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# Impact of the gut microbiota on rodent models of human disease

HansenAK *et al.* Gut microbiota and rodent models

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## Abstract

Traditionally bacteria have been considered as either pathogens, commensals or symbionts. The mammal gut harbors 1014 organisms dispersed on approximately 1000 different species. Nowaday diagnostics in contrast to previous cultivation technqiues allow identification of close to 100% of bacterial species. This has revealed that a range of animal models within different research areas, such as diabetes, obesity, cancer, allergy, behavior and colitis, are affected by their gut microbiota. Correlation studies may for some diseases show correlation between gut microbiota composition and disease parameters higher than 70%. Some disease phenotypes may be transfered when recolonizing germ free mice. The mechanistics aspects are not clear, but some examples on how gut bacteria stimulate receptors, metabolism, and immune responses are given in the paper. A more complicated understanding of the microbiota impact has it’s origin in both the more over all composition of the microbiota as well as in some newly recognized species, such as *Akkermansia muciniphila,* Segmented filamentous bacteriaand *Faecalibacterium prausnitzii,* which by themselves seem to have an impact on the expression of more or less disease in specific models. It is concluded that the impact the microbiota has on animal models is of a magnitude that cannot be ignored in future research, and therefore either models with specific microbiota must be developed, or the microbiota must be characterized in individual studies and incorporated in data evaluation.

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**Key words:** Animal models; Gut microbiota; Diabetes; Obesity; Cancer; Allergy; Behavior; Colitis

**Core tip:** Full characterisation of the gut microbiota of animal models has revealed that a range of animal models within different research areas, such as diabetes, obesity, cancer, allergy, behavior and colitis, are highly affected by their gut microbiota. The mechanistic aspects are not fully clear, but the impact the microbiota has on animal models is of a magnitude that cannot be ignored in future research, and therefore either models with specific microbiota must be developed, or the microbiota must be characterized in individual studies and incorporated in data evaluation.

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## INTRODUCTION

### *Host-microbiota relationship*

The gut is an ideal incubation chamber for bacteria adapted to the mammal body temperature and the anaerobic environment. Thousands of years of co-existence has led to such adaptation, and the mammal gut harbors 1014 organisms dispersed on approximately 1000 different species dependent on how cut-offs are set for similarity. Within the traditional approach to laboratory animal bacteriology, bacteria have been considered as either pathogens, commensals or symbionts, but there seems to be a need for a broader understanding of this. When first inside the gut, the bacteria will be fed and will be allowed to propagate, while the host organism will benefit from otherwise unavailable products of the microbial digestion. Generally, pathogenicity is not in the interest of the microorganism, because it induces a strong and eradicating immune response from the host, and even in the case of microbial victory in this battle, this may result in the death of the host and the need for the microbe to relocate to a new habitat. The host immune system on the other hand needs to protect the host from invasion without being that aggressive that it loses the microbe and thereby all the benefits of it.

### *Complexity of microbial impact on the host*

A more advanced understanding of the impact of the microbiota takes into consideration both the more over all composition and the balance between the members of the microbiota as well as some newly recognized species, which by themselves seem to have an impact on the expression of specific models. Some of these have a symbiotic effect while others push disease development in a more detriminal direction. However, it may also be observed that the same species may act in favour of the development of one disease, while still being more protective against another disease, and the mechanistic potential of the species may differ between different parts of the gut. For most of these bacteria it is the abundance of them rather than it is the qualitative presence or absence of them, which are responsible for their impact on the host[[1-4](#_ENREF_1)]. The microbiota is normally not very diverse in the upper part of the gut, *e.g.* in the ileum where there is a huge accumulation of lymphatic tissue available for stimulation[[3](#_ENREF_3),[5-10](#_ENREF_5)]. It gradually becomes more diverse as the gut contents passes thorugh the large intestine and becomes feces (Figure 1)[[3](#_ENREF_3),[5-11](#_ENREF_5)]. In both man and mouse a microbiota with a low diversity is indicative of an increased risk of developing inflammatory disease[[12](#_ENREF_12),[13](#_ENREF_13)]. Further, in animals a microbiota that is pretty similar in the upper part of the gut, may differ essentially in the lower part of the gut and vice versa[[3](#_ENREF_3),[14](#_ENREF_14)]. Finally, there might be essential differences between the impact of the various species at different ages of the animals, which may explain why some species may favor the development of one disease while protecting against another.

### *Modern microbiological identification techniques*

Over the last decades new methods based upon molecular biology diagnostics have been developed. Such methods, which includes multiple quantitative polymerase chain reaction (qPCR) assays[[15](#_ENREF_15)], pyrosequencing[[16](#_ENREF_16)] and metagenomic sequencing, allows identification of close to 100% of the gut’s operational taxonomic units (OTU), which include both cultivable and non-cultivable, bacterial species, and in principle also viral, eukaryotic and archea[[17](#_ENREF_17)], though they are seldomly specifically tested for at present. In contrast, previous cultivation techniques only allowed cultivation and identification of 10%-20% of the bacterial species present in the gut[[18](#_ENREF_18)]. These molecular biology based tools have enabled detailed correlation studies. Such studies have revealed that a range of animal models within a range of different research areas are affected by their gut microbiota[[19](#_ENREF_19)].

## GENERAL MECHANISMS BEHIND GUT MICROBIOTA IMPACT

As described below, the impact of the microbiota on animal models is well documented, while the mechanisms behind this is less clear. Some hypotheses, though, make more sense than other. As techniques for the full characterisation of the microbiota have been developed over the last decade, we are only in the very beginning of achieving an understanding on how the microbiota actually exerts its impact on the host, but some examples can be given.

### *Window of opportunity*

In the early life there is a window for the induction of *oral tolerance* in the gut[[20](#_ENREF_20)]. This seems essential to avoid inflammatory disease later in life[[21](#_ENREF_21)]. Molecular structures in bacteria known as *Microbial-associated molecular patterns* *(MAMP)* stimulate pattern-recognition receptors (PRR) in the host and thereby induce innate responses[[22](#_ENREF_22)]. Among the most important PRRs are the Toll-like receptors (TLR) present in different types on a range of different cell types[[22-29](#_ENREF_22)] (Figure 2). An important example of a MAMP is lipopolysaccharides (LPS), which are important parts of the cell wall of Gram negative bacteria[[30](#_ENREF_30)], such as Proteobacteria[[31](#_ENREF_31)] from which it is known to stimulate TLR4. Another important example is peptidoglycan found in the cell walls of Gram positive bacteria and known to stimulate TLR2[[32](#_ENREF_32)] and flagellin deriving from flagellated bacteria, which is known to stimulate TLR5[[33](#_ENREF_33)]. Therefore, as different types of MAMP’s stimulate different TLR’s dispersed on a variety of different cell types[[23](#_ENREF_23)], and as MAMP’s are also dispersed and shared between members of the microbiota[[22](#_ENREF_22)] there is a vast majority of innate host responses to bacteria.

### *Adult life stimulation*

Also the age of the animal makes a difference. For example, stimulation of TLR1, TLR2 and TLR4 in early life leads to higher production of interleukin (IL)-6 than stimulation later in life[[34](#_ENREF_34)].Germ free animals have more t helper cells type 2 (TH2) and less TH1 cells[[35](#_ENREF_35)], as the stimulation of the gut lamina propria dendritic cells, *e.g.* by polysaccharide A (PSA) from *Bacteroides fragilis,* induces IL-12 secretion, which favors TH1 on the cost of TH2[[36](#_ENREF_36)]. Host–bacterial interactions, probably mediated through glucagon-like peptide 2 (GLP-2), seem to control the gut barrier function[[37](#_ENREF_37)]. *Metabolic endotoxaemia* is the phenomenon occurring when excess intake of dietary fat increases plasma LPS levels[[38](#_ENREF_38),[39](#_ENREF_39)], which in mice is a sufficient molecular mechanism for triggering metabolic diseases such as obesity and diabetes[[40](#_ENREF_40)].

## EXAMPLES OF THE ANIMAL MODELS UNDER IMPACT OF THE GUT MICROBIOTA

### *Impact of germ free status*

The clearest documentation of a general microbial impact on rodent models are observed when comparing a conventional model with a microbiota with a germ free version. In several studies this has revealed essential differences in disease expression (Table 1)[[22](#_ENREF_22),[41-57](#_ENREF_41)]. Although germ free mice eat more they are leaner, and they have less body fat compared to conventional mice because they are less efficient in extracting energy from the diet[[50](#_ENREF_50)]. Germ free mice have increased expression of obesity-related peptides, such as glucagon-like peptide 1 (GLP-1) in the brain[[58](#_ENREF_58)], which is relevant as central GLP-1 reduces food intake in rats[[59](#_ENREF_59)]. Germ free mice also behave differently from microbiota harbouring mice and this behavior may be normalized by colonization[[43](#_ENREF_43)], although also for this phenotype there seems to be an important time window in early life[[60](#_ENREF_60)]. If germ free, mice with a mutant defect in the skin barrier suffer from a more severe B-lymphoproliferative disorder, because they express significantly higher levels of the proinflammatory cytokine thymic stromal lymphopoietin[[61](#_ENREF_61)]. Inflammatory bowel disease (IBD) occurs due to either a TH1/TH17 response (Crohn’s disease) or a TH2 response (ulcerative colitis) to gut commensals[[62](#_ENREF_62)]. Therefore, IBD under germ free conditions does not develop at all in *e.g.* Human Leucocyte Antigen subtypes B27 (HLA-B27) transgenic rats[[53](#_ENREF_53)] and IL-10 knockout mice[[56](#_ENREF_56)], and for the IL-10 knockout mice[[63](#_ENREF_63)] not even under barrier protected conditions (Table 1). IL-2 knockout mice may under germ free conditions show mild focal intestinal inflammation[[64](#_ENREF_64)] (Table 1).

### *Impact of fluctuations in the gut microbiota composition*

Within animal models of the metabolic syndrome there seems to be an association between the gut microbiota and at least some of the metabolic parameters. For example, in leptin-deficient obese mice there is a strong correlation between glycated hemoglobin levels and the composition of the gut microbiota[[1](#_ENREF_1)]. Further, these mice have significantly more Firmicutes and fewer Bacteriodetes members compared to their wild type and heterozygous litter mates[[10](#_ENREF_10)]. Their obese phenotype may be transferred with the microbiota by recolonizing germ free lean wild type mice[[65](#_ENREF_65)]. In C57 Black substrain 6 (C57BL/6) mice on both high and low calory diet continous oral ampicillin improves glucose tolerance[[66](#_ENREF_66),[67](#_ENREF_67)]. However, this effect is mainly due to an early life impact on the glucose tolerance, and the effect ceases immediately after termination of treatment, and hereafter the glucose tolerance may even decrease[[68](#_ENREF_68),[69](#_ENREF_69)]. Several studies describe a cross-talk between the brain and the gut through both the vagal system and the hypothalamus-pituitary-adrenal (HPA) axis[[70](#_ENREF_70)]. Stressing animal models changes their microbiota[[71](#_ENREF_71)], and it is well described that the composition of the gut microbiota, has an impact on responses in rodent stress tests[[72](#_ENREF_72),[73](#_ENREF_73)]. Innate immune system cytokines, such as IL-1, IL-6 and tumor necrosis factor α (TNFα), which may very well originate from a gut microbiota provocation, induce *“sickness behavior”*, change the priorities of the organism to enhance recovery and survival[[74](#_ENREF_74)], but metabolites formed by microbial decomposition in the gut may also have a direct impact on the brain[[75](#_ENREF_75)]. In mouse models of atopic dermatitis more than 70% of the variation observed in the local tissue cytokine response may be shared with the variation in gut microbiota[[76](#_ENREF_76)]. Changes in the structure of the microbial community seem to reduce the number as well as the size of tumors in azoxymethane/dextran sodium sulfate (AZO/DSS) colon cancer induced mice, and tumor induction may be achieved by colonizing germ free mice with a microbiota from induced mice[[77](#_ENREF_77)].

## EXAMPLES OF THE IMPACT OF SPECIFIC BACTERIAL SPECIES

### *Verrucomicrobioa*

*Akkermansia muciniphila* is a Gram negative bacterium, which in mice is the only species belonging to the phylum Verrucomicrobia[[78](#_ENREF_78)]. It interacts by its mucin degrading capabilities with enteroendocrine cells to modulate gut barrier function and it is capable of producing certain short chain factty acids (SCFA’s) with a direct action on the receptor G-protein receptor (GPR43)[[79](#_ENREF_79)]. Abundance is reduced in mice with obesity and type 2 diabetes[[80](#_ENREF_80)], and it gradually disappears as leptin deficient obese mice with age develop insulin resistance[[1](#_ENREF_1)]. In non-obese diabetic (NOD) mice it becomes more abundant when feeding mice a gluten-free diet, which is known to decrease incidence of type 1 diabetes[[81](#_ENREF_81)]. Early life treatment with vancomycin in NOD mice propagates it to become a dominating gut microbiota member, which reduces the incidence of type 1 diabetes[[3](#_ENREF_3)], but enhances susceptibility to allergic asthma[[82](#_ENREF_82)], which is in accordance with other studies showing allergy and diabetes to be counteracting one another in NOD mice[[83](#_ENREF_83),[84](#_ENREF_84)]. Induction of IBD in mice with dextran sodium sulphate (DSS) reduces the number of extracellular vesicles derived from *A. muciniphila*, and feeding DSS induced mice such vesicles reduces the severity of IBD[[85](#_ENREF_85)], which fits well with observations in humans[[4](#_ENREF_4)]. However, it is not only reducing severity of diseases, as it’s presence is correllated to a higher severity when infecting mice with *Salmonella typhimurium*[[86](#_ENREF_86)], and AZO/DSS colon cancer induced mice have an increased abundance of *A. muciniphila*[[77](#_ENREF_77)], which may be explained by it’s ability to downregulate the natural killer cell receptor, NKG2D, which is part of the anti-cancerogenic defence[[87](#_ENREF_87)].

### *Firmicutes*

Segmented filamentous bacteria (SFB’s) are clostridia-related Gram-positive bacteria[[88](#_ENREF_88)]. The term has been applied for decades for description of intestinal bacteria of a uniform morphology more precisely named as *Candidatus* *Arthromitus*[[89](#_ENREF_89)], but today the term refers to one single species, also known as *Candidatus* *Savagella*[[90](#_ENREF_90)]. SFB’s induce secretion of the pro-inflammatory cytokine IL-17 from TH17 cells[[91](#_ENREF_91)], which in the more adult mouse is correlated to a low number of regulatory T cells[[92](#_ENREF_92)]. The presence of SFB’s differ between mice from different vendors[[92](#_ENREF_92)], and SFB positive NOD mice have a significantly lower incidence of type 1 diabetes compared to negative ones[[93](#_ENREF_93)]. In the adoptive transfer severe combined immune deficiency (SCID) mouse model of IBD SFB’s are essential for the induction of severe inflammation[[48](#_ENREF_48)]. SFB’s and the induced TH17 is furthermore important in the defense against intestinal pathogens. *E.g.* mice infected with *Citrobacter rodentium*, a potent murine colon pathogen, exhibit severe symptoms if lacking SFB’s[[91](#_ENREF_91)].

IBD in IL-10 knockout mice is enhanced by *Enterococcus fecalis*[[94](#_ENREF_94),[95](#_ENREF_95)], which is probably linked to it’s production of gelatinase[[96](#_ENREF_96)].

*Faecalibacterium prausnitzii* is a clostridia-related bacterium[[97](#_ENREF_97)] linked to a protective effect against human Crohn’s disease[[98](#_ENREF_98)]. Oral feeding of *F. prausnitzii* reduces the severity of 2,4,6-trinitrobenzenesulfonic acid (TNBS) induced colitis in mice, and some studies indicate that this may also be the case in both multidrug resistance gene deficient (mdr1a knockout)[[99](#_ENREF_99)] and in the DSS induced mouse models of colitis[[100](#_ENREF_100)].

High abundances of *Lactobacillus* spp.and bifidobacteriaare strongly correlated to low levels of inflammation in mice[[101](#_ENREF_101)] and leptin in rats[[102](#_ENREF_102)], which also fits well with these bacteria acting protective against IBD in IL-10 knockout mice[[103](#_ENREF_103)], allergic sensitation in mice[[104](#_ENREF_104)], and myocardial infarction in rats[[102](#_ENREF_102)]. *Lachnospiraceae* seems quantitatively correlated to improved glucose tolerance in leptin-deficient obese mice[[1](#_ENREF_1)].

In stressed mice there is correlation between their Firmicutes levels and their responses in stress tests[[73](#_ENREF_73)], and ingestion of *Lactobacillus rhamnosus* in mice regulates their emotional behavior and central γ-aminobutyric acid (GABA) receptor expression via the vagus nerve[[72](#_ENREF_72)].

### *Bacteriodetes*

A high abundancee of the Gram negative family Prevotellaceae, maybe restricted to one unclassified genus, in the gut of leptin deficient obese mice correlates to an impaired glucose tolerance[[1](#_ENREF_1)], while it seems to be opposite in AZO/DSS induced colon cancer in mice, in which a high abundance of Prevotellaceae correlates to a low tumor burden[[77](#_ENREF_77)]. *P. copri*, which has been correlated to the development of arthritis in humans, seems to increase the severity of DSS induced colitis in mice[[5](#_ENREF_5)]. *Caspase-3* knockout mice exhibits a lower inflammatory response to DSS induction of colitis compared to wild type mice, but this protective effect of the mutation is decreased by cohousing knockout mice with wild type mice which significantly increases the abundance of *Prevotella* spp. in the knockout mice[[105](#_ENREF_105)].

*Bacteroides vulgatus* seem to enhance IBD in HLA-B27 transgenic rats[[106](#_ENREF_106)] and IL-10 knockout mice[[95](#_ENREF_95)], and also in the Bio Breeding (BB) rat, a spontaneous type 1 diabetes model, the fecal microbiota differ and contains an increased number of *Bacteroides* spp. prior to diabetes onset[[107](#_ENREF_107)].As in all other mammals, *Bacteroides* spp. form an important part of the Bacteroidetes fraction of the rodent gut[[16](#_ENREF_16)]. These Gram negative bacteria are important for the processing of complex molecules to simpler ones in the gut[[108](#_ENREF_108)], and complex glycans are their key source of energy[[109](#_ENREF_109)]. *B. fragilis* toxins cause symptoms of diarrea and IBD in germ-free mice[[110](#_ENREF_110)], and they strongly induce colonic tumors in multiple intestinal neoplasia (MIN) mice[[111](#_ENREF_111)]. On the other hand, *B. fragilis* PSA, which is important for the inflammatory gut response to pathogens[[36](#_ENREF_36)], also protects against *Helicobacter hepaticus* induced colitis in mice; probably due to the prevention of IL-17 secretion[[112](#_ENREF_112)]. Feeding the maternal immune activation (MIA) mouse model *B. fragilis* reduces symptoms of autism, which is probably linked to the normalisation of the levels of a specific gut metabolite[[113](#_ENREF_113)].

The abundance of *Alistipes* spp., a bacterium of the Rikinellaceae family, seems to increase when stressing mice by grid floor housing[[73](#_ENREF_73)].

### *Proteobacteria*

*Escherichia coli* has been shown to enhance IBD in HLA-B27 overexpressing rats[[106](#_ENREF_106)], although it is has also been shown that *E. coli* Nissle stabilizes the enteric barrier in mice[[114](#_ENREF_114)]. When reducing type 1 diabetes by pre-weaning treatment of NOD mice with vancomycin one of the observations in the puppies is a vast increase in the abundance of Proteobacteria[[3](#_ENREF_3)].

### *Actinobacteria*

*Bifidobacterium* spp. in rodents are known to have a positive impact on the regulatory and innate immunity[[101](#_ENREF_101),[115](#_ENREF_115)]. Perinatal supplementation of *B. longum* reduced TH1 and TH2 responses in allergen sensitized mice[[104](#_ENREF_104)]. On the other hand, they are also increased in abundance in gluten-fed NOD mice with a high incidence of type 1 diabetes compared to NOD mice on a gluten-free diet[[81](#_ENREF_81)].

## DISCUSSION

The knowledge achieved over the last decade on how the entire microbiota as well as some of its individual members have an impact on animal models of very different types, forces the scientific community to incorporate this in future production and quality assurance of animal models. It is not possible to regard these matters from a ‘Specific pathogen-free’ concept, as some of the species act in favour of the development of one disease, while against the development of another disease, *e.g.* when SFB’s both protect against type 1 diabetes and induces a TH17 response in favour of development of *e.g.* Crohn’s disease. Furthermore, the balance between the different fractions of the microbiota is also likely to make a difference, and finally, it is often quantitative rather than the qualitative presence that makes the difference. It is, therefore, likely that we will see more tailor-made rodent models, i.e. commercial breeders and research groups have aimed at producing animals with a microbiota specifically prone to showing exactly what they want to show. One obvious idea may be to breed such animals by selective breeding, which, however, does not seem to increase microbiota similarity, although the microbiota of offspring show a clear clustering with the mother’s microbiota[[116](#_ENREF_116),[117](#_ENREF_117)]. It is probably a more applicable tool to inoculate germ free mice with a tailor-made microbiota around weaning as they are conventionalized in SPF conditions[[118](#_ENREF_118)]. The window for induction of oral tolerance may in animal models also be turned around so that a low bacterial stimulation in the open phase of this window may be essential for development of target diseases in the model, and when stimulated later on, the nature of this stimulation is also essential as commonly used disease models in rodents are driven by specific subsets of T cells[[19](#_ENREF_19)]. Another alternative will be to characterize the microbiota composition for animals in sensitive studies and incorporate this in the data evaluation by chemometric or multifactorial statistical means. The impact of the gut microbiota on our animal models is of a magnitude that we cannot afford to neglect in the future.

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**Table 1 Examples of rodent models in which germ free status has a documented impact**

|  |  |  |
| --- | --- | --- |
| **Model** |  | **Disease** |
| Models with increased disease incidence or severity |
| β-lactoglobulin induced mouse[[51](#_ENREF_51)] | Allergy |
| NOD mouse[[42](#_ENREF_42)] | Type 1 diabetes |
| MyD88 KO NOD mouse[[42](#_ENREF_42)] |
| Restrained mouse[[43](#_ENREF_43)] | Stress |
| Models with decreased disease incidence or severity |  |
| Ovalbumin-specific TCR TG mouse[[44](#_ENREF_44)] | Allergy |
| Swiss-Webster mouse [[45](#_ENREF_45)] | Anxiety |
| Collagen induced rat[[52](#_ENREF_52)] | Arthritis |
| HLA-B27 TG rat[[53](#_ENREF_53)] |
| IL-2 KO mouse[[54](#_ENREF_54),[55](#_ENREF_55)] | Inflammatory bowel disease |
| IL-10 KO mouse[[56](#_ENREF_56)] |
| TCRα KO mouse[[57](#_ENREF_57)] |
| Dextran sulphate sodium induced mouse[[46](#_ENREF_46)] |
| SAMP1/Yit mouse[[47](#_ENREF_47)] |
| Adoptive T-cell transfer in the mouse[[48](#_ENREF_48)] |
| Carrageenan, LPS, or formalin induced mouse[[49](#_ENREF_49)] | Inflammatory pain |
| C57BL/6 mouse[[65](#_ENREF_65)] | Obesity |
| Type 2 diabetes |

NOD: Non-obese diabetic; MyD88: Myeloid differentiation primary response gene 88; KO: Knockout; TCR: T cell receptor; TG: Transgenic; HLA-B27: Human leucocyte antigen subtype B27; IL-2: Interleukin 2; SAMP1/Yit: Senecence accelerated mice prone line 1 Yakult; LPS: Lipopolysaccharide.

**Figure 1 The approximate composition of the gut microbiota in the ileum, caecum and faeces of mice[**[**3**](#_ENREF_3)**,**[**7**](#_ENREF_7)**,**[**8**](#_ENREF_8)**,**[**11**](#_ENREF_11)**,**[**16**](#_ENREF_16)**].**

**Figure 2 Examples on some theories on potential pathways for the impact that the gut microbiota may have on animal models of human disease.** Bacterial colonization may double the density of capillaries in the small intestinal epithelium thereby promoting intestinal monosaccharide absorption[[28](#_ENREF_28)]. Undigested food components may be fermented into SCFAs and subsequently act as signals for GPR of importance for the development of obesity[[26](#_ENREF_26),[29](#_ENREF_29)]. Bacteria may express several key enzymes relevant for hepatic lipogenesis[[27](#_ENREF_27),[50](#_ENREF_50)], and hepatic and muscular fatty acid oxidation[31]. Molecular structures in the cell walls of bacteria may act as MAMP, which stimulates TLR on the host cells to induce innate immune responses. The complex of TLR1, TLR2, TLR6 and TLR10 is expressed on a range of cell types such as enterocytes, macrophages, dendritic cells, natural killer cells, mast cells, T cells, B cells, neutrophilic cells and Schwann cells and may be stimulated by various MAMPs, *e.g.* peptidoglycan, from Gram positive bacteria cell types[[21](#_ENREF_21),[23-25](#_ENREF_23),[31-33](#_ENREF_31)]. TLR4 expressed by *e.g.* macrophages, dendritic cells, mast cells, natural killer cells and and enterocytes is stimulated by lipopolysaccharides Gram negative bacteria[[30](#_ENREF_30)], while flagellin from various bacteria may stimulate TLR5 expressed by *e.g.* mucosal dendritic cells and macrophages[[33](#_ENREF_33)]. Mucin-degrading *Akkermansia muciniphila* may reduce the mucus layer to increase TLR-stimulation[[79](#_ENREF_79)].SCFAs: Short chain fatty acids; GPR: G-protein receptors; MAMP: Microbial-associated molecular patterns; TLR: Toll-like receptors.