esterified by methanol) are fermented by intestinal bacteria.

Polysaccharides have a very high structural variety but only a small proportion of these polysaccharides can be degraded by host digestive enzymes. The human microbiome has a broad range of enzymes capable of catalyzing the degradation of indigestible polysaccharides. For this purpose, many intestinal bacteria have enzyme systems to degrade long-chained and branched carbohydrates into oligomers and monomers. These degradation products serve as an energy source not only for the microorganisms directly involved in the depolymerization process but also for other microorganisms lacking such enzymes.

### Bacterial fermentation in the digestive tract

Polysaccharides are the most important substrates for the intestinal microbiota. Therefore, the genes for carbohydrate degradation are more abundant in the intestinal microbiome than in other bacterial genomes or in the human genome [12]. The oxygen concentration is extremely low in the distal small intestine and the colon, so most intestinal bacteria are predominantly anaerobes. Fermentation provides them with the energy required for growth but this means that the available substrates cannot
be completely oxidized to carbon dioxide due to the lack of oxygen. For this reason, anaerobes obtain significantly less energy from substrates than aerobic organisms. 

Overview 1 shows substrates and reactions of bacterial fermentation in the colon.

Endogenous substrates

Indigestible, fermentable dietary carbohydrates as well as endogenous substrates produced by the host serve as energy source. Mucus (Box) is excreted into the intestine by the goblet cells. It covers the intestinal epithelium and functions as a protective barrier, ensuring that bacteria and other antigens are kept at a distance from the epithelium without affecting the uptake of nutrients.

The intestinal microbiome encodes a wide spectrum of enzymes allowing the utilization of glycoproteins from the mucus as an energy source. First, the carbohydrate residues are degraded, beginning at the end of the chain. As soon as proteases have access to the protein backbone, the protein portion of the mucus is also utilized. Akkermansia muciniphila is a bacterium that is capable of growing on mucus as a carbon and energy source [16]. This microorganism colonizes the human intestine within the first year of life [17]. In a recent study using mice, it was shown that the oral administration of A. muciniphila is able to improve some of the symptoms of the metabolic syndrome [18]. However, the underlying mechanisms are still not clear. Besides Akkermansia there are other intestinal bacteria that can utilize mucus, for example, Bacteroides acidifaciens, Bacteroides thetaiotaomicron, Mucispirillum spp. and several species of the Lactobacillaceae, Enterococcaceae and Ruminococcaceae [19].

In addition to the mucins, the digestive enzymes of the small intestine such as proteases, lipases, and nucleases also serve as substrates for the microbiota in the colon. Analyses of the intestinal contents of sudden-death victims revealed that the bacterial degradation of proteins mainly occurs in the distal colon, where the highest concentrations of degradation products characteristic of amino acid fermentation were found, in addition to acetate, propionate, and butyrate [20]. These include branched-chain fatty acids such as isovalerate and isobutyrate, the products of leucine and isoleucine degradation, as well as indoles and phenols originating from the aromatic amino acids phenylalanine and tyrosine. Further products are ammonia, sulfide and organic sulfur compounds [21].

Mucus – the intestinal barrier

Mucus is composed of glycoproteins, with MUC2 as the most important mucin in the intestine. Its protein backbone is O-glycosidically linked by serine and threonine residues to carbohydrate side chains. The mucin molecules are cross-linked to a network by disulfide bridges. Protein makes up around 20 % of the total mass of the mature glycoprotein, and the carbohydrate residues, around 80 % [14]. Depending on the type of mucin, there are varying proportions of N-acetylglucosamine, N-acetylgalactosamine, fucose, galactose, N-acetylneuraminic acid (sialic acid), and sulfated sugars as carbohydrate residues. Mucins not only protect the intestinal epithelium by covering it with a gelatinous layer but they also enable adhesin-producing bacteria to adhere to the mucus layer [15].
Intestinal bacteria and the immune system

Intestinal bacteria influence the immune system in many ways, as revealed by comparison of germ-free1 mice and conventional mice. In germ-free mice, the Peyer’s patches are underdeveloped compared with conventional mice. Moreover, the lamina propria of germ-free mice contains a decreased number of immunoglobulin A (IgA)-producing cells as well as CD4+ (cluster of differentiation) cells [22]. There are differences in mucosal immunity and in the structure of spleen and lymph nodes, which in germ-free animals are characterized by underdeveloped B- and T-cell zones and low levels of immunoglobulin. Colonization of the intestines of formerly germ-free mice leads to an approximation of these differences.

Despite the high microbial population densities in the intestine, especially in the colon, the immune system does not react to commensal bacteria in the intestine with a strong pro-inflammatory immune response, as caused by a bacterial infection.

Although commensal bacteria are ignored by the systemic immune system as long as they do not overcome the barrier between the intestinal lumen and host tissue, they are detected by the mucosal immune system.

The mucosal immune system

The barrier between the intestinal lumen and the mucosal immune system consists of a single-layered epithelium that is covered by gelatinous mucus. In the small intestine, the mucus has only one layer; in the colon with its high bacterial density there are two mucus layers. The mucus layer directly in contact with the epithelium, to which it is firmly attached, is impermeable to bacteria and thus free of bacteria [23]. Additional elements of the barrier are antibacterial substances such as lysozyme, angiogenin, α-defensin (cryptdin) and RegIII γ, a C-type lectin secreted by the Paneth cells in the small intestine [24–26], as well as IgA. Only small proportions of the intestinal bacteria overcome this barrier and reach the submucosa, where they are effectively killed by macrophages. Translocated bacteria are capable of survival for several days in dendritic cells, but only migrate as far as to the intestinal lymph nodes [22].

In addition, the regulatory T cells (Treg) in intestinal tissue are of major importance for immune homeostasis. Thus, the number of Treg in colon tissue of germ-free mice is considerably lower than in that of colonized mice, and the expression of the anti-inflammatory cytokine interleukin-10 (IL-10) in these cells is reduced [27]. The colonization of these mice with a selected bacterial community of 17 strains was sufficient to boost the number of Treg and the concentration of anti-inflammatory cytokines.

The mucosal immune system detects intestinal bacteria through so-called pattern recognition receptors (PRRs), which are mainly located in immune cells of intestinal tissues and in intestinal epithelial cells. They constitute basic components of the innate immune system but are also involved in the activation of acquired immunity [30]. The best known and most investigated PRR families, in addition to the toll-like receptors (TLR1–TLR11), are the nucleotide-binding oligomerization domain (NOD)-like receptors (NOD1 and NOD2). PRRs detect bacteria or viruses on the basis of conserved molecules. These molecules are, for example, compo-

1 Germ-free animals are completely free of bacteria. They are kept in a germ-free environment (in so-called isolators) in order to protect them from unwanted bacterial colonization.

Time point of colonization with microorganisms and the immune system

An early time point for microbial colonization following birth seems to be essential for certain functions of the immune system. Early contact of the host with microorganisms is associated with a lower incidence of immune-relevant diseases such as inflammatory bowel disease and asthma [28].

In germ-free mice, higher concentrations of invariant killer T-cells (iNKT) are found in the lamina propria of the colon and in lung tissue in comparison to colonized mice [29]. In models for inflammatory bowel disease or asthma, the elevated iNKT level correlated with a higher morbidity and increased expression of the chemokine CXCL16 (CXC motif chemokine 16; ligand of the chemokine receptor CXCR6). When the bacterial colonization of mice was allowed to take place directly after birth, the number of iNKT in the colon and lungs decreased as did the expression of CXCL16. This was paralleled by a lower morbidity of mice in which intestinal disease or asthma had been induced. However, when these mice were kept germ-free for up to five weeks after birth and then colonized, they exhibited the same pathology and likewise elevated numbers of iNKT as in the germ-free mice [29].
ponents of bacterial cells such as liposaccharides (TLR4, see below) and various lipopeptides (TLR1, TLR2, TLR6) as well as flagellin (TLR5), a component of the flagella of motile bacteria, and CpG sequence motifs in the bacterial DNA (TLR9) or virus-specific double-stranded RNA (TLR3). These and other molecules, referred to as pathogen-associated molecular patterns (PAMPS), are ligands of different PRRs, which are classified into different families of receptors.

Whereas the TLRs are located in the cytoplasmic membrane or in the endolysosome, NOD1 and NOD2 are found in the cytoplasm, where they are activated by the bacterial cell wall constituents peptidoglycan and γ-D-glutamyl-meso-diaminopimelic acid. TLRs and NODs act synergistically and are activated by their ligands, which leads to the induction of a pro-inflammatory signaling pathway [30].

Interaction of the immune system with intestinal bacteria

Commensal bacteria that reach the lamina propria are effectively killed by macrophages. The M(microfold) cells found in the epithelium are the main entry portal for intestinal bacteria. Peyer’s patches are clusters of lymph follicles beneath the M cells in the submucosa. Bacteria that reach the Peyer’s patches through the M cells are taken up by dendritic cells. The dendritic cells that contain bacteria and bacterial constituents interact with T and B cells and induce IgA-producing plasma cells. However, the dendritic cells carrying bacteria only reach the lymph nodes of the mesentery, where their further spread is stopped; this prevents the triggering of a systemic immune response [31]. The IgA secreted in the intestine makes up 70 % of the total immunoglobulin produced in the body. In human feces, between one- and three-fourths of fecal bacteria are coated with IgA [32]. The exact role of secretory IgA in the intestine has not yet been clarified because the binding of IgA to commensal bacteria does not result in their elimination but assumedly prevents their translocation into intestinal tissues [33].

Balance between defense and tolerance

The interactions described above illustrate the significance of intestinal microbiota for the maturation of the immune system and the maintenance of its functions. Disturbances in these interactions may be the cause for various diseases. On the one hand, the main role of the immune system is to combat and eliminate bacteria that succeed in overcoming the intestinal barrier. Bacteria that translocate from the lumen into intestinal tissues trigger an inflammatory response of the immune system. This response serves to eliminate the bacteria and prevent their further spread within the organism. On the other hand, the immune system must learn to tolerate or to ignore the presence of commensal bacteria and not to respond with an inflammatory response. Loss of tolerance to intestinal bacteria may lead to inflammatory bowel diseases. The host organism then reacts to the commensal intestinal bacteria with an inflammatory response that leads to destruction of intestinal tissues.

Intestinal bacteria and disease

In recent years there have been reports that different diseases may be linked to changes in the microbiome. However, it was only possible in few cases to show that changes in the microbiome caused the development of certain diseases. In most cases, only correlations between the occurrence of certain bacterial population patterns and a given disease were observed. Animal studies have revealed that microbiota transfer from diseased to germ-free mice resulted in transmission of the bacterial phenotype to the recipient. This applies at least to inflammatory bowel disease and obesity or the metabolic syndrome. For the most part, the underlying mechanisms are not understood.

Chronic inflammatory bowel diseases

Crohn’s disease and ulcerative colitis are recurring chronic inflammatory bowel diseases (IBD). In the past few decades, the incidence of these diseases has increased worldwide. In Europe, the highest prevalence reported per 100,000 persons is 505 cases for ulcerative colitis and 322 cases for Crohn’s disease [34].
It is now assumed that the basis for IBD lies in a disturbed interaction of host and environmental factors [35]. Some of the possible host factors include gene variants identified through association studies, such as those in NOD2, TLR2, TLR4 or in autophagy-related protein 16-1 (ATG16L1). These gene variants can cause an intestinal barrier dysfunction, triggered by environmental factors, and loss of the homeostasis between intestinal bacteria and the immune system (Figure 1).

**Differences in the microbiome**

A number of investigations have demonstrated differences between the microbiomes of healthy subjects and those of IBD patients. The latter not only displayed a diminished microbial diversity but also a shift in the relative proportions of certain members of the bacterial community [36–38].

Several studies have shown that the cell counts of *Faecalibacterium prausnitzii* are consistently lower in the intestines of IBD patients [37, 39, 40]. The oral administration of *F. prausnitzii* or its culture medium supernatant improved the symptoms of trinitrobenzene sulfonate-induced colitis. This anti-inflammatory effect was caused by the inhibition of nuclear factor kappa B (NFκB) activation and a reduced formation of IL-8 [41]. The anti-inflammatory properties may be ascribed to a hydrophobic peptide, which is secreted by this organism and can be isolated from the culture medium supernatant [42]. The oral administration of a recombinant strain of *Lactococcus lactis*, which expresses this peptide, caused an improvement in the symptoms of trinitrobenzene sulfonate-induced colitis in mice. Butyrate may also play a role, since *F. prausnitzii* is involved in its production and this compound also has an anti-inflammatory effect [43]. A protective effect is thus attributed to *F. prausnitzii*. However, it is not yet clear whether the reduced cell counts of *F. prausnitzii* in the intestines of IBD patients are the cause or the result of intestinal inflammation. Whereas the cell counts of intestinal *F. prausnitzii* in IBD patients are lower than those in healthy subjects, those of *Escherichia coli* are higher in patients with Crohn’s disease [44] or in IL-10−/− mice, an animal model for IBD [38]. One reason for the proliferation of *E. coli* in the intestine may lie in the capability of this bacterium to effectively adapt to conditions in the inflamed intestine [45, 46]. Foremost adherent and invasive strains of *E. coli* were found in lesions of the intestinal epithelium of patients with Crohn’s disease [47]. Species of *Salmonella* and *Campylobacter* have also been linked to the development of IBD because individuals previously diagnosed with gastritis caused by *Salmonella* or *Campylobacter* are at higher risk to develop IBD [48]. There are also indications for possible causative roles of *Mycobacterium avium* sub-species *paratuberculosis*, *Helicobacter* spp. and *Fusobacterium varium* [35]. Various clinical studies have reported the incidence of certain bacterial groups in association with IBD [40, 49]. However, the incongruent results of these studies and the observed positive or negative correlations do not allow any conclusions on the possible roles of bacteria. The reason for this is seen in the high inter-individual variability of the intestinal microbiota, so that the patterns of microbiota composition observed in IBD are inconsistent. In addition, it should be kept in mind that the differences in microbiota composition observed in IBD patients compared to healthy subjects must not be causal.

Despite this high variability, it is possible to differentiate between the microbiomes of healthy persons and those of patients with Crohn’s disease or ulcerative colitis by using principal components analysis, without identification of the causative bacteria [9].

The term dysbiosis, reanimated after years of disuse, is used to designate an altered pattern of microbiota composition. This term essentially refers to an abnormal bacterial colonization. However, as long as it is not possible to clearly diagnose dysbiosis, independent of additional symptoms, this term is very imprecise and not very help-ful.

**Dietary factors have an influence on IBD through the microbiome**

In the past decades, the increase in IBD has been accompanied by changes in lifestyle. Since diet is a very important lifestyle factor, there has been a search for correlations between the increased incidence of IBD and dietary factors. Especially in Japan has there been an ever-increasing tendency to shift from traditional toward a Western dietary pattern in recent decades. The ensuing increased consumption of animal fat and protein, of milk and milk products, as well as the reduced dietary intake of rice correlate with a concomitant increase in the incidence of IBD in the Japanese population [50]. Although diet cannot be ruled out as a factor directly impacting IBD development, it is nevertheless more likely that diet has an impact on IBD development by influencing the microbiome.

One possible mechanism how Dietary factors may promote IBD have been studied extensively and conclusively in animal models. Mice in which both alleles of the IL-10 gene have been knocked out (IL-10−/− mice) exhibit a significantly higher susceptibility to develop colitis [51]. IL-10 is an anti-inflammatory cytokine that suppresses the inflammatory response and proteolysis.
motes immune tolerance to intestinal bacteria. In animal experiments, two groups of Il-10⁻/⁻ mice were fed isocaloric high-fat diets. The diet of one group had a high content of polyunsaturated fatty acids (PUFA) content, whereas the diet of the other group contained predominantly saturated fats (milk fat) [52]. The Il-10⁻/⁻ mice with the milk-fat diet displayed not only a higher colitis incidence but also significantly more severe symptoms of inflammation than the mice on the PUFA diet. Further analyses revealed the saturated fats caused a shift in bile acid composition, especially toward a higher proportion of taurine (2-aminoethansulfonate)-conjugated bile salts. Due to the capability of the colitogenic intestinal bacterium *Bilophila wadsworthia* to use the sulfonate group of taurine as an electron donor, growth of the organism was stimulated and cell counts rose significantly (Fig. 2).

For the first time, these experiments verified a connection between a dietary factor and IBD-triggering intestinal bacteria. However, it is uncertain whether these results can be extrapolated to humans.

**Obesity and the metabolic syndrome**

**Differences in the microbiome**

First indications for a possible connection between intestinal microbiota and obesity were the result of a study comparing the microbiota of normal-weight mice and genetically obese mice [53]. Mice with a defective leptin gene (ob/ob) had a 1.8-fold increase in body weight and developed a nearly 8-fold amount of epididymal fat compared with corresponding wild-type mice. At the same time, differences in the cecal microbiota were observed. In the obese ob/ob mice the abundance of bacteria of the phylum Bacteroidetes was reduced to 50 % of that found in lean wild-type mice, and a higher level of Firmicutes was found in the obese mice than in the lean mice. A study with twelve adipose and five normal-weight test persons confirmed that these differences in fecal microbiota composition also apply in principle to humans [54]. When the obese persons were placed on a calorie-reduced diet, the intestinal Bacteroidetes/Firmicutes ratio shifted to that in normal-weight individuals, regardless of whether weight reduction was achieved through a fat- or a carbohydrate-reduced diet.

**Composition of microbiota is influenced by the diet**

The host diet is the main source of nutrients for intestinal bacteria, so diet-dependent shifts in the microbiota are not surprising. It has recently been shown that dietary changes in humans can cause detectable alterations in the microbiota within two days [55]. For intervals of four days each, ten test persons switched from their usual diets to two diets differing considerably in composition. One of the diets consisted mainly of meat, eggs, and cheese whereas the other one was rich in grains, fruit, vegetables and pulses. Compared with the original diets, the animal-based diet had a higher content of fat (69 versus 32 energy percent) and protein (30 versus 16 energy percent), and almost no fiber. In contrast, the plant-based diet was rich in fiber and had a lower fat and protein content than the previous diet. As a result of the animal-based diet, the abundance of bile-tolerant bacteria such as *Bilophila*, *Alistipes* and *Bacteroides* increased, whereas that of plant polysaccharide-utilizing bacteria such as *Ruminococcus*, *Roseburia* and *Eubacterium* decreased.

These experiments also point out that the diet constitutes a very important factor for the intestinal...
The intestinal microbiota because diet not only determines which substrates are available but also has an impact on the intestinal environment (concentration and spectrum of bile acids and fermentation products, pH value, redox potential).

Alteration of the microbiota composition by switching to a high-fat diet is not due to the bacterial utilization of fats as substrates: bacteria are unable to oxidize fats in the anaerobic conditions prevailing in the intestine. Instead, changes in the microbiota are due to an increased release of bile acids, which limit the growth of certain bacteria. The oral administration of cholic acid in rats induced an increase in the Firmicutes/Bacteroidetes ratio [56], similar to the effect previously observed in obese human subjects and mice [53, 54].

**Evidence for the influence of the microbiota on obesity**

Findings were quite surprising that the transplantation of intestinal microbiota from obese mice into germ-free mice resulted in transfer of the obese phenotype [57, 58]. Following transplantation of the fecal microbiota from a healthy human donor to germ-free mice (humanized microbiota), the microorganisms of the recipient mice closely resembled that of the donor. However, depending on the diet administered, the population structure of the intestinal bacteria in the recipient mice gradually changed. This revealed that, in the long term, diet is the decisive factor [59].

Thus, it can be concluded that an obesity-associated microbiota also can be altered by dietary measures.

A high-fat diet promoted the growth of Bacilli and Erysipelotrichi, which both belong to the phylum Firmicutes. Such an effect was not observed when a low-fat diet rich in plant carbohydrates was administered. Approximately two weeks after switching from the high-fat to the low-fat diet, the microbiota of the recipients of an obesity-associated microbiota closely resembled that of mice fed a low-fat diet during the entire test period [59]. This shows that a balanced diet promotes the development of microbiota beneficial to the host.

In summary, it can be concluded that high-energy diets influence the intestinal microbiome such as to enhance the development of obesity [59].

**Explanatory approaches to the interactions between obesity and the microbiota**

Owing to the ability of intestinal bacteria to synthesize fatty acids from indigestible fermentable carbohydrates, the host is provided with additional energy that otherwise would be lost. This may explain why colonized mice, despite their lower feed intake, have a higher body weight and more epididymal fat than germ-free mice [60]. Parallel to this, colonized mice had higher levels of serum leptin, insulin and glucose, and of triglycerides in the liver in comparison with germ-free mice. In agreement with higher glucose levels in the blood, glucose tolerance was diminished and, consistent with the rise in liver triglyceride values, the mRNA levels of the enzymes acetyl-CoA carboxylase and fatty acid synthetase involved in fatty acid synthesis were increased in comparison with the values in germ-free animals [60].

These findings were supported by studies involving identical twins discordant in body weight and body fat, i.e., one twin had an obese phenotype and the other twin, a lean phenotype. Also in these experiments did the transfer of microbiota to the germ-free mice result in transfer of the respective phenotype. Following cohousing of obese and lean recipients after transplantation of intestinal microbiota, the microbiota of the lean phenotype proved dominant by displacing the previously transplanted microbiota of the obese donor in the recipient, and the mice all stayed lean [61]. This finding is consistent with earlier observations which showed that this effect in turn can be modified by diet [59], and speaks for the transferability and diet-dependent modifiability of the microbiota-mediated effects on the host.

In view of these findings, one of the key questions is how the intestinal microbiome promotes or prevents the development of obesity and the symptoms of the metabolic syndrome.

Three mechanisms have been proposed [62] (for further details see • Overview 2, p. 226/227):

1. The intestinal microbiota degrades indigestible polysaccharides to carbohydrate monomers and ferments these mainly to short-chain fatty acids (SCFA). On the one hand the latter provide additional energy to the host and could thereby contribute to obesity development. On the other hand SCFA also possess regulatory functions that could antagonize obesity development [60] (• Figure 3).
2. Intestinal bacteria suppress the formation of angiotensin-like protein 4 (ANGPTL4), also referred to as fasting-induced adipose factor (FIAF). ANGPTL4 inhibits lipoprotein lipase, which plays an important role in the hydrolysis of triacylglycerides in plasma lipoprotein particles. This decrease in the ANGPTL4 level caused by the microbiota leads to an increased lipoprotein
lipase activity and in turn to a higher release of free fatty acids. These are utilized for fat synthesis in the adipocytes, where they are stored as triacylglycerides [60].

3. High-fat diets cause enhanced passage of bacterial lipopolysaccharides (LPS; component of Gram-negative cell wall) from the intestinal lumen into the blood. Consequence of this endotoxemia is a subclinical inflammation accompanied by a rise in blood glucose levels, lowered insulin sensitivity, and a higher proportion of body fat [63].

**Conclusion: the exact role of the intestinal microbiota is still not clear**

Finally, it may be concluded that the exact role of intestinal bacteria in the development of obesity has not yet been resolved. It is conceivable that there are various ways by which the intestinal microbiota can influence obesity development, i.e., there may be different mechanisms. The question still remains to which extent the intestinal microbiota actually contributes to obesity development. It is important to note that the development of an adipogenic microbiota is not accidental but occurs in response to an excessive intake of energy-rich diets. Apparently, obesity development is then further promoted by an adipogenic microbiota.

**The role of intestinal microbiota in other diseases**

There is evidence that intestinal bacteria are also involved in the development of the colorectal cancer, as well as in urolithiasis and autism.

**Colorectal cancer**

Intestinal bacterial could be involved in cancer development in the intestine, on the one hand because of their ability to produce genotoxic substances and tumor promoters, and on the other hand by influencing the immune system [90]. Epidemiological studies indicate that the consumption of red meat with its high heme content promotes the formation of nitroso compounds. These lead to the formation of DNA adducts, which may result in mutations and subsequently in the development of colorectal cancer [91]. Intestinal bacteria are responsible for the anaerobic environment of the intestine, which ensures that heme iron is kept in its reduced form, and this in turn promotes nitrosylation by nitrite or nitric oxide (NO) [92]. Intestinal bacteria such as *E. coli* are capable of reducing nitrite to nitrate. The latter can be reduced further by lactobacilli or bifidobacteria to NO [93]. Nitrate is utilized by plants as a nitrogen source and is stored in plants, especially under conditions of over-fertilization. Nitrate reaches the digestive tract when such plants are consumed. When a high-protein diet is consumed, higher concentrations of indoles and phenols are excreted in the urine. These compounds are products of the bacterial fermentation of aromatic amino acids in the colon [94]. Phenol enhances the nitrite-dependent *N*-nitrosylation of dimethyamine, a further product of bacterial amino acid degradation.

**Fig. 3: Role of the fermentation products propionate und butyrate in stimulation of intestinal gluconeogenesis as well as associated physiological effects**

During its transport through the portal vein, the propionate produced in the colon activates the fatty acid binding receptor 3 in portal vein neurons, thereby stimulating gluconeogenesis in intestinal tissue via a signal to the brain. The butyrate produced by bacteria also stimulates gluconeogenesis in intestinal tissue because its oxidation causes an increase in ATP levels and, in turn, cAMP levels. The latter ultimately stimulates intestinal gluconeogenesis resulting in an increased glucose concentration in the portal vein, which activates the glucose transporter SGLT3, whereby a signal is sent to the brain. This signal exerts a reduction of hunger and of fat storage, an increase of insulin sensitivity and a reduction of glucose production in the liver [77].
### OVERVIEW 2: POSSIBLE MECHANISMS TO EXPLAIN THE INFLUENCE OF THE MICROBIOTA ON THE DEVELOPMENT OF OBESITY AND THE METABOLIC SYNDROME

**To point 1: The contradictory role of short-chain fatty acids**

The degradation of indigestible but fermentable polysaccharides by intestinal bacteria leads to the formation of monomeric carbohydrates, which in turn are fermented primarily to short-chain fatty acids. Intestinal bacteria promote the uptake of the carbohydrate monomers produced. By activating the transcription factors carbohydrate-responsive element-binding protein and sterol regulatory element-binding protein-1, the resulting rise in glucose and insulin levels stimulates lipid synthesis, in which the bacterial product acetate can serve as a substrate [60].

The role of short-chain fatty acids in obesity development has not been fully clarified. On the one hand, short-chain fatty acids provide the host with additional energy and thus promote obesity. On the other hand, there are many indications that short-chain fatty acids counteract obesity development and its accompanying symptoms. Epidemiological studies have demonstrated an inverse correlation between fiber consumption and obesity [64].

Findings that support an obesity-promoting effect show that obese individuals, compared to normal-weight subjects, exhibit higher levels of fecal short-chain fatty acids, especially propionate [65]. This result is consistent with another study in which elevated levels of short-chain fatty acids correlated positively with risk factors for the metabolic syndrome [66]. These findings suggest that short-chain fatty acids contribute to increased body weight by providing additional energy. However, it should be noted that the presence of increased concentrations of short-chain fatty acids in the intestine does not necessarily reflect an increased uptake; theoretically, this could also be the result of diminished absorption. The concentration of fecal short-chain fatty acids apparently depends on the amount of carbohydrates consumed: the fewer the carbohydrates consumed by obese subjects, the lower the concentrations of short-chain fatty acids, especially butyrate, in the feces [67].

Short-chain fatty acids also play a regulatory role in energy metabolism. As ligands of the G-protein-coupled free fatty acid receptors 2 (FFAR2) and 3 (FFAR3), formerly GPR43 and GPR41 [68], their effects are considered more likely to counteract development of the metabolic syndrome. Adipocytes, immune cells and enteroendocrine L-cells of the ileum and colon are equipped with these receptors. When FFAR2 in enteroendocrine L-cells is activated, peptide YY (PYY) [69] and glucagon-like peptide (GLP)-1 [70] are released. In adipocytes, the activation of FFAR3 causes the release of leptin [71]. GLP-1 [70], PYY [73] and leptin [74] all suppress appetite. PYY also extends the intestinal transit time [75] and so improves nutrient resorption. This effect has been confirmed experimentally: the feces of FFAR3-deficient (Ffar3<sup>-/-</sup>) mice had a lower energy content that those of mice with intact FFAR3 (Ffar3<sup>-/-</sup>) [76]. Body weight and body fat of the Ffar3<sup>-/-</sup> mice were 30 % and 25 % lower, respectively, than those of Fhar3<sup>+/+</sup> mice. However, these differences were only observed in conventional mice but not in germ-free animals.

These findings suggest that the short-chain fatty acids produced by bacteria serve to activate FFAR3, in which case the release of PYY causes lengthening of intestinal transit time and thus allows an efficient uptake of nutrients. This effect contradicts findings, based on epidemiological studies, that there is an inverse correlation between fiber consumption and obesity [64]. Since FFARs have different effects, these must be assessed as a whole.

Recent research impressively demonstrates that the short-chain fatty acids produced by bacteria, propionate and butyrate in particular, unfold their positive effects on glucose metabolism and energy homeostasis by activating neuronal circuits [77]. These effects are mediated by intestinal gluconeogenesis, which is stimulated by butyrate and propionate via two complementary mechanisms. Independent of FFAR2, intestinal gluconeogenesis is activated by butyrate via elevation of the cAMP level, which activates the expression of gluconeogenesis genes. The higher cAMP levels originate in turn from elevated concentrations of ATP, the result of an increased utilization of butyrate as energy substrate in enterocytes [78]. Propionate on the other hand serves as an agonist of FFAR3, which promotes gluconeogenesis in the afferent periportal nervous system via a gut-brain neural circuit [77] (Figure 3).

In overweight subjects given a 10 g oral dose of an inulin-propionate ester to cause preferential release of propionate in the colon, the plasma concentration of propionate was nearly twice as high as in overweight control subjects given unesterified inulin [79]. The higher propionate level was accompanied by a short-term elevated release of GLP-1 and PYY and reduced energy intake. After 24 weeks, administration of the inulin-propionate ester in the intervention group caused reductions in weight gain and in abdominal and hepatic fat, compared to the control group. During this period, the concentrations of GLP-1 and PYY were no longer elevated in the group given the ester. The authors concluded that the underlying mechanisms responsible for this positive long-term effect of propionate are independent of GLP-1 and PYY [79].
To point 2: A rise in lipoprotein lipase activity enhances the release of free fatty acids

The colonization of germ-free mice with the microbiota of conventional mice caused a 60% increase in body fat within two weeks, compared with mice that remained germ-free, reduced levels of Angptl4 mRNA in intestinal tissue as well as an increase in lipoprotein lipase activity in white adipocytes and heart tissue [60]. Since germ-free Angptl4+/− mice have the same amount of body fat as conventional wild-type mice (Angptl4+/+) of the same age, and because the former mice gained much more body weight and body fat when fed a high-fat diet than the germ-free Angptl4−/− mice, it was concluded that this increase in body weight and body fat was mediated by the ANGPTL4 produced in intestinal tissues of the wild-type mice. An independent study confirmed the presence of elevated Angptl4 mRNA levels in the intestinal mucosa of germ-free mice, but the plasma concentrations of ANGPTL4 in these mice were not higher than in colonized mice, as revealed by Western blot analyses [80]. These results and the fact that ANGPTL4 also exerts other functions and plays a role in angiogenesis, for example, oppose the idea that ANGPTL4 serves as an inhibitor of lipoprotein lipase [81].

To point 3: Subclinical inflammation and bacterial lipopolysaccharides

Individuals with type 2 diabetes mellitus, in comparison to controls, exhibit not only an increased level of insulin and lipopolysaccharides (LPS) in plasma but also an elevated expression of TLR2 and TLR4, IL-6 and nuclear factor kappa B (NFκB) [82], components of the innate proinflammatory immune response. LPS, a conserved component of Gram-negative bacteria, activates as a ligand of TLR4 the release of proinflammatory cytokines [83]. LPS thus serves as a signal for activating an inflammatory response and for mounting a defense against bacteria. It is interesting to note that overeating also causes metabolic stress and activation of proinflammatory signaling pathways [84]. After consuming high-fat meals, test persons actually do exhibit a significantly higher LPS level in plasma [85]. This was also the case in mice fed a high-fat diet for four weeks: the LPS concentration increased two- or three-fold—a condition referred to as metabolic endotoxemia—an effect that was reversed under fasting conditions [63].

It was then suggested that LPS is the trigger for various symptoms of the metabolic syndrome. In fact, a continuous subcutaneous infusion of LPS in mice over a period of four weeks caused an increase in glucose and insulin levels in plasma and of body weight and body fat. This was accompanied by an intensified release of proinflammatory cytokines and signs of hepatic insulin insensitivity [63]. The authors of these studies came to the conclusion that high-fat diets promote the transit of LPS from the intestinal lumen into the blood. On the other hand, it is not very likely that a higher incidence of obesity correlates with a relative decrease in the concentration of Gram-negative Bacteroidetes and is linked to a relative increase in Gram-positive Firmicutes [54]. One of the roles of LPS in development of metabolic diseases is supported by an investigation in which germ-free mice were colonized with an LPS-producing Enterobacter cloacae strain, which had been isolated from an obese person [86]. Germ-free mice colonized with this strain developed obesity and insulin resistance on a high-fat diet, whereas mice that remained germ-free or were monoassociated with Bifidobacterium animalis did not show these symptoms. However, these differences became apparent only after more than ten weeks [86].

Although this study suggests that the deterioration of metabolic parameters triggered by Enterobacter cloacae was mediated by LPS, experiments in another gnotobiotic mouse model revealed that also in a Gram-positive bacterium incapable of LPS production may promote obesity. Clostridium ramosum (a member of the Erysipelotrichi mentioned above), is capable of causing an increase in body weight and body fat accompanied by elevated hepatic triglyceride values in gnotobiotic mice [87]. Mice in one group were colonized with a simplified microbiota consisting of eight bacterial species including C. ramosum whereas the other group was associated with the same species except for C. ramosum, which was absent in this group. Both groups were fed the same high-fat diet, which differed neither in energy content nor in digestibility. Differences in intestinal permeability and plasma LPS concentrations were not observed. However, the mice colonized with C. ramosum exhibited an increased gene expression of the glucose transporter 2 (Glut2) in the jejunum and of the fatty acid translocase (Cd36) in the ileum. This result suggests that the effect of C. ramosum is based on a stimulation of the efficiency of nutrient uptake. It could be ruled out that the short-chain fatty acids produced by bacteria or the elevated level of LPS played a role in enhancing obesity development on a high-fat diet [87]. The fact that C. ramosum contributes to obesity development is supported by data from human studies, which revealed an association between the incidence of the bacterium and parameters of the metabolic syndrome [88, 89].
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Fusobacterium nucleatum produces the adhesin FadA, which promotes formation and growth of colorectal tumors by binding to the calcium ion-dependent transmembrane glycoprotein E-cadherin, an adhesion protein, and influences cell communication via β-catenin (cadherin-associated protein β1) [100]. From 10- to 100-fold higher levels of FadA are found in colon tissues of patients with colorectal adenoma and adenocarcinoma, which correlates with the increased expression of inflammatory genes and oncogenes.

Bacteroides fragilis produces an enterotoxin with proteolytic activity, referred to as Bacteroides fragilis toxin (BFT) [101]. This zinc-dependent metalloprotease cleaves the extracellular domains of E-cadherin in intestinal epithelial cells, leading to the complete degradation of this glycoprotein. The β-catenin associated with the cytoplasmic domains of E-cadherin is thus released to activate the transcription and translation of the oncogene c-myc in the cell nucleus in interaction with the T-cell factor. As a result, cell proliferation is accelerated, increasing the tendency toward tumor formation [102].

Various components of the innate immune system influence the composition of the intestinal microbiota. Mice lacking NOD2, for example, develop intestinal inflammation and tumors as well as an altered intestinal microbiota [103]. When the intestinal microbiota of these mice is transplanted to germ-free wild-type mice, the recipients display an increased tendency toward intestinal inflammation and tumors. Also mice lacking caspase-1 or other components of the inflammasome, such as NOD-like receptor family pyrin domain-containing 6 (NLRP6), show an enhanced tendency toward intestinal inflammation and tumor formation [104, 105]. Transplantation of the microbiota of these mice to germ-free wild-type mice, the recipients display an increased tendency toward intestinal inflammation and tumors. Also mice lacking caspase-1 or other components of the inflammasome, such as NOD-like receptor family pyrin domain-containing 6 (NLRP6), show an enhanced tendency toward intestinal inflammation and tumor formation [104, 105].

Autism

Autism is a congenital, incurable impairment of perception and information processing. Clinical and epidemiological studies suggest that autists have an increased prevalence of inflammatory and other intestinal disorders [108] as well as a higher intestinal permeability and altered intestinal motility, in comparison to control subjects [109]. Autistic children differ in both their microbiota and their metabolome [110]. It is still unresolved whether these differences can be considered a consequence or cause of the disorder. However, it has been hypothesized that the altered intestinal permeability allows substances produced by the microbiota in the intestine to reach the brain and cause damage. In fact, it was shown in a mouse model for autism that the abnormal behavior of the offspring of such animals improved after they were colonized with a commensal B. fragilis strain, and this was accompanied by a reduced intestinal permeability and an altered serum metabolome profile [111]. In untreated animals, the concentrations of some metabolites were several times higher than those in the B. fragilis-colonized animals. The systematic administration of one of these metabolites, 4-ethylphenylsulfate, to healthy wild-type mice resulted in behavior similar to that seen in the autistic mice [111].

Urolithiasis

Oxalate is consumed together with foods such as amaranth, spinach and rhubarb on the one hand, and is also a product of human metabolism arising, for example, from the oxidation of glyoxylate. High levels of oxalate can cause formation of kidney stones of calcium oxalate (urolithiasis). Oxalate is mainly excreted in the urine; however, the oxalate-degrading intestinal bacterium Oxalobacter formigenes reduces the concentration of oxalate reaching the blood [112]. The absence of this bacterium has been linked to a
higher risk for the formation of calcium oxalate stones in the urinary tract [113].

Closing remarks

The intestinal microbiota constitutes a complex community of microorganisms whose composition is significantly influenced by diet. The immense metabolic potential of the intestinal microbiota as well as its interactions with the immune system have a variety of effects on the physiology of the host. Disturbances in the harmonious interactions of the microbiota and host may be the causes of various diseases. In recent years it has become increasingly clear that the role of the intestinal microbiota in health has long been underestimated. A healthy diet not only serves to provide nutrients for the host organisms but also to maintain a health-promoting intestinal microbiota.

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