Insights Into the Role of the Microbiome in Obesity and Type 2 Diabetes

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The worldwide prevalence of obesity and type 2 diabetes mellitus (T2DM) continues to rise at an alarming pace. Recently the potential role of the gut microbiome in these metabolic disorders has been identified. Obesity is associated with changes in the composition of the intestinal microbiota, and the obese microbiome seems to be more efficient in harvesting energy from the diet. Lean male donor fecal microbiota transplantation (FMT) in males with metabolic syndrome resulted in a significant improvement in insulin sensitivity in conjunction with an increased intestinal microbial diversity, including a distinct increase in butyrate-producing bacterial strains. Such differences in gut microbiota composition might function as early diagnostic markers for the development of T2DM in high-risk patients. Products of intestinal microbes such as butyrate may induce beneficial metabolic effects through enhancement of mitochondrial activity, prevention of metabolic endotoxemia, and activation of intestinal gluconeogenesis via different routes of gene expression and hormone regulation. Future research should focus on whether bacterial products (like butyrate) have the same effects as the intestinal bacteria that produce it, in order to ultimately pave the way for more successful interventions for obesity and T2DM. The rapid development of the currently available techniques, including use of fecal transplantations, has already shown promising results, so there is hope for novel therapies based on the microbiota in the future.

The rising prevalence of type 2 diabetes mellitus (T2DM) continues to be a growing concern worldwide. From 1980 to 2008 the number of people diagnosed with diabetes, of which 90% type 2, has increased from 153 (123–182) million to 347 (314–382) million (1). The proportional increase in prevalence of obesity (between 1980 and 2008, this has nearly doubled to more than half a billion people in the world) shows weight gain and changes in dietary habits to be the main contributing factors to this alarming trend. The resulting metabolic disorders like dyslipidemia and insulin resistance, both part of the metabolic syndrome, are a major risk factor for associated diseases such as cardiovascular pathology, nonalcoholic fatty liver disease, and different types of cancer (2,3). The main cause for the obesity and diabetes epidemic has been attributed to economic and lifestyle changes in the last decades, including the decrease in physical activity combined with a growing availability of food high in calories. However, it appears to be extremely difficult for people to voluntarily change their lifestyle drastically in order to lose weight. In this respect, evidence of a powerful regulating biological system resisting these cognitive signals in order to maintain body weight in a relatively strict range is substantial and growing (4). For this reason, obesity is now considered a disease, rather than a

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REVIEW

willful choice, which calls for further insight into the pathophysiological pathways, as this could lead to sorely needed novel therapeutic targets.

A recently discovered partaker in this process is the intestinal microbiome (2). The microbiome refers to the $>10^{14}$ bacteria that reside in the human intestine, comprising a bulk of genetic material larger than the human genome (5). Recently our knowledge of the microbiome in relation to the function of the human (small) intestine (Fig. 1) (6) has increased immensely due to the development of new analytical methods such as high-throughput metagenomic sequencing (7). This has enabled researchers to identify possible effects of the microbiome on human metabolism, including its potential role in metabolic disorders like obesity and T2DM. In this review, we aim to provide deeper insight of relevance to clinicians by discussing several topics in a "bench to bedside" approach within this emerging field.

DIAGNOSTIC VALUE OF INTESTINAL MICROBIOTA IN T2DM

Although bacteria are usually considered as pathogens, an essential symbiotic interaction between the human host and intestinal bacteria is the forging and maintenance of the immune system in the gut. The first recognition came from findings in germ-free (GF) mice of defects in the development and function of their immune system (8). Another crucial interaction of gut microbiota is their endogenous metabolic function that enables the digestion of food components such as plant polysaccharides, which are otherwise nondegradable (9). In this respect, it is interesting that studies in mice as well as humans have shown that gut microbiota differ in composition between obese and lean subjects (10,11). In a leptin-deficient *ob/ob* mouse model Ley et al. (10) found a difference in the ratio of *Bacteroidetes* and *Firmicutes*, the two dominant intestinal bacterial phyla. Compared with their lean counterparts, obese mice showed a decrease in *Bacteroidetes* and a corresponding increase in *Firmicutes* (10). When Ley et al. (10) compared gut microbiota of obese humans to lean controls, they found similar differences in this ratio (12). Other studies in mice have corroborated these results (13–16). However, other human studies have found contradicting data (17–19), and it is considered that part of this controversy results from both the variations in diet composition around the globe as well as different methods used to determine microbiota composition.

The involvement of the microbiome in energy balance was further demonstrated in a study where it was found that GF mice were leaner compared with conventionally raised counterparts, despite a higher food intake. Additionally, when transferring intestinal bacteria from normal mice to GF counterparts, an increase in body fat of 60% was observed within 10–14 days, even though food consumption was decreased (20). These results have led to the belief that the obese microbiome is more efficient at yielding energy from the diet (11,17). This was supported by findings that the total body fat of GF mice colonized with "obese microbiota" increased significantly compared with those colonized with "lean microbiota" (11). The technique used in these studies in mice is known as fecal microbiota transplantation (FMT). In humans, FMT can be regarded as a working tool to dissect association from causality for a number of diseases (21). The first clinical use was the successful treatment of patients with pseudomembraneous colitis, an unremitting infection with *Clostridium difficile* usually following the use of antibiotics (22). Since then, FMT has been found effective in other chronic gastrointestinal infections and inflammatory bowel diseases, its therapeutic potential being attributed to a restoring ability of the gut microbial balance by replacing pathogens with more beneficial bacterial strains (21,23). Considering the promising results of the effects of FMT on metabolism in mice, a current interest in the clinical use of FMT for humans is focusing on metabolic and cardiovascular disorders. We recently performed a double-blind randomized controlled trial in insulin-resistant males with metabolic syndrome, who received either autologous or allogenic feces infusion from lean donors (24). Beneficial metabolic effects were observed in the group receiving the lean donor transplantation, including a significantly improved peripheral (muscle) insulin sensitivity. This was accompanied by a significantly increased intestinal microbial diversity, along with a distinct increase in levels of butyrate-producing

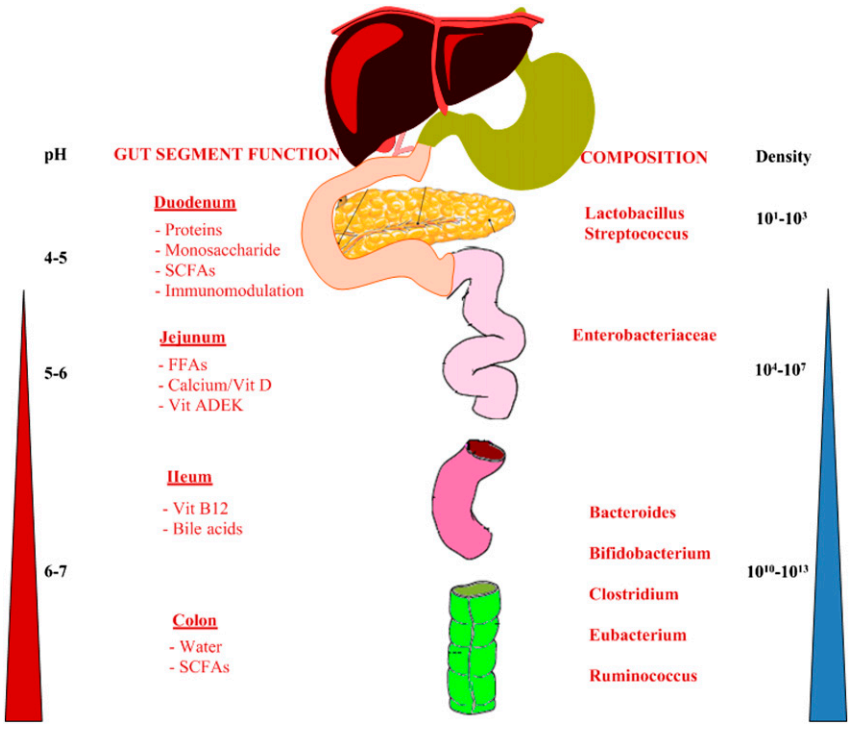

Figure 1—Differential functions of small and large intestine in relation to microbial density (6). In the proximal part of the small intestine (where only few intestinal bacterial strains reside), important metabolic functions take place such as uptake of dietary glucose, lipids, and proteins. More distally in the colon (where the majority of intestinal bacterial strains reside), water is absorbed from feces and SCFAs are produced via fermentation.

bacteria, such as *Roseburia* in the feces and *Eubacterium halii* in the small intestine. Interestingly, not all lean donors exerted the same beneficial effects in the obese host. Based on the small sample size, however, one should take into account that the reported effect might be due to a variation around a mean (meaning no clear effect of lean donor FMT when larger numbers of individuals are studied). On the other hand, these findings might indicate the presence of "super fecal donors," a concept that is currently being studied at our departments. The results from this relatively small cohort of patients with metabolic syndrome on the relation between microbial diversity and amount of butyrate-producing bacteria are in line with similar findings in two large metagenome-wide association studies (25,26), a type of study where clinical data are combined with metagenomic analysis. Both Karlsson et al. (25) and Qin et al. (26) independently found a decrease of butyrate-producing bacteria, namely *Roseburia* and *Faecalibacterium prauznitzii*, in the gut microbiota of patients with T2DM compared with healthy subjects. Moreover, we showed that increases in fecal concentrations of *Lactobacillus gasseri* and *Streptococcus mutans* (both inhabitants of the proximal intestine) as well as *Escherichia coli* were found to be predictive of the development of insulin resistance in postmenopausal obese Caucasian females in Sweden (see Table 1). It should be noted, however, that these correlations are not very strong and have not been reproduced in other cohorts; moreover, it is not known at this time whether these found changes in intestinal microbiota composition are secondary to altered gastrointestinal motility and small intestinal bacterial overgrowth often seen in T2DM. Nevertheless, such intestinal bacterial strains might function as early diagnostic markers in the clinic for better identification of those obese subjects that are prone to develop T2DM (26). To strengthen the predictive potential of particular patterns of microbial diversity and composition as well as pathogenic alterations of the microbiota composition, further research both in prospective cohorts and therapeutic phase I/II intervention trials with specific bacterial strains are urgently needed. In this respect, it is promising that an increasing number of companies are starting to appear that focus on the development of intestinal microbiome diagnostics and therapeutics (27,28).

Table 1—Intestinal bacterial species associated with and/or predictive of insulin resistance/T2DM development as future potential clinical diagnostic markers of T2DM

	Increase in T2DM	Decrease in T2DM
Intestinal bacterial phyla		
Firmicutes	x	
Bacteroidetes		x
Intestinal bacterial species	Increase in T2DM	Decrease in T2DM
Roseburia		x
Eubacterium halii		x
Faecalibacterium prauznitzii		x
Lactobacillus gasseri	x	
Streptococcus mutans	x	
E. coli	x	

PRODUCTS OF INTESTINAL BACTERIA IN T2DM PATHOPHYSIOLOGY

Butyrate and acetate and propionate are short-chain fatty acids (SCFAs) fermented by the intestinal bacteria from dietary fiber that play an important role in energy metabolism (Fig. 2) (29). These SCFAs are absorbed in the intestine, where particularly butyrate provides energy for the colonic epithelial cells, whereas the remaining SCFAs enter the (portal) venous system. Data from animal studies have suggested that propionate affects hepatic lipogenesis and gluconeogenesis, whereas peripherally acetate functions as substrate for cholesterol synthesis (17). The colonic mucosa primarily relies on the luminal presence of butyrate as energy source, and a lack of these SCFAs has been proposed to play an important part in the pathogenesis of intestinal disease and inflammatory bowel diseases (30). More specific, low concentrations of SCFAs have been found in ulcerative colitis patients (31) and treatment with SCFA enemas, especially butyrate, has been shown to reduce inflammation in this patient group (32). Interestingly, oral administration of sodium butyrate was found to be safe and well tolerated in humans with Crohn disease and ulcerative colitis (33,34); these studies showed a systemic anti-inflammatory effect and improved clinical improvement. In mice, oral butyrate has been demonstrated to improve insulin sensitivity and increase energy expenditure by enhancing mitochondrial function (35). Whether these beneficial effects apply to humans as well is currently being studied in our department. The underlying mechanisms of the potential positive influence of butyrate on metabolism are not clear. However, there is data on inhibiting effects of butyrate on histone deacetylases in mammalian cultured cells, which regulate gene expression by deacetylating histone proteins and transcription factors (36). This may contribute to increased expression of PGC-1a, a transcription coactivator associated with increased fatty acid oxidation and mitochondrial activity (35). Butyrate, being an SCFA, is oxidized in the mitochondria of colonocytes into acetyl-CoA and via the tricarboxylic acid cycle contributes to ATP production. Important catalyzing enzymes in this process have been shown to be downregulated in GF mice, resulting in a significantly decreased level of ATP in GF colonocytes. This indicates a potential stimulating role of the intestinal microbiota, particularly butyrate-producing microbes, in the expression of these enzymes and consequently mitochondrial function and energy metabolism (37). Another way in which SCFAs might influence the host's energy balance is by acting as specific signaling products. SCFAs bind to G protein–coupled receptors, namely GPR41 and GPR43, which are expressed in enteroendocrine cells in the intestinal epithelium (3,38). This leads to secretion of certain peptide hormones, like PYY, which are basolaterally released into the systemic circulation, enabling a form of communication between gut milieu and host. Conventional *Gpr41*$^{-/-}$ mice and GF *Gpr41*$^{-/-}$ mice colonized with members of the human gut microbiota stayed significantly leaner than their wild-type counterparts, whereas no differences were seen between wild type and GF *Gpr41*$^{-/-}$ mice. The latter indicates a

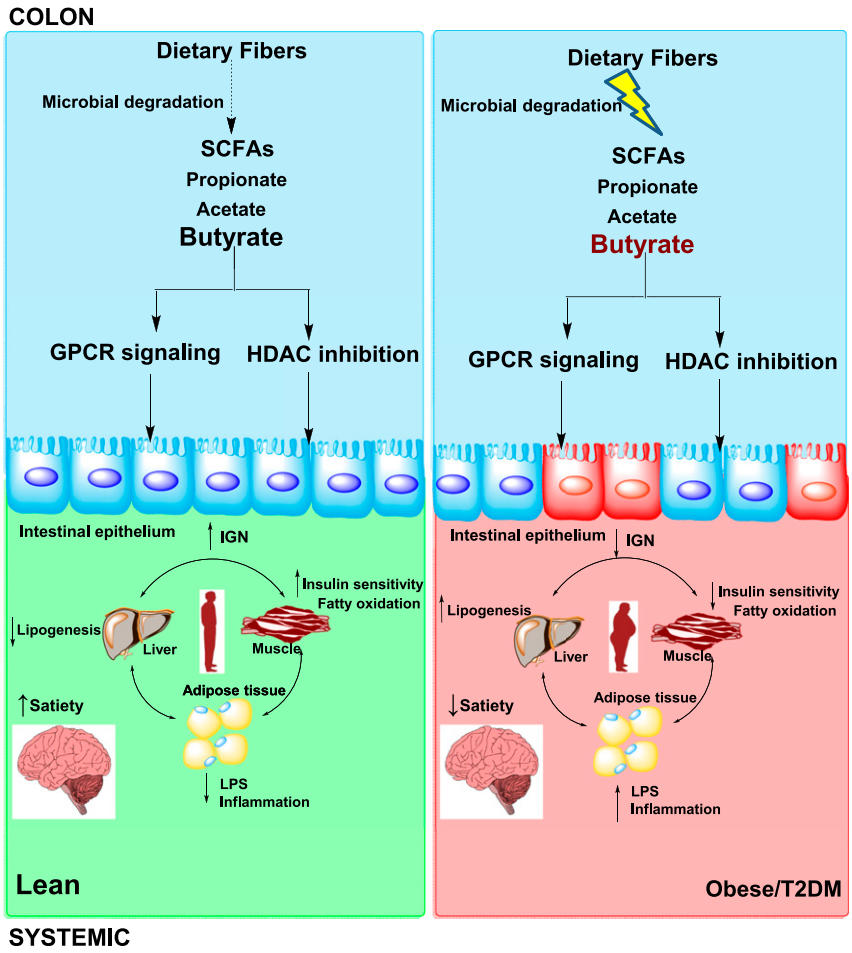

Figure 2—Role of gut microbiota–produced SCFAs in human glucose metabolism in obese subjects. Fermentation of dietary fibers by intestinal bacteria generates SCFAs, including butyrate, that have both metabolic and epigenetic effects. Obese insulin-resistant subjects are characterized by altered SCFA production compared with lean subjects. We hypothesize that in these subjects, this adversely affects satiety, hepatic glucose, and lipid production as well as inflammatory tone.

regulating role of GPR41 in energy homeostasis in relation to the intestinal microbiota and their metabolic products. Furthermore, *Gpr41*$^{-/-}$ deficiency was associated with a decrease in the gut-derived hormone PYY, resulting in a decreased extraction of energy from the diet associated with an increase in intestinal transit time (39).

Another function of butyrate, which may contribute to its possible beneficial role in the host's metabolism, is maintaining intestinal integrity. This contributes to the prevention of endotoxemia, a process resulting from translocation of endotoxic compounds (lipopolysaccharides [LPS]), of gram-negative intestinal bacteria. In the last decade, it has become evident that insulin resistance and T2DM are characterized by low-grade inflammation (40). In this respect, LPS trigger a low-grade inflammatory response, and the process of endotoxemia can therefore result in the development of insulin resistance and other metabolic disorders (41,42). Butyrate also seems to play a part in the recent discovery of the intestine's ability to produce glucose itself. Glucose released by intestinal gluconeogenesis (IGN) is detected by a portal vein glucose sensor that signals to the brain through the peripheral nervous system, thus positively influencing glucose metabolism and intake of food (43). De Vadder et al. (44) confirmed in rats the beneficial effects of SCFAs and IGN on glucose metabolism and subsequently showed that butyrate is involved by activating gene expression of IGN in mice. However, these findings still need validation in humans, and we are currently executing a study in which we have treated subjects with metabolic syndrome for 4 weeks with oral butyrate to study its effects on insulin sensitivity and microbiota composition.

INNOVATIVE STRATEGIES FOR NOVEL THERAPEUTICS IN T2DM

Interestingly, in animal models, butyrate has also been shown to both affect intestinal serotonin levels (45) and increase serotonin transporters (SERTs) in the hypothalamus (46). Furthermore, butyrate directly affects sympathetic tone and intestinal transit times (47) as well as physical activity (48). In line, it is known that serotonin itself can regulate intestinal permeability (49) besides being an important signaling neurotransmitter in the gut and brain involved in regulation of body weight and food intake by enhancing satiety (50). A reduction in cerebral SERTs, essential regulators of serotonergic transmission, is associated with obesity (51). In a human study, when healthy lean subjects received a hypercaloric snacking diet for 6 weeks, a significant 30% decrease of hypothalamic SERT binding was seen (52).

In this respect, it is interesting to note that studies have suggested a regulating influence of intestinal bacteria on serotonin (53,54). For example, bariatric surgery (Roux-en-Y gastric bypass [RYGB]) has been shown to significantly affect serotonin metabolism in both animals (51,55) and humans (56). Moreover, RYGB is regarded as a last resort but very successful treatment for morbidly obese patients, because next to inducing weight loss up to 50% of the original weight, it also decreases the risk of T2DM and cardiovascular pathology (57,58). RYGB has even been shown to resolve insulin resistance faster than the actual weight loss, underscoring a potential weight-independent effect on metabolism (41,58). As RYGB can alter the composition of the gut microbiota in both mice (59) and humans (60,61), this might be one of the contributing factors. When diabetic mice were colonized with feces of post-RYGB mice, they lost weight and showed improvement in glucose and lipid metabolism with specific changes in butyrate-producing bacteria (59). These findings suggest that the change in butyrate-producing microbiota after RYGB may play an important role in satiety as well as regulation of glucose and lipid metabolism.

It is thus becoming increasingly evident that the composition of gut

microbiota plays a role in the regulation of glucose and lipid metabolism. Specifically, there seems to be an association between butyrate-producing bacteria and beneficial effects on metabolism in both mice and humans (24,35). Furthermore, an alteration in the composition of the gut microbiota may be involved in the development of obesity and T2DM. Further studies are however needed to establish the causality of this and as to whether increasing intestinal SCFAs, including butyrate, has the same metabolic influence as SCFA-producing bacteria on human metabolism, including insulin sensitivity and inflammatory tone. Thus, in order to validate the hypothesis of butyrate-producing bacteria as role players and their products as signaling molecules in human glucose and lipid metabolism, double-blinded randomized controlled trials using either SCFA supplementation (given either orally or rectally) or FMT derived from different donors (e.g., on different diets) are needed. Priorities for further studies also include therapeutic intervention trials with specific types of single bacterial strains in order to elucidate particular beneficial patterns in gut microbiota composition.

Other areas of therapeutic interest in this respect are nondigestible but fermentable fibers, such as inulin, fructo-oligosaccharides, galacto-oligosaccharides, and lactulose. Food artificially enriched with these fibers has been termed a prebiotic when it is able to shift the composition of gut microbiota by stimulating the growth or activity of beneficial species (23). In this respect, carbohydrate-fermenting bacteria such as *Bifidobacteria* and *Lactobacilli* increase upon prebiotic treatment in different age-groups (62). The effects of prebiotics have been ascribed to an immune-mediated mechanism. As previously mentioned, high-fat dietary feeding is associated with endotoxemia, which in turn is linked to a reduced abundance of *Bifidobacteria* with a concomitant increase in gram-negative (LPS-containing) bacteria. In line, when prebiotic-containing oligofructose (OFS) were fed to mice on a high-fat diet, this restored levels of their *Bifidobacteria* and consequently reduced endotoxemia and improved glucose tolerance (63).

Another line of therapeutic approach is probiotics, which encompass food supplements enriched with strains of live bacteria, including species of *Bifidobacteria* and *Lactobacilli*, that are able to alter the gut microbiota beneficially for the host (28,64). In mice, antidiabetic effects have been shown following administration of probiotics containing certain *Lactobacillus* strains (65) with a concomitant reduction in endotoxemia (66). Due to the placebo effect of these products, proper double-blinded randomized controlled trials with accepted hard end points are needed in humans to address the potentially beneficial metabolic effects of probiotic strains in relation to the composition of the intestinal microbiota.

Although public health has benefited substantially from the discovery of antibiotics, its rapid increase in use is starting to raise health concerns. Next to the obvious issue of antibiotic resistance, its worldwide use might potentially be associated with the obesity epidemic (67). Although oral antibiotic treatment effectively eradicates pathogenic bacteria, the beneficial intestinal microbial community is also affected with possible dire metabolic consequences. Long-term intravenous vancomycin (aimed at gram-positive bacteria) in adult patients was linked to an increased risk of developing obesity (68), whereas amoxicillin (aimed at gram-negative and anaerobic bacteria) had only minor effects. In line, short-term oral administration of vancomycin (but not amoxicillin) significantly impaired peripheral insulin sensitivity via altered bile acid dehydroxylation in males with metabolic syndrome, which was associated with a changed gut microbiota composition (69). Moreover, even short-term courses of oral antibiotics were shown to have profound (irreversible) effects on intestinal microbial diversity and composition (70). The recent data linking use of antibiotics in early infancy to distinct long-term effects on intestinal microbiota diversity and the risk of childhood overweight (71) are even more alarming but not surprising. In the last 50 years, the use of subtherapeutic antibiotic therapy in farm animals has become widely used as it increases growth and therefore food production. In mice, treatment with subtherapeutic doses of antibiotics alters the composition of the intestinal microbiota and therefore affects metabolic pathways, particularly concerning SCFA metabolism (72). These findings emphasize the causal relationship between metabolism and the gut microbiome, and a more cautionary use of antibiotics seems to be more justified than ever.

However, the simplest solution to restoring pathological disturbances in the composition of the gut microbiota may be a change in dietary habits. Diet has been shown to strongly affect the composition of the microbiome (73). When obese humans were put either on a fat-restricted or carbohydrate-restricted low-calorie diet, an increase in the abundance of *Bacteroidetes* and a decrease in *Firmicutes* was reported (12). In another study, diet-induced weight loss versus weight-stabilization interventions in obese humans increased intestinal microbial gene richness and was associated with a reduced systemic inflammation (74). These data corroborate with another controlled diet intervention study in 98 human subjects showing that certain dominant gut microbial communities, or "enterotypes," correlated with specific kinds of diets (73). For example, *Bacteroides* was associated with a protein-rich diet, whereas *Prevotella* correlated with a fiber-rich diet; moreover, gut microbiota composition could be altered within 24 h whereas enterotype remained stable during the 10 days of the study. Based on this rapid and dramatic plasticity of intestinal microbiota composition, there is a specific need to determine intestinal microbiota composition in a standardized way (e.g., sequencing several fecal samples per person over a specific time point while taking dietary intake and medication use into account).

CONCLUSIONS

The determination of the intestinal microbiome in obesity and T2DM has led to an exponential increase of scientific research in this area. In this review, we tried to cover several topics in order to provide clinically relevant insight. A multitude of studies has revealed various potential mechanisms, ranging from endocrine and metabolic pathways to mechanisms on a cellular and genetic level. Our understanding of environmental factors affecting the microbiome, such as our diet, repetitive infections, and the use of antibiotics, is improving and will hopefully

contribute to finding a solution for the global obesity epidemic.

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