Therapeutic Potential of Butyrate for Treatment of Type 2 Diabetes

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Metagenomics studies have shown that type 2 diabetes (T2D) is associated with an altered gut microbiota. Whereas different microbiota patterns have been observed in independent human cohorts, reduction of butyrate-producing bacteria has consistently been found in individuals with T2D, as well as in those with prediabetes. Butyrate is produced in the large intestine by microbial fermentations, particularly of dietary fiber, and serves as primary fuel for colonocytes. It also acts as histone deacetylase inhibitor and ligand to G-protein coupled receptors, affecting cellular signaling in target cells, such as enteroendocrine cells. Therefore, butyrate has become an attractive drug target for T2D, and treatment strategies have been devised to increase its intestinal levels, for example by supplementation of butyrate-producing bacteria and dietary fiber, or through fecal microbiota transplant (FMT). In this review, we provide an overview of current literature indicating that these strategies have yielded encouraging results and short-term benefits in humans, but long-term improvements of glycemic control have not been reported so far. Further studies are required to find effective approaches to restore butyrate-producing bacteria and butyrate levels in the human gut, and to investigate their impact on glucose regulation in T2D.

Keywords: type 2 diabetes (T2D), microbiota, butyrate, metabolic disease, short chain fatty acids (SCFAs)

INTRODUCTION

Type 2 diabetes (T2D) is a global concern and is projected to affect 700 million people by 2045 (1). Although lifestyle interventions (including diet, exercise and weight loss) are the first option for managing T2D, patients are often prescribed medications. A variety of drugs are already available, but side effects (such as pancreatitis and gastrointestinal complications) (2), and lifelong dependency on drugs entail a significant burden on the patients and on the healthcare system globally. Therefore, novel individualized therapies are being developed, focused on safety and personalized management of T2D.

‘Gut microbiota’ is a term used to describe the trillions of microbes that live in the gastrointestinal tract. The gut microbiota has been identified as a virtual organ interacting locally with the gut and systemically with other organs in the host to facilitate multiple physiological processes (3). The interest in understanding the composition and function of the gut microbiota has increased exponentially over the last two decades. From the initial studies addressing the possibility to culture and profile gut
microbiota communities (4–7), the field has developed to describe the core human gut microbiota (8–12), its tremendous genetic potential (100 times larger than the human genome) (10, 13) and variations according to geographical location (14, 15), ethnicity (16, 17) and disease status (18), including T2D (19, 20). How an altered gut microbiota can impact metabolic health is debated, but metabolomics investigations have shown that the gut microbiota contribute to the variation of blood metabolites in humans (21), with important implications for metabolic regulation (22). Trimethylamine oxide (23), imidazole propionate (24) and indolepropionic acid (25) are examples of microbial metabolites associated with development or protection against metabolic diseases, and their specific roles in metabolic diseases as well as signaling mechanisms are currently under investigation.

Butyrate is one of the short chain fatty acids (SCFAs) produced as end-products of intestinal microbial fermentations (26, 27). Butyrate is absorbed rapidly in the gut and acts as signaling molecule in receptor-mediated signaling in numerous cell types (28). Microbial butyrate production in the human gut has been known for decades (29) before the large sequencing efforts of the gut microbiota started, but it was only in the last decade that metagenomics surveys consistently revealed in multiple independent human cohorts a decrease of butyrate-producing bacteria in individuals with T2D (19, 20). As restoration of butyrate-producing bacteria and butyrate levels might provide new treatment options for T2D, here we review recent literature on the association of butyrate and butyrate-producing bacteria with T2D, and discuss the therapeutic potential for management and treatment of this disease (Figure 1).

**FIGURE 1** | Several independent metagenomics studies have detected a decrease of butyrate producers in stools from individuals with prediabetes and type 2 diabetes (T2D) compared to individuals with normal glucose tolerance (NGT). While the loss of butyrate producers is robust and associated also with obesity and other cardiometabolic comorbidities (18), consistent patterns for increased microbial features have not been found, possibly due to the redundancy of the gut microbiota and stochasticity in gut microbiota alterations (30); only few gut microbiota species (mostly opportunistic pathogens) have been observed as increased in a limited number of studies. Thus, butyrate and butyrate producers have been selected as potential targets for the development of novel therapeutic strategies for T2D, such as direct butyrate administration, administration of butyrate producers and/or bacteria able to promote intestinal butyrate production [probiotics and next-generation probiotics (NGPs)], interventions with dietary fibers and fecal microbiota transplant (FMT).
TYPE 2 DIABETES AND BUTYRATE-PRODUCING BACTERIA

T2D has been associated with compositional and functional shifts in the gut microbiota. One of the striking features that was consistently observed in multiple cohorts across diverse geographical locations is the reduction of butyrate-producing bacteria in individuals with T2D. The first observations came from shotgun metagenomics studies of fecal communities in Chinese (20) and Swedish (19) individuals, and showed decreased abundance of several butyrate producers such as Roseburia and Faecalibacterium prausnitzii; these findings were later extended to individuals with T2D in Indian (31) and African (32) populations. Lower abundance of F. prausnitzii has also been found in the mesenchymal adipose tissue of obese individuals with T2D compared to normoglycemic controls matched for body mass index (33), reflecting the lower intestinal abundance of this bacterium in T2D independent of obesity, but linked to the metabolic status. The positive association between microbial potential for butyrate production and normoglycemia is supported by the results of a Dutch study that combined fecal microbiota metagenomics and human genome sequencing, and showed that higher potential for butyrate production driven by host genetics was linked to improved insulin response to an oral glucose load in normoglycemic individuals (34). Furthermore, one recent study has shown that also the diurnal oscillation of the gut microbiota is altered in T2D, and in particular that the abundance of several bacteria, among which Roseburia and F. prausnitzii, lost rhythmicity, and that the arrhythmicity might be important for risk classification and prediction of T2D (35). However, it is important to mention that other gut commensals (e.g. Akkermansia, Bifidobacterium and Bacteroides) are also decreased in T2D and might play additional roles for the modulation of gut barrier function, inflammation and metabolism, as reviewed elsewhere (36, 37).

The initial studies on the associations between gut microbiota and T2D did not account for medications used for T2D treatment; however, it is now established that numerous non-antibiotic drugs can influence the gut microbiota (38). Metformin is the first line of treatment in newly discovered T2D patients and was first demonstrated to influence gut microbiota composition in diet induced obese mouse models, where it resulted in enrichment of Akkermansia muciniphila (39, 40), a gut commensal showing beneficial effects on metabolism (41). Similar results were later observed also in large human cohorts (including both treatment naïve and metformin-treated T2D patients), in which shotgun sequencing of the fecal microbiota showed enrichment of butyrate producers, and in some cases also of A. muciniphila (42–45). By using germ-free (GF) mice, it was also shown that part of the antidiabetic effects of metformin can be explained by the microbiota shifts. GF mice are devoid of any microbiota, and are an efficient model to understand the functional significance of compositional changes in the gut microbiota: fecal microbiota transplant from metformin-treated individuals to GF mice were able to transfer the improved glucose tolerance phenotype to the recipients (45). In addition, gut microbiota has also been shown to interact with metformin and the diet to promote microbial synthesis of agmatine, specifically in individuals with T2D, an effector molecule able to regulate host lipid metabolism (46). Overall, these results indicate a possible beneficial role of the microbial shifts induced by metformin for the improvement of the glycemic and metabolic status in T2D. However, these studies also indicate that potential confounders [e.g., medications, dietary supplements and obesity (47)] should be considered in gut microbiota analyses for the identification of possible microbial signatures of T2D.

T2D is a chronic metabolic disorder that can remain undetected for a number of years. Prediabetes precedes T2D, and often presents with intermediate hyperglycemia, such as impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or combined glucose intolerance (CGI) (48). Microbiota profiling in a Danish cohort diagnosed with prediabetes revealed lower abundance of butyrate-producing bacteria compared with age- and sex-matched individuals with normal glucose regulation (49). In addition, a metagenomic study in a T2D-treatment-naïve Swedish cohort has shown that microbiota composition is altered in IGT and CGI, and is characterized by a reduction in the abundance of butyrate-producing bacteria and the terminal genes for butyrate synthesis (43). These studies indicate that the butyrate-producing potential of the gut microbiome is depleted already in the prediabetes state, and suggest that replenishment of butyrate producers or butyrate levels might be important to delay or prevent progression to T2D. In contrast with the consistent finding of reduced abundance of butyrate-producing bacteria in T2D, different studies report extensive variation for the levels of butyrate in feces and/or blood of individuals with T2D compared to controls. In the large Dutch cohort, fecal butyrate levels did not correlate with either butyrate production potential or the selected anthropometric and glycemic traits, thus suggesting that fecal butyrate levels might not be representative of butyrate production and absorption (34). However, results from two smaller cohorts have showed decreased levels of SCFAs (including butyrate) in individuals with T2D as well as significant correlation with metabolic parameters (50, 51). Therefore, as butyrate is volatile and quickly absorbed and consumed by the colonic epithelium, static measurements in fecal and blood samples might not be sufficient to reveal an actual reduction of butyrate levels in T2D. Radioactive tracers might offer a better solution to trace the origin and absorption of butyrate, but would not be applicable to large-scale human cohort studies.

BUTYRATE – PRODUCTION, ABSORPTION AND PHYSIOLOGICAL ROLES

The SCFAs acetate, propionate and butyrate are produced by microbial fermentations in the gut (52). Most of the butyrate
Salmonella consume oxygen and prevent colonic invasion by pathogenic in the induction of proliferator-activated receptor-gamma in colonocytes, which results in studies have shown that butyrate contributes to maintain the colonic human gut microbiota express HDAC inhibitory activity to regulate the expression of genes by epigenetic mechanisms. Particularly interesting therapeutic target. Produced butyrate is an important source of energy for colonic epithelium (59) and slower intestinal transit (60). Supplementation of butyrate reverses these phenotypes (i.e. prevents autophagy and efficiency results in reversible adaptive mechanisms to cope with nutrient deficiency. In addition, mouse studies have shown that butyrate contributes to maintain the colonic environment anaerobic through the activation of the peroxisome proliferator-activated receptor-gamma in colonocytes, which results in the induction of β-oxidation (57). This process has been shown to consume oxygen and prevent colonic invasion by pathogenic Salmonella and Escherichia species (57). Because of its effects on colonocytes, butyrate levels in the gut might be important not only for signaling to the host (as described below) but also for the composition of the gut microbiota. Therefore, butyrate might be a particularly interesting therapeutic target. Butyrate also serves as histone deacetylase (HDAC) inhibitor to regulate the expression of genes by epigenetic mechanisms. Supernatants from cultures of butyrate-producing bacteria from the human gut microbiota express HDAC inhibitory activity to class I and II HDACs (61). T2D is associated with epigenetic changes in multiple organs (62) and it seems plausible that microbially-produced butyrate could be one of the contributing factors. In a non-obese diabetic mouse model, it has been observed that the reduction of intestinal butyrate associated with T2D caused an increase in colonic HDAC activity resulting in production of reactive oxygen species and alteration of colonic permeability (63). Furthermore, supplementation of sodium butyrate has been shown to ameliorate palmitate-induced insulin resistance in muscle cells by promoting hyperacetylation of insulin receptor substrate-1 in an in vitro study (64). Consistent with this study, supplementation of sodium butyrate to mice modulated mitochondrial chromatin structure (65) and lowered HDAC activity in skeletal muscle resulting in improved insulin sensitivity (66). Finally, sodium butyrate treatment has been shown to suppress HDAC activity also in mouse liver, leading to reduced gluconeogenesis and improved glucose homeostasis (67). These animal studies suggest that reduction of butyrate in T2D may alter gene expression by epigenetic mechanisms leading to insulin resistance, which can be reversed by butyrate supplementation. However, this observation awaits validation in human cohorts.

Butyrate (as well as the other SCFAs) also acts as a signaling molecule and is identified as a ligand for G-protein coupled receptors (GPCRs), such as FFAR3, FFAR2 and GPR109A. Human orthologs of FFAR2 have similar affinity for acetate and propionate followed by butyrate, while FFAR3 has higher affinity for propionate and butyrate compared to acetate (68). Niacin is identified as the most potent ligand of GPR109A, though butyrate also shows weak binding (69). These receptors are located in distal regions of the intestine and in adipocytes (70). In the intestinal lumen, the concentrations of SCFAs are supramaximal and therefore it is thought that these receptors are localized on the basolateral side of the intestinal epithelium (70). FFAR3 and FFAR2 are present on hormone-producing enteroendocrine cells (EECs): exposure to SCFAs stimulates EECs differentiation (71), and binding of SCFAs to FFAR3 and FFAR2 results in altered gene expression and secretion of gut hormones, such as peptide YY (PYY) and glucagon like peptide-1 (GLP-1) (72, 73). GLP-1 is one of the gut hormones that profoundly affects glucose regulation by promoting post-prandial insulin secretion, and GLP-1 based drugs are approved for the treatment of T2D (74). Supplementation of butyrate along with inulin have been shown to increase GLP-1 levels in individuals with T2D with significant improvement in the glycemic status (75). However, it is important to note that the in vitro studies mentioned here report different effects after stimulation with a mix of SCFAs or with individual SCFAs, indicating that the effects on EECs are not exclusively mediated by butyrate. Nevertheless, in human cell lines, butyrate selectively stimulates PYY secretion through mechanisms largely driven by HDAC inhibition (76).

In addition to signaling in the gut, activation of SCFAs-binding receptors might be important also in the adipose tissue. Selective chemical agonism of GPR109A in individuals with T2D
decreased fasting glucose, but not Hb1Ac, through inhibition of lipolysis in adipocytes as demonstrated by the decreased circulating levels of non-esterified fatty acids in the patients (77, 78). Consistent with these results, overexpression of FFAR2 in adipose tissue protected mice from gut microbiota-dependent diet-induced obesity (79). Additionally, a role for butyrate in the stimulation of thermogenesis in brown and white adipose tissue has been demonstrated in rodents (66), with potential relevance for the regulation of glycaemia. However, oral supplementation of butyrate in individuals with T2D did not alter brown adipose tissue activity (80).

Finally, butyrate signaling might also be important for islets function. *In vitro* experiments have shown that addition of butyrate in culture media reduced streptozotocin-induced islet cell death (81). Moreover, supplementing HFD with 5% butyrate in a T2D mouse model prevented β-cell expansion and fat accumulation in the pancreas (82). In contrast, an oral dose of 4g butyrate to individuals with type 1 diabetes for a month did not improve β-cell function or islet autoimmunity (83).

Therefore, SCFAs (including butyrate) may play important roles in metabolic control, particularly via regulation of EECs and adipocyte lipolysis. However, specific targeting of the GPCRs in humans might be difficult due to their complex chemistry and diverse functions in different tissues (84). Additionally, applications might be limited by the lack of concordance between mouse and human studies that can possibly be explained by differences in dose, route and duration of administration, discrepancy between experimental models for diabetes and human diabetes, and specific intestinal environments in the different hosts. Nevertheless, the animal studies suggest that butyrate influences the regulation of glucose metabolism through multiple pathways that, if further characterized and validated in humans, can possibly be harnessed for the development of therapeutic strategies (Figure 2).

**RESTORATION OF BUTYRATE IN TYPE 2 DIABETES**

Restoration of the intestinal levels of butyrate might be a novel strategy for the treatment of T2D, that could also be added to conventional therapy with lifestyle management and glucose-lowering drugs. In recent years, a number of studies have attempted to replenish butyrate levels and butyrate-producers in the gut using different approaches as discussed below: direct supplementation of butyrate or butyrate-producing bacteria, dietary supplementation of fibers to feed microbial butyrate production, and fecal microbiota transplantation.

**Supplementation of Butyrate**

Butyrate can be supplemented as sodium conjugate or as tributyrin (a triglyceride in which glycerol is esterified with three butyrate molecules). In mice with diet-induced obesity, oral supplementation of 400mg/kg of butyrate improved glucose tolerance and increased the expression of phosphorylated adenosine monophosphate kinase (AMPK) as well as glucose transporter-4 in the adipose tissue, and reversed some of the gut microbiota alterations caused by the high-fat diet (HFD) (85). In another mouse study, addition of 5% butyrate to HFD increased energy expenditure, improved insulin sensitivity, and induced adaptive thermogenesis in BAT followed by increased AMPK activity and mitochondrial biogenesis in muscle cells (66). As an alternative, tributyrin supplementation in diet-induced obese mice

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**FIGURE 2** | Dietary fiber is fermented by the gut microbiota to produce short chain fatty acids, including butyrate. Butyrate is efficiently absorbed by colonocytes and is utilized as energy source. Butyrate-mediated activation of the peroxisome proliferator-activated receptor-gamma (PPAR-γ) induces β-oxidation and consumption of oxygen, thus facilitating the establishment of anaerobic conditions that are required for the growth and function of several anaerobic gut commensals (57). In the specialized enteroendocrine cell (EEC), butyrate binds free fatty acid receptors (FFAR) FFAR2 and FFAR3 and regulates gut hormone release (73). Butyrate also acts as histone deacetylase (HDAC) inhibitor to regulate gene expression in EEC (78) and enterocytes (63). After absorption and utilization by colonocytes, the residual butyrate is first drained into the portal circulation, and then into the peripheral systemic circulation (29). In the systemic circulation, butyrate may regulate thermogenesis in brown adipose tissue (68) and β-cell function in pancreas (82).
was also shown to improve glucose tolerance and inflammatory status (86), indicating that direct supplementation of butyrate might have beneficial effects on both metabolic and inflammatory parameters relevant for the pathophysiology of T2D.

With convincing results in mouse studies, butyrate supplementation was also tested in individuals with and without metabolic syndrome who were given 4g sodium butyrate in capsules for a period of 4 weeks (80). In this study, butyrate supplementation did not increase butyrate levels either in feces or plasma, but it improved both peripheral and hepatic insulin sensitivity in individuals without metabolic syndrome. In another study, oral butyrate supplementation at the same dose improved the inflammatory status in individuals with metabolic syndrome, but no effect on insulin sensitivity was measured in this study (87). Additional studies might be required to determine effective doses of butyrate in humans, or other methods of administration and delivery of butyrate to the colonic epithelium that mimics the production by the gut microbiota.

**Butyrate-Producing Bacteria**

Live bacteria that provide health benefits when consumed are generally called probiotics, and traditional *Lactobacillus* probiotics have demonstrated some efficacy for hyperglycemia and insulin sensitivity in human cohorts (88–91). In two independent studies, supplementation of *Lactobacillus paracasei* or *Bifidobacterium bifidum* to healthy individuals increased fecal butyrate levels (92, 93), indicating that traditional probiotics may modulate the activity of butyrate producers. However, the intestinal microbes that have been found as decreased in T2D in metagenomics studies are not traditional probiotics, and are being explored to produce next-generation probiotics (NGPs) (94, 95). For the butyrate producers, oral administration of *Clostridium butyricum* to mice lacking the leptin receptor, or to mice on HFD injected with streptozotocin to induce diabetes, was shown to improve oral glucose tolerance and insulin levels, and to increase the abundance of butyrate producers and fecal butyrate levels (96).

In another study, oral administration of *Eubacterium hallii* to mice lacking the leptin receptor improved insulin sensitivity and increased energy expenditure (97). However, administration of *E. hallii strain L2-7* (now reclassified as *Anaerobutyricum soehngenii*) to individuals with insulin resistance improved insulin sensitivity only in individuals with a specific gut microbiota at baseline (98), reflecting both the resilience of the human gut microbiota and the ecological interactions of commensal microbes in the communities that might be species-specific. To produce effective NGPs, advanced data-driven metagenomics approaches (99) and specific isolation efforts might be required to develop synthetic microbial communities targeted to produce butyrate.

**Dietary Fiber**

Since butyrate-producing bacteria feed upon dietary fiber, dietary supplementation with fiber may provide a feasible option to increase the levels and the activity of the bacteria, and increase the intestinal levels of butyrate. In a randomized clinical study by Zhao et al., supplementation of a mix of dietary fibers to individuals with T2D improved glycemic parameters, accompanied by increased abundance of acetate- and butyrate-producing bacteria and increased fecal levels of acetate and butyrate (100). In another study, combining a mix of butyrate-producing species (*E. hallii, Clostridium beijerinckii* and *C. butyricum*), with other gut bacteria (*A. muciniphila* and *Bifidobacterium infantis*) and inulin as fermentable fiber modestly increased butyrate levels and improved oral glucose tolerance and glycated hemoglobin levels in individuals with T2D (101). Finally, dietary supplementation of inulin along with sodium butyrate in capsules for 45 days improved fasting glucose and waist-to-hip ratio in individuals with T2D (75). These studies clearly indicate that dietary fiber itself or in combination with NGPs or butyrate can improve glucose control in T2D. However, strategies to maintain patient compliance and investigations of long-term effects of these supplements are still warranted. Additionally, as it is now evident that the baseline gut microbiota is a strong predictor of success for dietary interventions (102, 103), probiotic administrations (89) and microbiota transplantations (104), stratification of individuals with T2D based on their microbiota may help to achieve better metabolic outcomes.

**Microbiota Transplantation**

Microbiota transplants from mouse models (105) and humans (45, 106) into GF mice have successfully demonstrated the transmissibility of donor’s phenotypes. Therefore, human-to-human fecal microbiota transplants (FMT) [that have shown unprecedented success for the treatment of *Clostridium difficile* infections (107)] have recently been attempted for the treatment of T2D. When insulin resistant individuals were administered with duodenal infusion their own fecal microbiota (autologous) or fecal microbiota from a healthy lean donor (allogenic), the allogenic group displayed an improved insulin sensitivity (104, 108). The improved phenotype was observed 6 weeks after transplant in both studies, but not 18 weeks post-FMT (104). Change in butyrate producers (such as *Roseburia, Eubacterium* and *Butyriciclostridium*) in feces and in the small intestine was observed in both studies, but increase in butyrate levels were observed only in one (108). In another study, daily cellulose supplementation after a single-dose oral FMT in individuals with metabolic syndrome improved insulin sensitivity 6 weeks after treatment compared to baseline. The authors found that this outcome was linked to higher GLP-1 secretion and better engraftment of the donor microbiota; however, they did not report significant changes for either the proportions of butyrate producers or fecal butyrate levels (109). Overall, the studies on FMT indicate that, while this procedure may improve insulin sensitivity in the short-term, the host gut microbiota is resilient enough to drift away the foreign microbial community in the long-term. In addition, while FMT is generally associated with mild side-effects, major adverse events have also been reported (110). These observations question the feasibility and applicability of FMT as a way of treatment for T2D.
**BUTYRATE AS THERAPY – CONCLUSIONS**

Butyrate has long been known as a microbial fermentation product of dietary fibers in the gut, and references of butyrate-producing bacteria isolated from dietary sources emerged already in late 1940 (111). The recent association of T2D with reduction in late 1940 (111). The recent association of T2D with reduction of butyrate-producing bacteria has spurred interest to explore the therapeutic potential of butyrate for the treatment of T2D but, while the results of experimental studies overall look promising, human interventions have only shown positive outcomes in the short term, and might have important limitations. In particular, current studies based on the metagenomic profiling of DNA are not able to determine the activity of butyrate producers in the human gut. Probiotics, NGPs and fiber supplementations might be successful strategies to increase butyrate-producing bacteria and improve hyperglycemia and insulin resistance, but their effects might be dependent on the individualized gut microbiota; for example, ingestible electronic capsules able to monitor microbial fermentations directly in the gut (112) could be used to characterize intestinal conditions, responses to fibers and microbiota profiles linked to homeostatic butyrate production. Furthermore, robust methods for the measurement of butyrate, tracer studies and live-detection of butyrate-producing bacteria [for example, by flow cytometry (113)] might help to strengthen the association of butyrate with T2D and identify new potential NGPs or synthetic microbial communities for butyrate-based management of T2D.

**AUTHOR CONTRIBUTIONS**

TA conceptualized the manuscript. TA and VT wrote the manuscript. All authors contributed to the article and approved the submitted version.

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