The role of gut microbiota in human metabolism

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Publication date
2013

[Link to publication](#)

Citation for published version (APA):
Vrieze, A. (2013). *The role of gut microbiota in human metabolism*.

CHAPTER 3

THE THERAPEUTIC POTENTIAL OF MANIPULATING

GUT MICROBIOTA IN OBESITY AND

TYPE 2 DIABETES MELLITUS

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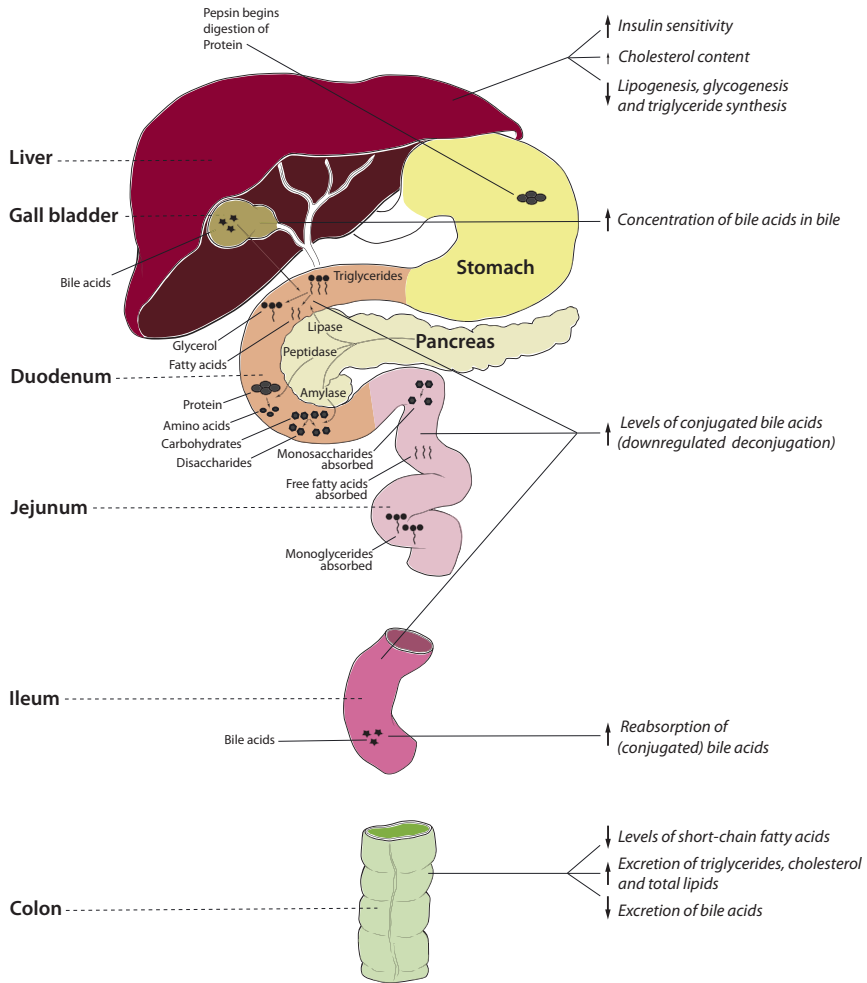Erik S. Stroes, Max Nieuwdorp

Abstract

Obesity and type 2 diabetes mellitus (T2DM) are attributed to a combination of genetic susceptibility and lifestyle factors. Their increasing prevalence necessitates further studies on modifiable causative factors and novel treatment options. The gut microbiota has emerged as an important contributor to the obesity- and T2DM -epidemic proposed to act by increasing energy harvest from the diet. Although obesity is associated with substantial changes in the composition and metabolic function of the gut microbiota, the pathophysiological processes remain only partly understood. In this review we will describe the development of the adult human microbiome and discuss how the composition of the gut microbiota changes in response to modulating factors. The influence of short-chain fatty acids, bile acids, prebiotics, probiotics, antibiotics and microbial transplantation is discussed from studies using animal and human models. Ultimately, we aim to translate these findings into therapeutic pathways for obesity and T2DM in humans.

Introduction

The medical community is currently facing an unprecedented epidemic of obesity and type 2 diabetes mellitus (T2DM) (1;2). These metabolic disorders are believed to be caused by a combination of genetic susceptibility and lifestyle changes (1-3). Recently, interest has been drawn towards the role of the intestinal microbiota as a potential novel contributor to this epidemic.

Until recently, knowledge of the intestinal microbiota was limited mainly due to the lack of analytical methods to identify microorganisms. The introduction of culture-independent approaches based on 16S ribosomal RNA-analysis (rRNA) and it corresponding gene has profoundly changed the landscape allowing to identify a large number of previously undetected microbes in a wide variety of ecosystems and has lead to a major shift in research on the ecology of microbes including those harbouring the intestinal tract. The application of these 16S rRNA based approaches in researched focusing on the intestinal ecosystem has resulted in renewed insights into the pathophysiological roles of the intestinal microbiota in cardiometabolic diseases. In initial studies, obese mice as well as humans were characterized by an altered composition of gut microbiota in compared to their lean counterparts (4;5). These distinct differences in gut microbiota have been shown to result in a greater capacity to harvest energy from the diet, thereby contributing to obesity (5;6). In support, germ-free mice were protected from obesity, insulin resistance, dyslipidemia and fatty liver disease/non alcoholic steatosis hepatis (NASH) when fed a high-fat Western diet (7;8). In contrast, following the colonisation with microbiota from conventionally raised mice, body fat content in the originally germ-free mice increased up to 60% in 14 days. Moreover, insulin resistance increased, despite a reduced food intake (9). Food ingredients otherwise indigestible for the hosts were microbially fermented and absorbed afterwards (6). Furthermore, the presence of gut microbiota resulted in an increase in hepatic lipogenesis and suppression of the lipoprotein lipase (LPL)-inhibitor angiopoietin-like 4 (ANGPTL4), which was formerly known as fasting-induced adipocyte factor (FIAF) (7;9). On the basis of these and other observations, the gut microbiota is hypothesized to influence total body energy homeostasis (figure 1).

Pepsin begins
digestion of
Protein

↑ *Insulin sensitivity*

↑ *Cholesterol content*

↓ *Lipogenesis, glycogenesis
and triglyceride synthesis*

Liver

Gall bladder

↑ *Concentration of bile acids in bile*

Bile acids

Stomach

Triglycerides

Lipase

Glycerol

Peptidase

Pancreas

Fatty acids

Duodenum

Amylase

Protein

↑ *Levels of conjugated bile acids
(downregulated deconjugation)*

Amino acids

Carbohydrates

Disaccharides

Monosaccharides
absorbed

Free fatty acids
absorbed

Jejunum

Monoglycerides
absorbed

Ileum

↑ *Reabsorption of
(conjugated) bile acids*

Bile acids

↓ *Levels of short-chain fatty acids*

↓ *Excretion of triglycerides, cholesterol
and total lipids*

Colon

↓ *Excretion of bile acids*

Legend

↑ Increased
↓ Decreased
↑ Mildly increased

Figure 1. Studies comparing germ-free to conventionally raised animals have revealed important effects of gut microbiota on bile acid, fatty liver (NASH) disease as well as glucose, lipid and short-chain fatty acid metabolism. On the right side of this figure is depicted how metabolism in germ-free animals differs from metabolism in conventionally raised animals. Moreover, absorption of different food-derived nutrients in different parts of the intestine is depicted.

This review portrays modulators that influence the composition of the gut microbiota. We will also describe how the specific changes in gut microbiota affect metabolism and how these findings could be translated into novel therapeutic pathways for obesity and T2DM.

The arising of an adult human microbiome: role of the epithelial lining

It is estimated that the human adult intestines contain more than 10^{14} bacteria from over 1000 species. The genetic material of the intestinal microbes, collectively named the microbiome, exceeds the magnitude of the human genome over one hundred times (10-13). Medical literature therefore refers to the gut microbiota as an 'exteriorized organ' (13). The phyla that account for the vast majority of all gut microbes include the Gram-negative *Bacteroidetes, Proteobacteria* and *Verrucomicrobia* as well as the Gram-positive *Firmicutes* and *Actinobacteria* (10;13-15). Despite its enormous diversity and complexity, it has been demonstrated that the microbiota can be divided into three enterotypes driven by different groups of species that are suggested to contribute to the preferred community composition (14). How does this exteriorized organ develop, realizing a fetus is considered to be predominantly sterile? At birth, the intestine is immediately colonized with bacteria from the mother and the environment (12;16;17). Within several years after this initial colonization, an adult-like microbiome has succeeded. External insults during life can (temporarily) change the composition of the adult microbiome. Examples of these are dietary changes, bacterial infections and the use of microbiota-modulating agents (e.g. antibiotics). Despite continuous external stimuli, the composition of a humans gut microbiota remains relatively stable over time in healthy adults (10;12;13).

Host-characteristics may contribute to the selection of a specific gut microbiota. Epithelial cells throughout the gastrointestinal tract are covered by an integrated layer of glycocalyx and mucus gel (18). This layer is continuously renewed and varies between different parts of the intestine. Its functions are the selective binding and

nourishing of commensal intestinal microbes and trapping and clearing of invasive pathogens, thereby affecting the composition of the gut microbiome (19). Vice versa, bacteria are capable of adapting to the surrounding mucus- and glycocalyx-layer. Sonnenburg et al. colonized germ-free mice with one of the Bacteroides-species, *B. thetaiotaomicron,* which prevented its own wash-out from the intestines by assembling on partially digested food particles, on shed elements of the mucus gel layer and on exfoliated epithelial lining cells. In addition, *B. thetaiotaomicron* showed the capacity to digest endogenous (mucus-derived) glycans when exogenous polysaccharides were absent (20). Similarly, *Akkermansia muciniphila* was isolated as the only cultured *Verrucomicrobium* species that exclusively grows on mucus, has a large glycobiome and is found in healthy intestinal mucosa (21). *Escherichia coli* on the other hand seems to be capable of deploying specific pili to bind to mucus or mannose sugars on intestinal epithelium (14). Bearing the variability and changing capacities of the mucus and glycocalyx layer in mind, the symbiotic 'system' of host and microbiota at least partially might determine the composition of the gut microbiota and its observed enterotypes in humans (14;20).

The interaction between diet and gut microbiota composition; a role for short chain fatty acids

Diet is one of multiple factors that can determine the composition of the gut microbiota (22). Although dietary-induced changes in gut microbiota occur within a short time frame (within 1 to maximally 3-4 days after a diet switch), the changes are readily reversed (23-25). In animal models, the ratio of the most prominent intestinal bacteria, the *Bacteroidetes* and *Firmicutes*, is altered in response to dietary changes (10;13). High-fat Western diets in mice resulted in an increase of the abundance of *Firmicutes* and a decrease in the abundance of *Bacteroidetes* (23;24;26-28). To date, human studies have not been able to fully confirm these observations. Ley et al. reported a similar response in the presence of *Bacteroidetes* and *Firmicutes* when obese subjects were put on a low-calorie diet (29). In contrast, others reported no relationship between the *Bacteroidetes/Firmicutes*-ratio and low-carbohydrate diets

or BMI (14;30). Hence, more data are needed to demonstrate the presence as well as the value of the *Bacteroidetes/Firmicutes*-ratio in humans.

While diet may alter gut microbiota composition, it is only partly clear how this in turn might influence metabolism. Intestinal microbes are capable of generating short-chain fatty acids (SCFAs, i.e. acetate, butyrate, proprionate) by fermenting dietary carbohydrates that humans cannot digest themselves (5;14;31;32). Acetate is the dominant SCFA type in humans (31;32). SCFAs like proprionate can be utilised for de novo glucose or lipid synthesis and serve as an energy source for the host (5). A recent study showed that germ-free mice are devoid of SCFAs, indicating the importance of gut microbiota for production of SCFAs in the intestine (33). From these observations it would appear that following diet-induced changes in the composition of the gut microbiota, the availability of SCFAs in the intestines changes, thus directly modulating energy absorption. In line, therapeutic modulation like alpha-glycosidase inhibitors, primarily used as oral antidiabetics, also have the capacity to influence plasma SCFA-concentration, underscoring the potential importance of SCFA-production (34).

Indirect pathways by which SCFA might influence metabolism are suggested. An enteroendocrinological pathway is proposed in which SCFAs stimulate the secretion of peptide YY (PYY), a hormone related to obesity (35;36). Of all SCFAs, butyrate in particular seems to function as a signalling molecule, although the precise role is unclear. High-fat diets supplemented with butyrate prevented and reversed insulin resistance in dietary-obese mice. The effects of butyrate action were related to a promotion of both colonic epithelium (37) and whole body energy expenditure via a modulation of mitochondria function (38). At the same time, butyrate-producing bacteria and fecal butyrate concentrations decline with diets containing decreasing amounts of carbohydrates (30). Apparently, butyrate can beneficially affect metabolism in pathophysiological states.

3

The complex relationship between gut microbiota and bile acid metabolism

After having travelled through the small intestine, 95% of all liver-secreted bile acids are reabsorbed in the ileum to be taken up by the liver: the enterohepatic cycle (EHC) (39). Only a small part of the bile acid pool escapes the EHC and travels towards the large intestine to be excreted in the feces. This excretion is accompanied by microbial deconjugation of glycine- and taurine-conjugated bile acids (40;41). Following the discovery that germ-free rats tend to accumulate more cholesterol than their conventionally raised counterparts (42), Wostmann showed that in the absence of gut microbiota, the bile acid concentration in bile was 3 times increased and cholesterol absorption was 25% greater (43). The cholesterol accumulation was thought to be due to an increase in intestinal bile acid reabsorption (43;44). Follow-up studies supported Wostmanns hypothesis by showing that germ-free animals have elevated levels of conjugated bile acids throughout the intestine with no deconjugation and strongly decreased fecal excretion (41;45). More recently, these results were confirmed in experiments with mice treated with antibiotics. Three days of treatment with ampicillin increased biliary bile acid output threefold while fecal output decreased 70% (46). The authors concluded that increased expression of the sodium-dependent bile acid-importer SLC10A2 underlies this response. In addition to the increased expression of SLC10A2, Miyata et al. observed a striking decrease in expression of fibroblast growth factor 15 (FGF 15) (47). This autocrine hormone is a product of intestinal bile acid-stimulated nuclear hormone farnesoid X receptor (FXR), which is apparently deactivated in the absence of ileal microbiota. FGF 15 is involved in the cross-talk between liver and intestine and under normal physiological conditions serves to down regulate hepatic bile acid synthesis in the postprandial phase. Clearly the presence of intestinal microbes influences this signalling pathway and may explain the ampicillin-induced increase in expression of 7-alpha-hydroxylase (CYP7A1), the key enzyme in bile acid synthesis (47).

Compared to conventionally raised animals, the bile of germ-free rats and mice contains relatively more hydrophilic muricholic acid (MCA) than amphiphatic cholic acid (CA) (43;48). The ratio of both primary bile acids may play a role in the regulation of the endogenous production and absorption of cholesterol by the liver, by determining the hydrophobicity and thus level of cholesterol solubilisation in mixed micelles of the total bile acid pool (49). Therefore, the decrement of the CA/MCA-ratio in germ-free animals changes cholesterol homeostasis. Claus et al. tried to determine which mechanism caused this change (49). They hypothesized that the enzyme CYP8B1 is a contributing factor, as it is responsible for the production of CA, whilst MCA may be produced independently of this enzyme (48;49). Germ-free mice indeed showed lower expression levels of CYP8B1 (48-50). Activation of hepatic FXR by the increased bile acid pool in the portal blood may be the cause of this phenomenon. However, activation of FXR would also be expected to result in decreased expression of CYP7A1 and this has not been observed (49). Clearly, the relationship between activity of the gut microbiota and bile acid homeostasis is complex. Competition between intestinal derived FGF 15 propagated signalling and hepatic FXR may underlie this complex behaviour.

Besides FXR, bile acids may signal via a newly discovered signal transduction pathway, mediated by the membrane receptor TGR5. This receptor is strongly expressed in brown adipose tissue and the small intestine. In mice, oral gavage with exogenous bile acids led to increased energy expenditure in brown adipose tissue via activation of TGR5 (51-53). As such, obesity and insulin resistance were prevented. In addition, as L-cells in the distal ileum and colon express TGR5 in high amounts, bile acid–induced stimulation of TGR5 results in glucagon-like peptide (GLP-1) release and stimulation of insulin secretion (52;54).

It appears there is a complex interaction between bile acids and gut microbiota. Nevertheless, it is irrefutable that gut microbiota can influence human metabolism via the bile acid pathway.

3

Gastrointestinal surgery and gut microbiota

Bariatric surgery is one of the most efficient procedures to treat morbid obesity. It results in drastic weight loss and improvement of metabolic and inflammatory status, but very little is known about the effect of this type of surgery on the gut microbiota (55). Roux-en-Y gastric bypass (RYGB) is the bariatric major intervention. Detailed studies were able to link both the preoperative metabolic state and the postoperative changes to gut microbiota composition. Before surgery, high levels of an H2-producing subgroup of *Bacteroidetes*, the *Prevotellacaea*, next to high levels of H2-utilizing *methanogens* (*Archaea*) were observed (56). The transfer of H_2 between both bacteria increases the energy uptake by the intestine, as *methanogens* are capable of removing fermentation intermediates (H2) from polysaccharides. This results in greater amounts of SCFAs present to be absorbed across the intestinal epithelium (57). Moreover, a small pilot study showed that after surgery the amounts of both *methanogens* and *Prevotellacaea* were lowered and the initial predominance of *Firmicutes* was mitigated (56). A recent animal study partially confirmed these human results. RYGB in non-obese rats diet resulted in decreased amounts of *Firmicutes* and *Bacteroidetes* but a 52-fold higher concentration of *Proteobacteria* in comparison with sham-operated rats (58). With regards to the inflammatory state in obesity, the butyrate-producing *Faecalibacterium prausnitzii* species was shown to negatively associate with inflammatory markers before and after RYGB indicating its contribution to a healthy intestine (55).

Although bariatric surgery is directly aimed towards improving metabolic disturbances, other surgical interventions within the gastrointestinal tract also seem to influence gut microbiota composition. Patients with short bowel syndrome for instance are characterized by nutrient malabsorption and aberrant SCFA-metabolism (59). An invasive therapy for this patient group is a small bowel transplantation (SBT). After SBT, a temporary ileostomy is created to allow monitoring of the transplanted tissue. During the presence of an ileostomy, the ileal microbiota shifts dramatically with increased colonization by the facultative anaerobic bacteria *Lactobacilli*, belonging to the *Firmicutes*-phylum, and *Enterobacteria* whereas under physiological

conditions and after closure of the ileostomy, *Bacteroidetes* and *Clostridia* dominate the ileum (60). Booijink et al. compared ileal effluent microbiota with fecal microbiota in patients with a Brooke ileostomy. Their results confirmed previous data as they found a greater concentration of Lactobacilli and a decrease concentration of Bacteroidetes in the ileal microbiota. More importantly, they found a lesser diversity and stability of ileal microbiota when compared to fecal microbiota (61). In conclusion, surgical interventions in the gastrointestinal tract have profound effects on gut microbiota composition and SCFAs in particular. Nevertheless, the surgically induced malnutrition status in case of an RYGB outweighs the finetuning effect of the gut microbiota in reversing obesity and T2DM.

Prebiotics

Prebiotics (mostly oligosaccharides) are non-digestible but fermentable food ingredients that selectively stimulate the growth or activity of one or multiple gut microbes that are beneficial to their human hosts (62;63). The human intestinal tract can not digest the food ingredient, but the intestinal microbes ferment the food particles. Effects of prebiotics have been claimed in all ages, ranging from infants to elderly (62). Bacteria of the *Bifidobacterium*, belonging to the *Actinobacteria* – and in some cases *Lactobacillus spp.-* particularly are known for a response to the administration of certain prebiotics (62;63).

The effects of prebiotics on metabolism in part arise by reducing a diet-induced inflammatory state. High-fat diets have the ability to increase lipopolysaccharide (LPS)-containing gut microbiota and subsequently downregulate the amount of *Bifidobacteria*. The accompanying inflammatory state, called metabolic endotoxaemia, is accompanied by insulin resistance and weight gain (64;65). In a physiological situation, *Bifidobacteria* are capable of lowering LPS-levels (65;66), Prebiotics containing oligofructose (OFS) specifically stimulate the growth of these intestinal bacteria (62;67;68). Administering OFS totally restored *Bifidobacteria*-levels and normalized plasma endotoxin-levels. It diminished the metabolic

endotoxemia, thus leading to improved glucose tolerance, increased satiety and weight loss in human subjects (64;69;70). Besides modulating endotoxemia, OFS can alter metabolism in various other manners. Cani et al. showed that effects of OFS were mediated via a GLP1-dependent pathway. High-fat fed diabetic mice on OFS treatment exerted improved glucose tolerance, diminished body weight and a decrease in endogenous glucose production. Either adding the GLP-1 receptor antagonist exendin 9-39 (Ex-9) or using GLP-1 knockout mice resulted in a complete lack of the OFS mediated beneficial effects (71), thus demonstrating the causal role of GLP-1 in this pathway in animals. Attempts to translate these findings to human subjects are ambiguous showing that OFS tends to dose-dependently decrease energy intake and increase PYY plasma concentrations (70;72), but reported effects on satiety are conflicting (72;73). Finally, oligofructose fermentation directly affects butyrate synthesis from extracellular acetate and lactate implicating the therapeutic potential of prebiotics (74).

Thus, there is emerging evidence to support the hypothesis that prebiotics can influence gut microbiota composition and as such, metabolic disturbances. The amount of evidence however is limited and a definite beneficial effect on metabolic disturbances remains to be demonstrated in large prospective randomized controlled trials.

Probiotics

Probiotics are food-supplements that contain living bacteria such as *Bifidobacteria, Lactobacilli, Streptococci* and non-pathogenic strains of *E. coli* (75;76). When adequately administered, they confer beneficial effects to the host due to changes in the gut microbiota that are both transient and diminish gradually with time after cessation (77-83). In 2006, Goossens et al. compared the effects of consuming *Lactobacillus Plantarum* on the microbial colonisation of feces and biopsies from the ascending colon and rectum (80). Within fecal samples, the amount of *Lactobacilli* was significantly increased. However, the biopsies did not confirm a growth of *Lactobacilli.* These data suggest that probiotics influence the intestinal lumen rather than the gut-

epithelium, possibly explaining the transient effect of probiotics. Surprisingly, Van Baarlen et al. recently described changes in the expression of up to thousands of genes in duodenal biopsies after administration of 3 types of *Lactobacilli* (84).

Alterations in the gut microbiota as a result of probiotics are widely acknowledged but evidence showing that probiotic administration directly affects inflammatory state has only recently been shown in humans (85;86). In contrast, studies on the effects of probiotics on characteristics of T2DM are mostly performed in animal models, reporting beneficial effects by various strains of *Lactobacilli* on characteristics of T2DM (87-89). Naito recently described both anti-diabetic as anti-inflammatory effects of *Lactobacillus casei* in diet-induced obese mice (90). In addition to these anti-diabetic effects, diet-induced obese mice and diet-induced overweight rats showed a reduction in body weight gain after they were fed specific *Lactobacilli* (91;92). Although these animal findings are interesting, translation of these beneficial effects of *Lactobacilli* in humans merits caution. First, none of the above mentioned studies directly assessed the relationship between the observed metabolic changes and the gut microbiota composition. Second, *Lactobacilli* are widely used within the farming industry, resulting in weight gain in animals (93;94) and this weight-modulating role has also been confirmed both in children and adults (95-97).

Synbiotics

Studies on probiotics have shown that every probiotic only benefits from a selected number of carbohydrates (98). Amylase-resistant starch for example increases the number of intestinal *Lactobacilli and Bifidobacteria*, whilst decreasing the number of *Enterobacteria* (99). From 40 types of *Bifidobacteria*, the species *Bifidobacterium lactis* showed the most growth when combined with this type of starch (100). These observations have led to the production of preparations containing both bacteria (probiotics) and saccharides (prebiotics): synbiotics (101). As the prebiotic-part of synbiotics is specifically selected on the basis of its ability to stimulate the growth of the probiotic-part, the effects of the probiotics are enhanced. In certain cases, these combinations show synergistic effects. *Lactobacillus reuteri* was more resistant to

bile salts when combined with soygerm powder (102). In addition, the combination of fructooligosaccharides (FOS) with *Lactobacillus Acidophilus* not only led to an expected increased amount of *Lactobacilli*. It also caused a growth of *Bifidobacteria,* to an even higher extent than the growth of *Lactobacilli* (103). Thus, although a possible relationship seems logical, new studies should focus on directly relating probiotic-/synbiotic-induced changes in metabolism to changes in gut microbiota.

Antibiotics

Antibiotics normally are prescribed to remove or prevent a bacterial colonization in the human body, without specifically targeting certain types of bacteria. As a side effect, antibiotics can influence the composition of the gut microbiota up to 2 years after administration (104-106). The decrement of intestinal bacteria after using antibiotics causes alterations in several bacteria-induced metabolic pathways. Tetracycline-antibiotics resulted in improved glucose tolerance and insulin sensitivity in diabetic rats and obese mice (107-109). Further exploring this relationship, ampicillin and norfloxacin caused a reduction in hepatic steatosis (NASH), liver triglyceride production and lipogenesis in ob/ob mice. In line, glucose levels normalized and insulin sensitivity increased. Obesity itself was not a contributing factor to the state of improved glucose tolerance as these results were independent of weight changes (110).

Two mechanisms are proposed to bridge metabolism and antibiotic-induced changes in the gut microbiota composition. The first is a reduction in low-grade inflammation. Tumor Necrosis Factor(TNF)-α and LPS were shown to negatively correlate with insulin sensitivity (110). Cani et al. further elucidated the role of LPS as they demonstrated that antibiotics lowered LPS-levels, which inhibited metabolic disorders from developing. Glucose intolerance, body weight gain and fat mass development all were reduced (111). The second possible explanation is that antibiotics directly influence SCFA-levels. When female mice were administered vancomycin orally, microbial fermentation of carbohydrates diminished, with a subsequent decrease of

fecal SCFA-excretion. In line with what was expected, fecal samples contained more (unfermented) oligosaccharides (112). Previous *in vitro* experiments showed similar results (113). Although one might hypothesize that antibiotics result in a diarrhoea induced detrimental nutritional status and thus weight loss, recent observations have suggested otherwise. Early exposure to oral antibiotics was found to be associated with overweight in children (114). Even more interesting is the recent report of an association between intravenous administrated vancomycin and weight gain in adults subjects, most likely via a *Lactobacilli*-mediated pathway given the intrinsic resistance of *Lactobacilli* for vancomycin (114;115).

The present data offer an intriguing view into the potential beneficial effects of antibiotics on human metabolism. Future interventions however should be introduced deliberately, as antibiotic-resistance is an increasing problem and antibiotics are associated with *Clostridium difficile* diarrhoea (116).

Recent interest: microbial transplantation

First described in 1958 (117), the transplantation of fecal microbiota remains a controversial treatment. Until recently, this therapy was limited to cases of pseudomembraneous colitis, a bacterial infection often following antibiotic treatment (116). Fecal transplant aids in curing this disease by restoring the gut microbiota of the diseased. This has raised interest in the result of fecal transplantation on the modulation of metabolism. Previous studies reported an increase of body fat up to 60% and an increase in insulin resistance after feces was transplanted from conventionally raised mice to germ-free (9). When comparing different feces donors, lean versus obese, the latter caused a greater rise in body fat within germ-free recipients (6;23). Fecal transplants were even tested between different mammals. Gnotobiotic mice, mice conventionalized with human feces, have shown a preservation of structure and diversity of the gut microbiota transplanted from humans. At the same time, the cholesterol metabolism and adiposity of the donor were preserved (24;118;119).

As aforementioned data are hampered by methodological concerns, we recently performed a study on fecal transplantation in humans (FATLOSE-trial) (120). In this double-blind RCT we studied the therapeutic effects of allogenic lean donor feces infusion on insulin resistance in male patients with metabolic syndrome. We observed beneficial alterations in glucose metabolism after lean donor transplantation, thus offering a new view on the metabolic deviations in obese subjects and a rationale for novel therapeutic intervention methods directly affecting gut microbiota-induced metabolism. A follow-up study, the FATLOSE 2-trial, has been initiated to study the long-term effects of single versus multiple lean donor fecal transplants on metabolic measurements.

Conclusions

The gut microbiota may be a major player in maintaining human metabolism homeostasis. Although interest in humans has only recently developed, it becomes clear that we first need to unravel the underlying mechanisms in the causality of human obesity and T2DM in order to know how to influence these gut microbiota-driven processes. Despite our increasing knowledge on genetic pathways underlying human obesity and T2DM, specific data on the human gut microbiome are scarce. In this review, we tried to answer which potential gut microbiota-driven pathways would be worth to study in humans and could hopefully render novel treatment options in the near future.

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