The role of gut microbiota in human metabolism

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CHAPTER 8

FECAL TRANSPLANT: A SAFE AND SUSTAINABLE CLINICAL THERAPY FOR RESTORING INTESTINAL MICROBIAL BALANCE IN HUMAN DISEASE?

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Abstract

Recent studies have suggested an association between intestinal microbiota composition and human disease, however causality remains to be proven. With hindsight, the application of fecal transplantation does indeed suggest a causal relation between interfering with gut microbiota composition and a resultant cure of several disease states. In this review, we aim to show the available evidence regarding the involvement of intestinal microbiota and human (autoimmune) disease. Moreover, we refer to (mostly case report) studies showing beneficial or adverse effects of fecal transplantation on clinical outcomes in some of these disease states. If these findings can be substantiated in larger randomized controlled double blind trials also implementing gut microbiota composition before and after intervention, fecal transplantation might provide us with novel insights into causally related intestinal microbiota, that might be serve as future diagnostic and treatment targets in human disease.

Introduction

The average human bowel is home to trillions of bacteria, which outnumber the cells of their human host by a factor of ten to one, and collectively their genes outnumber human genes by one hundredfold (1). Although the composition of the gut microbiota varies after birth, it becomes relatively stable after the age of 2 years and onward into adult life. Metagenomic research has currently suggested that up to four major bacterial phyla (*Bacteroidetes, Firmicutes, Actinobacteria and Proteobacteria)* consisting of thousands of mostly anaerobic species inhabit the human gut with a steep, stomach acid-driven, proximal-distal gradient (1). Together this intestinal microbiota form an exteriorized organ complementing and interacting with the human intestinal mucosa. On the other hand, the intestinal mucosa continuously encounters a wide variety of antigens derived from food, commensal organisms and occasional pathogens and together with the immune system needs to balance between protective reactions against harmful pathogens and tolerance against commensal bacteria and dietary antigens to maintain intestinal homeostasis (1).

Intestinal microbiota transfer (also called fecal transplantation or fecal bacteriotherapy) has only recently gained increasing popularity with its success for treating *Clostridium difficile* infection in the last decade (2). However, the use of bowel-derived (of enteric) material for treatment of disease goes back more than 2500 years, when traditional Chinese medicine already used human fecal suspension or infant feces (for esthetic reasons called “yellow soup”) given orally to treat several gastro-intestinal related illnesses, including chronic diarrhea and food poising (3). Although intestinal microbe infusions used in veterinary practice in the Western world can be dated back to the 17th century as a treatment for ruminal acidosis (4), application in humans took until 1958, when Eiseman described the first report on the efficacy of fecal transplantation in the treatment of chronic (broad spectrum antibiotics induced) diarrhea (5). The above- mentioned therapies were given with great sense of logic reasoning, but with little biological substantiation. With the current developments in next generation culture-independent tools, such as 16S ribosomalRNA gene sequencing, broader insights into the composition of the

gut microbiota have now rendered significant associations of intestinal microbiota composition and human (predominantly autoimmune) disease states (6). This is of specific interest as a number of bacteria-derived proteins have now been identified as superantigens that can act as either B cell or T cell activators (so called molecular mimicry) thus eliciting nonspecific activation of lymphocytes, including self-reactive lymphocytes (7). Interindividual variations in gut microbiota might therefore lead to variations in immune activation, and possibly directly to autoinflammatory conditions. Following Koch's postulates to dissect causality from correlation in human (infectious) disease (8), application of fecal transplantation in these (autoimmune) diseases might be regarded an interesting research tool to identify causally involved intestinal microbial species as initiators of both gastrointestinal and systemic. In this review, we will thus aim to demonstrate whether specific human diseases correlate with altered intestinal microbiota composition and increased intestinal permeability. Moreover, we will present current (mostly case report-based) evidence on efficacy of fecal transplantation as treatment modality in these (autoimmune) diseases. Finally, with respect to safety we will describe optimalized screening protocol for fecal donors, way of infusion and the potential clinical applicability of fecal transplantion.

Neurology and intestinal microbiota composition

It has been long recognized that a bidirectional relationship between the brain and intestine exists (9). A good example is multiple sclerosis (prevalence 150 per 100,000 subjects with more females than males affected, age of onset 20–40 years), an autoimmune chronic inflammatory disease associated with degradation of fatty myelin sheaths around the axons of the brain and spinal cord, resulting in neurologic debilitating symptoms (10). Recent studies have identified a crucial role for intestinal bacteria species which via an IL-17-dependent process are able to trigger autoimmune-mediated demyelination (11). Although no studies are yet available on the intestinal microbiota composition in patients with multiple sclerosis, it is known that these subjects are characterized by an increased intestinal permeability (12), thus facilitating bacterial translocation from the intestine into the bloodstream. Albeit in a small group of patients fecal transplantation was successful on clinical symptoms relief in a recently presented case report (13, also see figure 1).

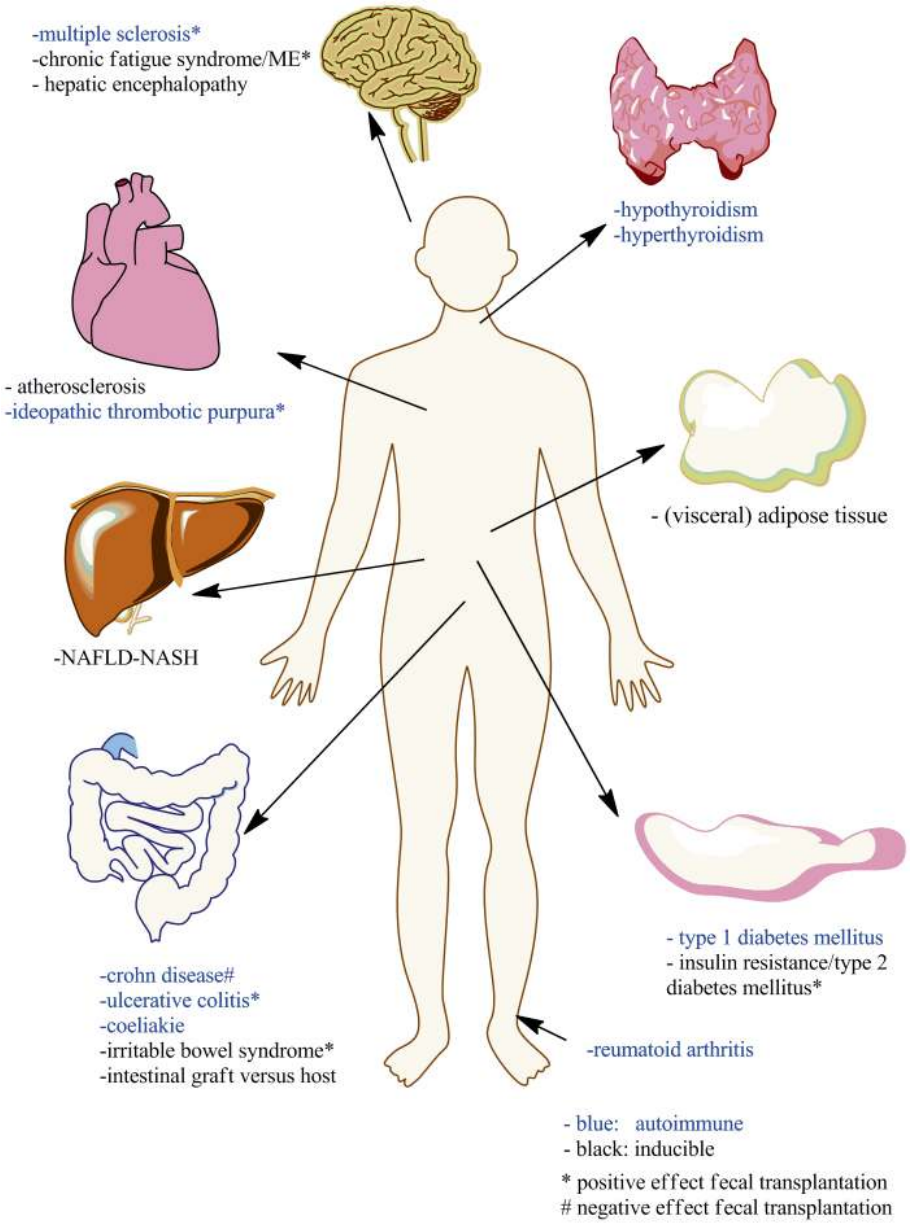

Figure 1. This depicts all (autoimmune) disease known to be associated with microbial dysbalance (blue: autoimmune and black: inducible disease) and current reports of fecal transplantation used in these disease states.
(beneficial effect denoted by *; adverse or negative effect denoted by #)

Along this line, chronic fatigue syndrome (or myalgic encephalomyelitis, prevalence around 3,000 cases for every 100,000 adults with more females than males affected, age of onset 29-35 years) is characterized by chronic myopathy and arthralgia, headaches and mental and/or physical exhaustion (14). Increased intestinal permeability was found to be present in these patients with chronic fatigue syndrome and correlated with disease severity (15). Moreover, a recently presented retrospective case series comprising 60 subjects suggested that fecal transplantation was able to reduce disease severity grouping the majority of patients (16). No prospective randomized studies are currently available to underscore the clinical validity of these findings.

Endocrinology and intestinal microbiota composition

Hashimoto's thyroiditis (prevalence 200 in 100000 people with more females than males affected, age of onset 60 years) is a thyroid autoimmune disease characterized by intrathyroidal mononuclear cell (lymfocytic) infiltration and the production of autoantibodies against thyroglobulin and thyroid peroxidase resulting in decreased thyroid hormone production (17). Although no data are available on altered intestinal microbiota composition in patients with Hashimoto's thyroiditis, increased intestinal permeability has been shown to be present (18). Also, conflicting data on the association between specific subsets of gut microbiota (Yersinia enterocolitica) and the development of Hashimoto's thyroiditis has been reported (19;20). Along this line, no data on gut microbiota composition are available in hyperthyroidism (Graves' hyperthyroidism, prevalence 400 per 100,000, more females than males, age of onset 20–50 years) which is thought to originate by thyroid autoantibodies that activate and block the TSH-receptor, thereby stimulating thyroid hormone synthesis (21). However, it is not unlikely that translocation of intestinal microbiota may play a role in the development of TSH receptor autoantibodies, as in vitro findings suggest that activated orbital fibroblasts from subjects with Graves' ophthalmopathy can function as sentinel cells (like lymphoid tissue) upon bacterial activation (22). Up to now, no case reports are available with respect to fecal transplantation and thyroid disease.

With respect to the pancreas, type 1 diabetes mellitus is an autoimmune disease associated with progressive beta cell destruction and subsequent insulin dependence in the first 2-3 decades of life (prevalence 19 per 100,000, more males than females age of onset 5-25 years), which is associated with an increased morbidity and mortality risk (23). Altered intestinal microbiota composition and increased intestinal permeability have been shown to be present in DM1 patients (24;25). In this regard, recent mouse studies suggest that interaction in the small intestinal lamina propria between the intestinal microbes and the innate immune system (most likely T-helper cell type 17 (Th17) is a critical epigenetic factor in the development of type 1 diabetes mellitus (26). Indeed, a very recent paper by Korsgren links the increased incidence of T1D during the last decades to differences in the intestinal bacterial flora (27). These data also suggest that bacteria entering the pancreatic ductal system could trigger β-cell destruction and thus induce type 1 diabetes mellitus. During this process, tolerance to gastrointestinal commensals is lost and microbiota-specific T cells are activated that affect beta cell function (28). Based on these findings, we have recently initiated a randomized controlled double blinded trial using fecal transplantation to investigate the effect on beta cell insulin secretion capacity in subjects with recently diagnosed type 1 diabetes mellitus. This work is in line with our previous work in insulin resistant (type 2 diabetes mellitus) subjects who are also characterized by increased intestinal permeability (29) and altered intestinal microbiota composition (30). In a double blind randomized controlled trial in treatment-naive insulin resistant male subjects, we were the first to show that fecal transplantation induced changes in specific (small) intestinal butyrate producing bacteria were associated with (temporarily) improved insulin sensitivity (31).

Gastroenterology and intestinal microbiota composition

Often diagnosed in conjunction with type 1 diabetes mellitus, celiac disease (prevalence 500-1000 per 100000, more prevalent in women than in men, age of onset 6-18 months upon introduction of grain products and a second peak at 20-40 years) is an autoimmune disease that presents with gastrointestinal complaints of bloating and chronic diarrhea, weight loss, osteoporosis and fatigue (32). Recent data have suggested altered (small) intestinal microbiota composition in subjects

with celiac disease (33), but at this moment no data on fecal transplantation in celiac disease have been reported. Inflammatory bowel disease (IBD) comprises Crohn's disease (30 per 100,000, age of onset 15-30 years, equal in males and females) and ulcerative colitis (200 cases per 100000, age of onset 15-30 years, equal in male and females) and both are regarded to have an autoimmune background (34;35). Whereas ulcerative colitis confines to the colon and rectum, Crohn's disease can be present in both small and large intestine as well as present with extraintestinal manifestations (eg arthritis and pyoderma gangrenosum); increased intestinal permeability has been described for both disease conditions (36;37). Anti-inflammatory corticosteroids and anti TNFa therapy and (if needed) bowel resection are the cornerstone of treatment; interestingly, like other autoimmune disease (eg hyperthyroidism) cessation of smoking has beneficial effects on remission (34;35). Intestinal microbiota composition and diversity are altered (30-50% reduced) in both ulcerative colitis (decreased *Akkermansia Muciniphila*) and Crohn's disease (decreased *Faecalibacterium prausnitzii*) (38;39). A recent casereport suggests I remission of the chronic inflammation of the colonic mucosa by fecal transplantation in subjects with ulcerative colitis (40) and a randomized controlled double blind trial (TURN) trial is currently ongoing at the AMC department of Gastroenterology aimed at treating dysbiosis present in UC patients with fecal transplantation . However, adverse effects (abdominal pains, bloating and no clinical improvement) were reported in a small group of patients with Crohn's disease treated with donor fecal transplantation (41). Although these data need to be reproduced in a larger trial, these findings warrant caution on the universal use of fecal transplantation in autoimmune disease with a different genetic background. Finally, a most interesting but largely unexplored research area comprises intestinal graft versus host disease after bone marrow transplantation, which was found to be associated with changes in intestinal microbiota composition and diversity (42), which makes it a likely candidate for pilot experiments with fecal transplantation.

With respect to non-autoimmune intestinal diseases, irritable bowel syndrome (IBS) and chronic constipation (prevalence 10-15%, age of onset 20-50 age, more females than males) are also reported to have decreased microbial diversity and

altered intestinal microbiota (decrease in Bacteroidetes phylum and more specific in Faecalibacterium species (43). In a retrospective cohort of 30 IBS patients, fecal transplantation had a success rate of 60% on clinical symptom relief (44). However, most scientific data regarding the clinical therapeutic efficacy of fecal transplantation is available for chronic *Clostridium difficile* infections. It is estimated that in the USA about 500.000 cases are seen annually (45) and this infection is also associated with decreased microbial diversity and altered composition (46). We recently reported in an open label, randomized controlled trial that a single fecal transplantation (via duodenal tube) was superior to vancomycin treatment in patients with recurrent Clostridium difficile-associated diarrhea (47). Moreover, we found normalized gut microbiota composition and increased numbers of Bacteroidetes species and decreased numbers of *Proteobacteria* after fecal transplantation using fresh healthy donor stool samples.

Finally, along this line altered microbiota diversity and composition (*Alcaligenaceae and Porphyromonadaceae*) were found in subjects with hepatic encephalopathy, which directly correlated with the level of cognitive function (48). To date, there are no ongoing trials showing superior effects of fecal transplantation on cognitive function as compared to lactulose or antibiotic treatment in this patient group.

Cardiovascular disease and obesity in relation to intestinal microbiota composition

The presence of fatty liver disease is strongly associated with obesity and cardiovascular disease (49). An increasing amount of data implies a causal link between the (small) intestinal microbiota and non alcoholic fatty liver disease (NAFLD) (50;51). It is now thought that development of NAFLD and subsequent nonalcoholic steatohepatitis (NASH) is mediated by bacterial endotoxins such as lipopolysaccharides (LPS) derived from intestinal bacteria (eg Proteobacteria), which are found in relatively large numbers in the human intestine of obese subjects (52). In line, recent data suggest that translocation of gram negative bacteria is able to induce a local inflammatory response and subsequent macrophage influx in the visceral adipose tissue (53). In this study, Amar et al. showed in an obese mouse model of type 2 diabetes mellitus that adipose tissue macrophages contain bacterial genomic material (originating from the intestine) and this was linked to low grade systemic inflammation. In humans, visceral

adipose macrophage infiltration is also related to the level of insulin resistance and endothelial dysfunction in obese subjects, underscoring a role for the innate immune system and intestinal microbiota in this detrimental phenomenon (54;55).

In line, atherosclerosis is nowadays regarded an autoimmune disease (56) and increased intestinal permeability in subjects with atherosclerosis was suggested in the Bruneck study showing that plasma endotoxin levels above the 90th percentile were associated with a threefold increase in cardiovascular event risk (57). Also, intestinal bacterial DNA (e.g. *Porphyromonas gingivalis*) has been isolated from human carotid plaque material (58;59). Moreover, gut microbiota and their catabolic products are thought to be involved in the chronic inflammatory status associated with cardiovascular disease. In a landmark study by Hazen et al, plasma levels of the bacterial products (choline and TMAO) were directly correlated to the percentage of foam cells in atherosclerotic lesions of mice as well as cardiovascular events rate in humans (60). Another autoimmune disease associated with enhanced cardiovascular disease risk is reumatoid arthritis (prevalence 200-300 per 100,000, more females than males affected, age of onset between 40 and 50), a chronic autoimmune inflammatory disease, generally leading to significant joint destruction and deformation. Anti-inflammatory corticosteroids, methotrexate and anti TNFa therapy are the backbone of treatment to reduce disease progression. Also, rheumatoid arthritis is characterized by increased intestinal permeability (61) and altered intestinal microbiota (e.g. increased Porphyromonas gingivalis) (62).
Along this line, immune (idiopathic) thrombocytopenic purpura is an autoimmune disease associated with increased bleeding tendency due to chronically lower platelets (5-10 per 100000, female to male 2:1, age of onset 56–60 years) (63). Treatment usually confers steroid regimens, splenectomy or stimulation of platelet production (thrombopoietin stimulation), and a relation with helicobacter pylori was reported (64). As a recently presented case report suggested beneficial effects of fecal transplantation on thrombocyte levels in a subject with idiopathic thrombocytopenic purpura (65), this calls for further investigation of gutmicrobiota induced molecular mimicry in immune (idiopathic) thrombocytopenic and rheumatoid arthritis (7).

Methodology of fecal transplantation

Historically, different routes are chosen to deposit fecal material in the bowel. Strategies range from fecal enemas (comprising the majority of cases), infusion via duodenal or gastric tube, through colonoscopy and self-administration via the rectum (66). There currently is no consensus on the best method of infusion, as it is difficult to compare the vast amount of case series and case reports which have different protocols and strategies. We have shown that duodenal infusion is an effective modality to infuse feces, comparable with the high success rates via colonscopy reported in case reports (47;66). Although both modalities have their specific (mostly theoretical) risks, our experience is that infusing feces through duodenal tube is less invasive and less strenous than through colonoscopy. Taking the pathophysiology of specific diseases into account (e.g. insulin resistance and celiac disease that originate in the small bowel) (1), we prefer infusion through a duodenal tube route. The potential adverse events from a fecal transplantation can be separated in procedure related adverse events, or complications related to donor feces infusion itself and if feces from a healthy donor is used with the risk of contracting a disease that can be spread through fecal material. With respect to procedure related safety (67) and in line with the available case reports we have performed more than 120 fecal transplantations via small intestinal tube infusion without any (serious) adverse events. Diarrhea on the day of infusion is reported frequently in most patients, followed by infusion related belching or cramping in a minority of patients. With respect to intestinal preparation, we usually use about 2 liters of cetomacrogol solution (via oral ingestion) which is administered to all patients either one day before or on the day of procedure, regardless the route of administration. According to our protocol, freshly produced donor stool (200-300gram dissolved in 500cc of sterile saline) is used preferably within 6h of passage. Water and other diluents (e.g. yogurt or milk) have also been described as vehiculum, with a trend towards improved outcome using larger volumes of prepared solution (68).

In regard to screening of potential donors, it is of the utmost importance that individuals with an increased risk of (sexually) contractible diseases and/or subjects who recently received blood transfusions are excluded in order to reduce transmission of (otherwise unknown) pathogens. We use a questionnaire (adapted from the Dutch

Blood Transfusion questionnaire used for potential blood donors) to ask questions regarding travel history, sexual behaviour, previous operations, blood transfusions, recent skin piercings and all other interventions that might contribute to carriage of an infectious disease (see Table 1). Any risk of a recently contracted infectious disease that is still in its window phase (HIV, Hepatitis) warrants exclusion of the potential donor. Although the potential risk of transferring an infectious disease is probably less using a relative as donor, a thorough investigation prior to screening is nevertheless preferred.

Table 1. Screening criteria of donor providing stool samples for fecal transplantation.

1.	No diarrhea or irritable bowel complaints; GI malignancy or polyposis coli
2.	Normal BMI (18-25 kg/m2)
3.	No family history of autoimmune diseases (type 1 diabetes, Hashimoto hypothyreoidism, Graves hyperthyreoidism, rheumatoid arthritis, inflammatory bowel diseases e.g. Crohn's disease, Colitus ulcerosa or coeliakie)
4.	No HIV, HAV, HBV, HCV, active CMV, active EBV (donor and acceptor are matched for EBV/CMV immune status)
5.	No unsafe sex practice or use of illicit drugs
6.	Screening of fecal bacterial pathogens (Salmonella, Shigella, Campylobacter, Yersinia, Helicobacter pylori antigen), viruses (rotavirus) or parasites (ova and parasites, Giardia antigen, cryptosporidium antigen)
7.	Negative *C. difficile* stool test and/or current communicable (intestinal) disease
8.	Any medication use including PPI and antibiotics in the last 3 months
9.	No travelling to areas with endemic diarrhea in the last 3 months
10.	No immunosuppressive or chemotherapeutic agents

Apart from the risk on contracting infectious disease, the risk of transmitting other diseases, especially the ones described in this review merit attention. Knowing the (family) history of a potential donor with regard to autoimmune diseases, malignancies and intestinal colon polyps, as well as any other condition that can

possibly be transferred through feces is important. This provides an opportunity to select only donors who are unlikely to transfer a microbiome that might lead to problems in the future. Until recently, there has not been literature on using standardized frozen stool samples, but outcomes do not seem to be affected by this processing procedure in a recent case series (69). As described above, the use of thoroughly screened standardized frozen stool batches in fecal transplantation might have several logistical advantages over the use of fresh donor stool (69). Especially as there is no difference in efficacy using either donor stool from family members or unrelated subjects, the installment of a (local) standardized donor bank with frozen feces might be feasible (70;71).

In conclusion, we have tried to summarize human (autoimmune) disease states in which alterations in gut microbiota composition are reported as well as for which fecal microbial transplantation case reports were available. Based on the high prevalence of the abovementioned (autoimmune) diseases in conjunction with the high disease burden for both patients and community, fecal transplantation seems to be a safe and promising technique (with a broad therapeutic horizon ranging from infectious, metabolic and autoimmune disease) that behoves further clinical investigation. The currently available data on fecal transplantation efficacy in specific diseases is predominantly based on case reports and small trials produced by specific research groups. There is an increasing need for larger well-designed, double blinded, randomized trials to test efficacy as well as determination of intestinal microbiota composition changes before and after fecal transplantation while taking reproducibility and safety into account. Moreover, fecal transplantation is thought to introduce a complete, stable community of novel intestinal bacteria, replacing the disrupted native gut microbiota in patients. With the current insight that intestinal microbiota seem to interact and function in a network of specific bacterial groups (so called core gut microbiome) (72), identified intestinal microbiota species in specific (autoimmune) disease states could also serve as treatment target with novel probiotic strains. This was already put forward by Elie Metchnikoff's work more than a century ago, stating that useful microbes could be used to replace harmful ones in the human intestine (73). One could hypothesize that in autoimmune disease treatment with these novel probiotic strains might dampen the antigenic mimicry reaction to keep self-reactive T cells at bay in order to prevent clinical progression (7).

When preparing such a standardized probiotic product, we have to overcome several hurdles including preservation of beneficial characteristics of specific bacterial strains when cultured in large quantities and maintaining viability of these bacterial strains while passing through the acid milieu of the stomach. With demonstrating efficacy in randomized controlled trials using clearly defined clinical endpoints, the ultimate goal would be to not only a) show causality for the role of intestinal microbiota in human disease, but also b) identify and influence the culprit (small) intestinal microbe(s) involved in specific diseases. Undoubtedly, when successful this will entice research focusing on specific intestinal bacterial strains as novel diagnostic and therapeutic treatment modalities into clinical research.

Practice Points:

* Intestinal microbiota are increasingly recognized as partakers in human (autoimmune) disease, however it is difficult to discern cause from consequence.
* Fecal transplantation can (temporarily) alter intestinal microbiota composition and subsequent human disease states such as insulin resistance and *Clostridium difficile* diarrhea.
* Applying a proper fecal donor screening-protocol (minimizing risk of transmissible disease) together with executing randomized controlled double blind trials are pivotal for determining the therapeutic role of fecal transplantation in clinical practice.

Research agenda:

* The causative role of intestinal microbiota in (autoimmune mediated) disease needs to be defined.
* Double blind randomized controlled studies are necessary to define the efficacy of fecal transplantation in human pathophysiology.
* Also, detailed studies on gut microbiota composition before and after fecal transplantation are needed to find novel therapeutic treatment targets (e.g. probiotics) that can replace fecal transplantation in the long term.

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Duality of interest

The authors declare that there is no conflict of interest associated with this manuscript.

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