# ROLE OF INTESTINAL BACTERIA IN GUT INFLAMMATION AND OBESITY

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## SUMMARY

It has increasingly been recognized that the gut microbiota has a major impact on host physiology. It is therefore not surprising that various diseases have been linked to perturbations in the gut microbiome. Obesity, which is often accompanied by metabolic disorders, and inflammatory bowel disease (IBD) are associated with shifts in the microbial communities inhabiting the digestive tract. It is not completely clear whether differences in the microbiome observed between healthy and diseased subjects are consequences or causes of these disorders. The transferability of the obese phenotype by microbiota transplantation suggests a role of the intestinal microbiome in disease development. Similarly, the observation that germfree mice in contrast to conventional mice are protected from colitis in certain animal models also supports a role of the gut microbiota in the development of IBD. However, the mechanisms underlying disease promotion by intestinal bacteria are far from being understood. This review summarizes both human and animal studies that aimed to more precisely define the role of intestinal bacteria in obesity development. A number of explanations for the role of intestinal bacteria have been proposed but which bacteria-derived molecules are involved and what their targets are in the host has only been uncovered in a few cases. Available evidence indicates that there is more than one way in which the intestinal microbiota may contribute to disease development and that it is necessary to use complementary approaches to unravel the underlying molecular mechanisms.

## INTRODUCTION

The microbial communities inhabiting the gastrointestinal tract support important physiological host functions and thereby contribute to disease prevention. Disruption of the microbiota has been associated with various diseases including inflammatory bowel disease (IBD) and obesity (*Ley* et al., 2005, 2006b; *Qin* et al., 2010). Effects of the intestinal microbiome on the host can be attributed to its immense catalytic potential and to its interactions with the host immune system. Conversely, genetic traits, initial colonization as well as lifestyle and environmental factors influence the composition and activity of the gut microbiota. Diet is a key factor in the development of the gut microbiota because it is the main substrate source for the microbial community (*Blaut*, 2013). Therefore, dietary changes may lead to shifts in microbiota composition and in the spectrum of microbial fermentation

products (*David* et al., 2014). Obesity and associated diseases are clearly connected to dietary habits, but it is also clear that the gut microbiota affects host energy metabolism (*Backhed* et al., 2004). The microbiome of obese subjects has recently been reported to be less rich in bacterial organisms and genes compared with non-obese individuals (*Le Chatelier* et al., 2013). However, even though various bacterial species were found to be associated with the obese phenotype the exact role of these organisms is still obscure.

IBD is a complex disease involving genetic and environmental factors. Intestinal bacteria are thought to play a major role in the development of IBD because certain mouse models for IBD do not develop disease symptoms as long as they are germfree (*Sellon* et al., 1998). IBD patients and healthy subjects can be distinguished based on their microbiota composition (*Qin* et al., 2010). However, so far it has not been possible to identify a single bacterial species or groups of bacteria that definitely account for the disease. Moreover, the microbial patterns observed in IBD patients may be consequence rather than cause of the disease. A number of host gene variants have been identified as possible contributors to IBD development, but they only explain a small proportion of cases. Various bacterial species have positively or negatively been associated with IBD. For example, IBD patients harbour reduced cell numbers of *Faecalibacterium prausnitzii* (*Frank* et al., 2007; *Sokol* et al., 2009; *Joossens* et al., 2011). In contrast, adherent and invasive *E. coli* strains (*Darfeuille-Michaud* et al., 2004), *Salmonella, Campylobacter* (*Gradel* et al., 2009) *Mycobacterium avium* subspecies *paratuberculosis, Helicobacter* spp. and *Fusobacterium varium* have been proposed to contribute to IBD (*Hold* et al., 2014).

This indicates that in both obesity and IBD different bacteria may cause similar disease symptoms even though the underlying mechanisms are different. There is an urgent need for a more precise understanding of how bacteria contribute to disease development, in particular for the identification of the bacterial molecules involved as well as their targets in the host organism.

## REVIEW AND DISCUSSION

### Role of intestinal bacteria in obesity and metabolic disease

Obesity may be considered a lifestyle disease because it is caused by an excessive caloric intake in conjunction with a lack of physical activity. Owing to its increasing incidence in many countries obesity is considered an epidemic, in particular because it is mostly associated with many other disorders such as hypertension, diabetes, dyslipidaemia, and non-alcoholic fatty acid liver disease. This development causes considerable costs for public health systems and is therefore an important research topic in medical research.

Jeffrey Gordon and colleagues were the first scientists to propose that obesity affects microbiota composition. Both genetically obese mice homozygous for a mutation in the leptin gene (*ob/ob*) (*Ley* et al., 2005) and obese human subjects (*Ley* et al., 2006a) displayed a shift in the gut microbiota compared to respective controls. Obesity was associated with an increase in the proportion of the Firmicutes at the expense of the Bacteriodetes. Even though this pattern could not be observed by other investigators (*Duncan* et al., 2008;

*Schwiertz* et al., 2010) (possibly due to the use of different methods for microbiota analysis) differences in gut microbiota composition between lean and obese human subjects or mice have been observed repeatedly (*Turnbaugh* et al., 2006, 2008, 2009a; *Jumpertz* et al., 2011). There is also experimental evidence that diet rather than the obese phenotype affects microbiota composition. This conclusion was drawn from an experiment, in which two genetically distinct mouse strains, which were fed the same high-fat diet, displayed essentially the same microbiota composition even though one of the strains was obese while the other one was not (*Hildebrandt* et al., 2009). This experiment suggests that diet is critically involved in microbiota composition. From a microbiological point of view this is not really surprising because consumption of a high-fat diet for example causes an increased excretion of bile acids into the digestive tract which, in turn, lowers the cell counts of bacteria that are sensitive to bile acids while bacteria that are resistant to bile acids are not affected or even proliferate in such conditions. Administration of the bile acid cholate to mice leads to increased cell numbers of Firmicutes at the expense of Bacteroidetes, in particular to an increase in Clostridia and Erysipelotrichi (*Islam* et al., 2011).

The most intriguing observations in this field relate to the transferability of the obese phenotype by transplantation of the microbiota from an obese donor mouse or human subject to germfree mice (*Turnbaugh* et al., 2006, 2009b; *Ridaura* et al., 2013). It is not quite clear which features render a gut microbiota obesogenic. What are the bacterial factors that cause this effect in the recipient mice and what are the targets in the host organism? It has been proposed that an obesogenic microbiota improves energy harvest from the diet.

However, how this is brought about is not yet clear. Several possible explanations have been proposed:

1. The gut microbiota leads to an increased formation of short chain fatty acids (SCFA) which can be utilized for energy generation, lipogenesis and/or gluconeogenesis. In agreement with a role of SCFA in obesity development, obese subjects were reported to have higher faecal SCFA concentrations compared to normal-weight subjects (*Schwiertz* et al., 2010). On the other hand, high faecal SCFA concentrations indicate that the diet consumed was rich in fermentable fibre. Fibre-rich diets usually have a lower energy density compared to highly digestible diets and the intake of diets rich in dietary fibre correlates with a lower incidence of obesity and symptoms of the metabolic syndrome (*Slavin*, 2005). This can be explained by the regulatory function of SCFA, which are ligands of the G-protein coupled receptors FFAR2 (Free Fatty Acid Receptor 2) and FFAR3 (formerly GPR43 and GPR41) found in ileal and colonic entero-endocrine L cells, adipocytes and immune cells (*Brown* et al., 2003). Following the activation of these FFARs by SCFA, leptin is secreted by adipocytes (*Xiong* et al., 2004) and peptide YY (PYY) and glucacon like peptide 1 (GLP-1) are secreted by entero-endocrine cells (*Tazoe* et al., 2008; *Tolhurst* et al., 2012). Leptin and PYY reduce appetite (*Wren and Bloom*, 2007) and GLP-1 stimulates insulin production, improves insulin sensitivity and promotes satiety.

2. High-fat diets lead to low-grade inflammation caused by increased permeability of the intestinal epithelium for bacterial lipopolysaccharides (LPS) and increased concentration of LPS in blood; this is

referred to as metabolic endotoxaemia (*Cani* et al., 2007). In agreement with this proposed mechanism association of germfree mice with an LPS-containing *Enterobacter cloacae* strain, which had been isolated from an obese Chinese patient, developed low-grade inflammation and obesity to greater extent than germfree mice in response to a high-fat diet (*Fei and Zhao*, 2013). However, the authors of a very recent study did not find any indications for an impaired gut barrier in response to high-fat diet feeding even though glucose tolerance was impaired and signs of low-grade inflammation in adipose tissue were observed (*Kless* et al., 2015).

3. Intestinal bacteria promote the formation of Angiopoietin-like protein 4 (ANGPTL4) also called Fasting-induced adipose factor (FIAF) in the intestine (*Backhed* et al., 2004). ANGPTL4 is a downstream target of the nuclear peroxisome proliferator-activated receptor family and an inhibitor of lipoprotein lipase (LPL). Inhibition of LPL by ANGPTL4 results in reduced plasma triglyceride levels and decreased deposition of released fatty acids as triglycerides in adipose tissue (*Lichtenstein* and *Kersten*, 2010). Germfree mice display lower intestinal ANGPTL4 mRNA levels than conventional mice and in accordance with this explanation less body fat (*Backhed* et al., 2004). Increased ANGPTL4 mRNA levels in intestinal mucosa of germfree versus conventional mice were confirmed in another study, but the plasma protein levels of ANGPTL4 in germfree mice were not higher than those in the conventional mice (*Fleissner* et al., 2010). This finding is in conflict with a critical role of ANGPTL4 in obesity development.

Mice colonized with *Lactobacillus paracasei* strain F19 displayed higher serum ANGPTL4 levels and reduced fat accumulation (*Aronsson* et al., 2010), suggesting that intestinal bacteria do not necessarily reduce circulating ANGPTL4 levels as proposed (*Backhed* et al., 2004).

4. The gut microbiome contributes to obesity development by enhancing nutrient uptake. In agreement with this explanation, Hooper *et al*. (2001) observed that mice mono-associated with *Bacteroides thetaiotaomicron* had higher mRNA levels of genes involved in nutrient uptake such as $Na^+$/glucose co-transporter (SGLT1), co-lipase and liver fatty acid-binding protein (L-FABP) compared to germfree mice. A recent study in gnotobiotic mice suggests that *C. ramosum*, a member of the Erysipelotrichi, promotes obesity development in mice fed a high-fat diet by enhancing nutrient uptake (*Woting* et al., 2014). This is in accordance with a recent human study, which reported an association of obesity with low bacterial gene content and presence of *C. ramosum* (*Le Chatelier* et al., 2013). Furthermore, symptoms of the metabolic syndrome in diabetic women were reported to correlate with faecal *C. ramosum* (*Karlsson* et al., 2013). We compared mouse groups that differed in their microbiological status and that were fed a high-fat diet (HFD) or a low-fat diet (LFD) (*Woting* et al., 2014). The first group of mice was associated with a simplified human intestinal (SIHUMI) microbiota of eight bacterial species, including *C. ramosum*, the second group with SIHUMI except *C. ramosum* (SIHUMIw/oCra), and the third group with *C. ramosum* only (Cra). After feeding the mice the HFD for 4 weeks, there was no

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difference between the mouse groups in energy intake, diet digestibility, gut permeability, and parameters of low-grade inflammation but the SIHUMI and Cra mice fed the HFD gained significantly more body weight and body fat and displayed higher food efficiency than the SIHUMIw/oCra mice and the germfree mice, respectively, also fed the HFD. Glucose transporter 2 (*Glut2*) and fatty acid translocase (*CD36*) mRNA levels in small intestinal mucosa were significantly higher in the obese SIHUMI and Cra mice than in the less obese SIHUMIw/oCra mice. The data suggest that in this animal model up-regulation of small intestinal glucose and fat transporters contribute to their increased body fat deposition (*Woting* et al., 2014). It remains to be shown whether this is a general mechanism and to which extent it may contribute to obesity compared to the other mechanisms that have been proposed.

**Role of intestinal bacteria in inflammatory bowel disease (IBD)**

IBD encompasses two major forms of inflammatory conditions of the digestive tract: Crohn's disease and ulcerative colitis. It is now generally accepted that IBD arises as a result of a disturbed interaction of genetic and environmental factors. Various gene variants have been identified that predispose the host organism to IBD. Examples include Nucleotide-binding oligomerization domain (NOD) 1 and 2, Toll-like receptor (TLR) 1 and 2 or Autophagy-related protein 16-1 (ATG16L1). These gene variants may lead to a disturbed barrier function of the gut epithelium and in consequence to a loss of homeostasis between intestinal microbiota and immune system. The intestinal microbiota and nutrition are considered the most important environmental factors in the development of IBD (*Hold* et al., 2014). As mentioned above for metabolic disease the exact role of intestinal bacteria in IBD development is not really clear, in particular whether specific bacteria are involved or whether there is a disturbance in the microbiome, which is not exactly defined. The latter is referred to as dysbiosis. The microbiomes of patients suffering from Crohn's disease or ulcerative colitis differ from those of healthy subjects (*Qin* et al., 2010). However, first of all it is not clear whether the microbiota patterns observed in patients are cause or consequence of the disease and second it has so far not been possible to deduce the mechanism(s) underlying IBD development. IBD is associated with a reduction in diversity and shifts in certain microbial populations (*Manichanh* et al., 2006; *Frank* et al., 2007; *Wohlgemuth* et al., 2009). Several studies concurrently report reduced cell numbers of *Faecalibacterium prausnitzii* in IBD patients (*Frank* et al., 2007; *Sokol* et al., 2009; *Joossens* et al., 2011). *F. prausnitzii* is considered to be protective against IBD because oral administration of this organism or of spent *F. prausnitzii* growth media improved symptoms of a colitis induced by trinitrobenzosulphonate (TNBS); it blocks Nuclear Factor kappa B (NFκB) and the formation of Interleukin (IL)-8 (*Sokol* et al., 2008). In contrast to *F. prausnitzii*, cell numbers of *E. coli* are increased in both Crohn patients and interleukin 10-deficient (IL-10^{-/-}) mice (*Darfeuille-Michaud* et al., 1998; *Wohlgemuth* et al., 2009). The *E. coli* strains detected in epithelial lesions of Crohn patients turned out to preferentially belong to the group of adherent entero-invasive *E. coli* (AIEC) (*Darfeuille-Michaud* et al., 2004). There are also indications
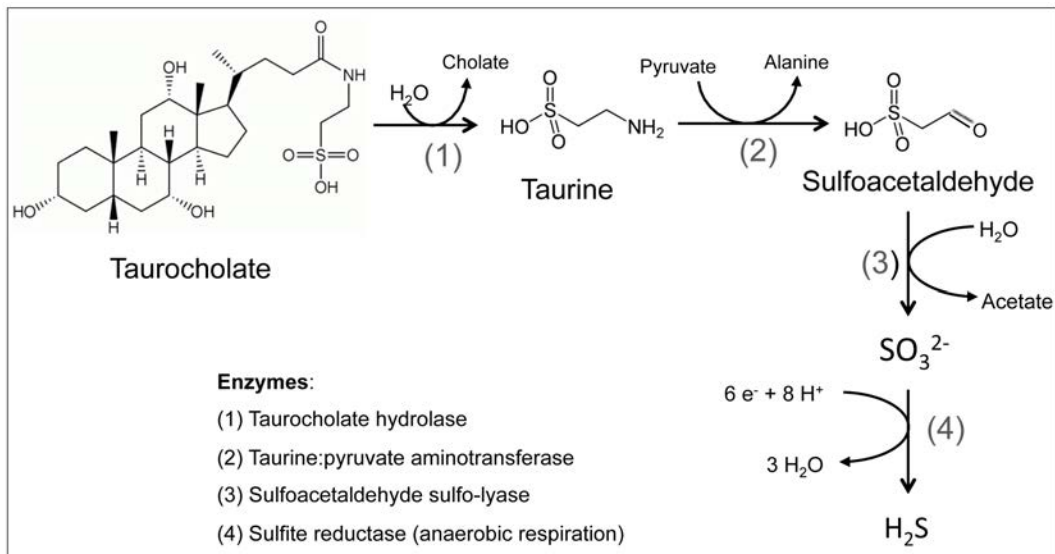
**Figure 1**: Pathway of taurocholate conversion by *Bilophila wadsworthia*.
This pathway enables the organism to generate sulphite, which is used as an external electron acceptor to generate ATP by anaerobic respiration (*Devkota* et al., 2012).

for a role of *Mycobacterium avium* subspecies *paratuberculosis*, *Helicobacter* spp. and *Fusobacterium varium* in IBD development (*Hold* et al., 2014). However, these and other differences in gut microbiota are not generally observed which may be due to the high inter-individual variability of the gut microbiota.

In the recent decades there has been an increase in the incidence of IBD. This development was accompanied by changes in lifestyle. As nutrition is a very important lifestyle factor, which also affects the gut microbiome, various studies investigated possible correlations between diet and the incidence of IBD. In particular Japan in the last decades underwent considerable changes in nutrition, from a traditional Japanese diet to a more Western-oriented diet. The latter is characterized by an increased consumption of fat, animal protein, milk, and milk products as well as a reduced intake of rice. Interestingly, these changes coincide with an increase in the incidence of

IBD in the Japanese population (*Asakura* et al., 2008). Even though a direct effect of diet cannot be excluded it appears reasonable to assume that possible dietary effects are mediated by the gut microbiome. One animal study provides an interesting example on how diet could promote the onset of IBD. Two groups of IL-10$^{-/-}$ mice, which are prone to developing colitis, were fed two isocaloric high-fat diets that did not differ in macronutrient composition but just in the type of dietary fat. One diet contained milk fat (MF), i.e. saturated fatty acids, whereas the other one contained poly-unsaturated fatty acids (PUFAs). Colitis incidence, inflammatory score, and pro-inflammatory markers were considerably increased in the MF diet-fed mice compared to the PUFA diet-fed mice (*Devkota* et al., 2012). The authors demonstrated that this effect was caused by changes in the spectrum of bile acids, specifically by increased concentrations of taurocholate (Figure 1). The latter stimulates the growth of
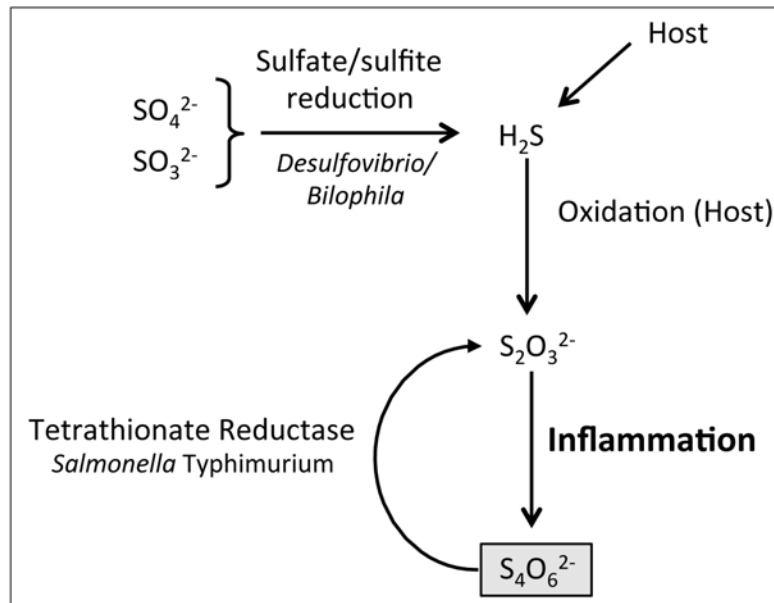
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**Figure 2**: Formation of tetrathionate ($S_4O_6^{2-}$) which *Salmonella enterica* Typhimurium utilizes as an electron acceptor.
Tetrathionate is only formed under inflammatory conditions in the gut. It gives *S*. Typhimurium a growth advantage leading to an outgrowth of the organism in the inflamed intestine (*Winter* et al., 2010).

the pro-inflammatory *Bilophila wadsworthia*, a common isolate from infected organs or tissues such appendix, biliary tract and liver (*Finegold* and *Jousimies-Somer*, 1997; *Summanen* et al., 1995). *B. wadsworthia* belongs phylogenetically to the Desulfovibrionaceae within the δ-Proteobacteria, but unlike other genera of this family, *B. wadsworthia* is not capable of sulphate reduction. This organism rather converts the sulphonic group of taurine to sulphite, which in turn stimulates the growth of *B. wadsworthia* in the intestinal tract owing to the organism's ability to utilize sulphite as an electron acceptor (Figure 1). These experiments showed for the first time a causal relationship between diet and IBD development. However, it has to be emphasized that it is unclear whether these observations are of any relevance to the human situation.

Tetrathionate has been identified as a molecule that stimulates the growth of *Salmonella enterica* serovar Typhimurium (*Winter* et al., 2010). As a response to infection by this organism host neutrophils produce reactive oxygen species. They react with thiosulfate ($S_2O_3^{2-}$) giving rise to tetrathionate ($S_4O_6^{2-}$). Thiosulfate is a detoxification product formed by epithelial cells through the oxidation of hydrogen sulphide, which can be produced by both intestinal bacteria and the host (Figure 2). However, tetrathionate formation is only observed under inflammatory conditions suggesting that the pathogen, by inducing an inflammatory response, creates favourable conditions that promote its own growth. *S*. Typhimurium is capable of utilizing tetrathionate as an external electron acceptor enabling the organism to gain energy from substrates that without an external electron acceptor cannot be utilized. *S*. Typhimurium's ability to utilize tetrathionate as an electron acceptor confers a substantial selective growth advantage on

this pathogen whose proliferation causes an aggravation of intestinal inflammation.

Whereas *B. wadsworthia* and *S.* Typhimurium have pro-inflammatory properties, *Akkermansia muciniphila,* a member of the phylum Verrucomicrobia, is considered a beneficial organism (*Derrien* et al., 2008). However, in a recent gnotobiotic animal study we showed that mice associated with a background microbiota of eight bacterial species (SIHUMI) plus *A. muciniphila* (SIHUMI-A) developed a more severe gut inflammation when challenged with *S.* Typhimurium (SIHUMI-AS) as compared to SIHUMI mice without *Akkermansia* but also challenged with *S.* Typhimurium (SIHUMI-S) or as compared to unchallenged mice (SIHUMI or SIHUMI-A) (*Ganesh* et al., 2013). Pro-inflammatory cytokines and *S.* Typhimurium cell numbers in mesenteric lymph nodes of SIHUMI-AS mice were considerably higher than in SIHUMI-S mice. Interestingly, the number of mucin filled goblet cells in caecal tissue of mice was much lower than in SIHUMI-S, SIHUMI-A or SIHUMI mice. The proportions of the microbial community members in SIHUMI-AS mice differed very much from those of the other three communities: *S.* Typhimurium accounted for 94% of total bacteria in the SIHUMI-AS mice but for only 2.2% in the SIHUMI-S mice suggesting that the comcomitant presence of *A. muciniphila* and *S.* Typhimurium led to a severe disturbance of the microbial community. We proposed that *A. muciniphila* exacerbates *S.* Typhimurium-induced intestinal inflammation by its ability to disturb host mucus homeostasis (*Ganesh* et al., 2013). However, this has not yet been demonstrated experimentally and requires clarification.

## CONCLUSION

The intestinal microbiome has been linked to various diseases including obesity, metabolic disease and IBD. However, the underlying molecular mechanisms are mostly not understood or unproven. Gnotobiotic and knockout animal models are valuable tools for testing hypotheses derived from correlations between metagenomics/ metabolomic data and disease symptoms. In a few instances it has been possible to identify dietary components or host-derived molecules that enhance the growth of certain intestinal bacteria and thereby directly promote or aggravate intestinal inflammation. In a complex disease such as the metabolic syndrome intestinal bacteria probably play more than one role in disease development. It will be necessary to scrutinize proposed hypotheses to come to a more precise understanding of how bacteria contribute to disease development.

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