

Intestinal Microbiota and the Efficacy of Fecal Microbiota Transplantation in Gastrointestinal Disease

Olga C. Aroniadis, MD, and Lawrence J. Brandt, MD, AGA-F

Dr Aroniadis is a gastroenterology fellow at Montefiore Medical Center in the Bronx, New York. Dr Brandt is the emeritus chief of gastroenterology at Montefiore Medical Center and a professor of medicine and surgery at the Albert Einstein College of Medicine of Yeshiva University in the Bronx, New York.

Address correspondence to:
Dr Lawrence J. Brandt
Montefiore Medical Center
111 East 210th Street
Rosenthal Pavilion
Bronx, NY 10467;
Tel: 718-920-4846;
Fax: 718-798-6408;
E-mail: lbrandt@montefiore.org

Abstract: Fecal microbiota transplantation (FMT) refers to the infusion of a fecal suspension from a healthy person into the gastrointestinal (GI) tract of another person to cure a specific disease. FMT is by no means a new therapeutic modality, although it was only relatively recently that stool was shown to be a biologically active, complex mixture of living organisms with great therapeutic potential for recurrent *Clostridium difficile* infection and perhaps other GI and non-GI disorders. The published revelations about the human microbiome are bringing the strength of science to clinical observation and enhancing the understanding of not only disease but also how much of a person's daily function and health depends on the microorganisms living in intimate relationship with each cell in the body.

Transplantation of stool for the treatment of gastrointestinal (GI) disease was first reported in 4th-century China by Ge Hong, who described the use of human fecal suspension by mouth for patients who had food poisoning or severe diarrhea.¹ In the 16th century, Li Shizhen described oral administration of fermented fecal solution, fresh fecal suspension, dry feces, and infant feces for the treatment of severe diarrhea, fever, pain, vomiting, and constipation.¹ In the 17th century, fecal microbiota transplantation (FMT) began to be used in veterinary medicine, both orally and rectally, and was later termed "transfaunation."²

The first "modern" use of FMT in humans was for the treatment of pseudomembranous colitis caused by *Micrococcus pyogenes* (*Staphylococcus*). It was given as fecal enemas and was reported in 1958 in a 4-patient case series by Eiseman and colleagues.³ Use of FMT for *Clostridium difficile* infection (CDI) was also by enema and first reported in 1983 by Schwan and colleagues.⁴ Until 1989, fecal retention enema was the most common technique for FMT⁵; however, alternative methods of administration have been used subsequently, including fecal infusion via nasogastric tube (1991),⁶ gastroscopy and colonoscopy (1998, 2000),^{6,7} and self-administered enemas (2010).⁸ To date, well over 500 cases of FMT have been

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reported worldwide and include approximately 75% by colonoscopy or retention enema and 25% by nasogastric (or nasoenteric) tube or gastroduodenoscopy.^{9,10}

Intestinal Homeostasis

The mechanism by which FMT results in cure of CDI has been poorly understood until now, as we have begun to comprehend the complex role that intestinal microbiota play in the maintenance of intestinal homeostasis and in disease.¹¹ The majority of microbiota are anaerobic, and although more than 50 bacterial phyla have been described, only 4 predominate in the mammalian GI tract: Bacteroidetes, Firmicutes, Actinobacteria, and Proteobacteria. Of these, Bacteroidetes and Firmicutes account for more than 90% of the bacteria in the human GI tract.¹² It is estimated that approximately 4000 bacterial species reside in human GI tracts, comprising as many as 10^{14} bacterial cells, a number 10 times greater than the number of cells in the human body.¹³ Per gram of contents, there is a marked and progressive distal increase in the number of bacteria: 10^1 in the stomach, 10^3 in the duodenum, 10^4 in the jejunum, 10^7 in the ileum, and 10^{12} in the colon.¹¹ This longitudinal heterogeneity of the microbiota population has a predominance of Firmicutes and Proteobacteria (notably *Helicobacter pylori*) in the stomach, Firmicutes and Actinobacteria in the small intestine, and Bacteroidetes and the Lachnospiraceae family of Firmicutes in the colon.¹⁴

A symbiotic relationship and complex interplay between the host immune system and the microbiota are essential in achieving intestinal homeostasis. Intestinal microbiota play a vital role in protecting the intestines against colonization by exogenous and injurious endogenous pathogens by competing for nutrients, creating epithelial barriers to inhibit attachment of pathogens, and modulating the host immune system.¹⁵ For example, *Escherichia coli* competes with enterohemorrhagic *E coli* for organic acids, amino acids, and other nutrients.^{16,17} It has been suggested that commensal bacteria can more effectively compete with metabolically related pathogens as opposed to metabolically unrelated pathogens because they need similar nutrients.¹⁵ In addition, commensal bacterial strains such as *Bacteroides thetaiotaomicron* catabolize mucin to produce fucose, which inhibits virulence factor expression by pathogenic *E coli*.¹⁸ Pathogenic bacteria have adapted to this competition by using alternative nutritional resources, directly killing commensal competitors and inducing inflammation, which increases epithelial cell turnover and results in production of nutrients that promote pathogen growth.

Commensals confer further protection by promoting mucosal barrier function. The mucus layer that coats the colon forms a strong physical barrier and interferes

with the ability of pathogens to attach to intestinal epithelium.¹⁵ Although the inner layer of mucus in the small and large intestines is devoid of commensal bacteria,^{19,20} the mucus layer in germ-free mice is much thinner than that of conventionally raised mice, which suggests that commensals do play a role in mucus production.²¹

It has been shown that the intestinal microbiota also can enhance epithelial barrier function. For instance, short-chain fatty acids, particularly acetate produced by commensal *Bifidobacterium* species, act on the epithelium to inhibit the translocation of Shiga toxin produced by *E coli* O157:H7.²²

The microbiota also can limit pathogen colonization by activating the host immune system, specifically intestinal macrophages, neutrophils, and innate lymphoid cells as well as T-helper cells, immunoglobulin A-producing B cells, and plasma cells.¹⁵ Additionally, in the presence of pathogenic bacteria, the microbiota can upregulate the production of certain cytokines, for example interleukin (IL)-1b and IL-22, which result in an inflammatory response and promote immunity, respectively.^{23,24}

It is miraculous that the GI tract can coexist in harmony with the dense carpet of bacteria that overlies its mucosa without inducing an excessive immune reaction and that the intestinal microbiota mediate such antigenic tolerance. For example, intestinal dendritic cells are conditioned to a tolerogenic phenotype by intestinal epithelial cells that are stimulated by *Lactobacillus* species and certain *E coli* strains.²⁵ *B thetaiotaomicron* prevents activation of the proinflammatory transcription factor NFk β ,²⁶ and *Aeromonas* and *Pseudomonas* promote intestinal alkaline phosphatase, which dephosphorylates and inactivates the lipopolysaccharide found in the outer membrane of gram-negative bacteria, thus protecting against septic shock.²⁷ Disease, however, can result from an exaggerated immune response to commensal bacteria.

Clostridium difficile Infection

The pathogenesis of CDI is now believed to begin with disruption of the normal balance of colonic microbiota, usually as a consequence of antibiotic use or other stressors. Patients with recurrent CDI (RCDI) have decreased phylogenetic richness and a reduction of Bacteroidetes and Firmicutes phyla in their stool compared with patients who have just 1 episode of CDI.²⁸ In a small study of 3 controls, 4 patients with 1 episode of CDI, and 4 patients with RCDI, Chang and colleagues showed that the stool of patients with RCDI had roughly one-third the number of phylotypes as the stool of control subjects and one-quarter to almost one-half the number of phylotypes as the stool of patients with an index episode of CDI.²⁸ Furthermore, the average Bacteroidetes content of stool from control subjects compared with that of patients with an index episode of

CDI was 36% vs 57%, and the average Firmicutes content was 58% vs 40%. A perturbed microbiome was observed in patients with RCDI, consisting of 100% Firmicutes in 1 patient, approximately 63% Proteobacteria and 37% Firmicutes in another patient, and approximately 72% Verrucomicrobia with approximately 10% Firmicutes and 18% Bacteroidetes in a third patient.

FMT is thought to provide its therapeutic benefit by reestablishing a balanced microbiota with its attendant "colonization resistance."²⁹ Studies using terminal restriction fragment length polymorphism analyses and gene sequencing techniques have shown that the bacteria of the recipient's stool closely resembles that of the donor approximately 2 weeks after FMT and is dominated by Firmicutes and Bacteroidetes.^{30,31} This alteration persists for up to 4 months after transplantation and perhaps longer.³¹

Stable engraftment of intestinal bacteria following FMT also was demonstrated in a study using a previously frozen and then thawed fecal bacterial product from a healthy donor.³¹ Post-FMT samples of recipient stool in this study displayed an abundance of Bacteroidetes and Firmicutes to resemble donor stool, whereas Proteobacteria and Actinobacteria were less abundant (<5%) compared with pre-FMT stool samples.³¹ Quantitative differences in groups of intestinal bacteria also were reported in a study of patients with RCDI who underwent FMT via nasoduodenal tube.³² Specifically, increased numbers of Bacteroidetes and *Clostridium* clusters IV and XIVa were noted after FMT (by a factor of 2-4 for both groups), as were decreased numbers of Proteobacteria (by a factor of up to 100).

In the colon, primary bile acids (cholic acid and chenodeoxycholic acid) are metabolized by microbiota to secondary bile acids (deoxycholic acid and lithocholic acid), which inhibit the growth of *C difficile* and recently has been proposed as one of the mechanisms whereby FMT results in cure and prevents CDI recurrence.³³ It has been shown that fecal samples of patients with RCDI have a high concentration of primary bile salts, whereas secondary bile salts are nearly undetectable.³³ In contrast, post-FMT fecal samples and non-CDI donor feces contain mostly secondary bile acids.³³

Recurrent *Clostridium difficile* Infection

The incidence of CDI has increased to epidemic proportions over the past 10 to 15 years. In the United States, from 1996 to 2003, CDI nearly doubled from 98,000 to 178,000 cases and represented 31 to 61 per 100,000 hospital discharges,³⁴ while the unadjusted case-fatality rate rose from 1.2% in 2000 to 2.3% in 2004.³⁵ It is now estimated that between 500,000 and 700,000 cases of CDI occur annually in US hospitals and long-term care facilities, with an estimated hospital excess cost of care of approximately \$3.2 billion.³⁶

Currently, first-line treatment for CDI includes cessation of the culprit antibiotic, if possible, and treatment with metronidazole or vancomycin depending on disease severity.^{36,37} Fidaxomicin (Dificid, Cubist) is a noninferior alternate to vancomycin. Most patients with CDI respond to this treatment, but recurrence rates are 15% to 30%.³⁷ Patients who have 1 recurrence have up to a 40% chance of a second recurrence, and after their second recurrence, up to 65% of patients will have a third.³⁸ Recurrences are usually treated with additional courses of metronidazole, oral vancomycin, or fidaxomicin or with prolonged therapy with vancomycin given in pulsed-tapered regimens. Alternate antibiotic regimens (eg, a rifaximin [Xifaxan, Salix] chaser after vancomycin) are many but have been presented only in small case series.

The high recurrence rates of CDI prompted the need for alternative therapies, and FMT offers a rational and straightforward approach. The current literature on FMT for RCDI is largely comprised of single-center case series and case reports,^{4,8,39-48} 1 meta-analysis,⁴⁹ and 1 systematic review.¹⁰ In all, approximately 92% of patients were cured of their RCDI, with a range of 81% to 100%.^{4,8,10,39,41-50} A multicenter long-term follow-up study of patients who underwent colonoscopic FMT for RCDI reported an overall ultimate cure rate of 98%.⁵¹ Patients in this study had symptoms for an average of 11 months before FMT, and most (74%) reported resolution of diarrhea within 3 days.⁵¹

The only randomized controlled trial in this area to date assigned patients with RCDI to receive an abbreviated course of vancomycin (500 mg 4 times daily for 4 days) followed by bowel lavage and FMT via nasoduodenal tube, a "standard" vancomycin regimen (500 mg orally 4 times per day for 14 days), or a "standard" vancomycin regimen with bowel lavage.³² The study was prematurely stopped by the institutional review board when it was deemed unethical to continue because of the superior cure rate in patients who received FMT. Thirteen (81%) of 16 patients in the FMT group experienced resolution of RCDI after the first infusion. Two of the 3 remaining patients were cured after a second infusion using feces from a different donor (overall cure rate of 94%). Resolution of RCDI only occurred in 4 (31%) of 13 patients who received vancomycin alone and in 3 (23%) of 13 patients who received vancomycin with bowel lavage.

Immediate symptom resolution and long disease-free intervals after FMT for RCDI also have been documented in other reports^{2,10,41,42} and may result from the durable effect of FMT on repopulating the colon with normal commensal organisms.^{30,31} A systematic review of FMT, including all methods of administration and comprising 317 patients from 8 countries and 27 case series and reports, reported an overall cure rate for RCDI of 92%.¹⁰ FMT via colonoscopy or enema has proved to be more

successful for RCDI than FMT via the nasoenteric route (esophagogastroduodenoscopy or nasoenteric tube), with the latter giving an overall resolution rate of 80%.¹⁰

Severe and/or Complicated Clostridium difficile Infection

To date, the efficacy of FMT for the treatment of severe and/or complicated CDI has only been reported in 1 small, multicenter study of 13 patients who had failed traditional antibiotic regimens and subsequently underwent FMT.⁵² Eighty-four percent of patients had severe CDI, and 92% had complicated CDI. Patients were followed for an average of 15 months. The overall cure rate was 92%. Diarrhea and abdominal tenderness resolved rapidly an average of 4.5 and 3.3 days after FMT, respectively. Disease-free intervals of up to 42 months were reported. Adverse effects of FMT were minimal and included abdominal cramping and bloating.

Inflammatory Bowel Disease

Specific infectious agents, such as *Mycobacterium paratuberculosis*, have been suggested to have etiologic links to Crohn's disease; however, isolation of a causative pathogen is awaited in ulcerative colitis (UC).¹¹ One widely accepted hypothesis suggests that inflammatory bowel disease (IBD) results from continuous antigenic stimulation by nonpathogenic commensals that leads to an exaggerated sustained immune response in genetically predisposed persons.¹¹ In patients with IBD, intestinal mononuclear phagocytes respond robustly to microbial products and commensal bacteria, resulting in production of large amounts of pro-inflammatory cytokines (eg, tumor necrosis factor- α and IL-23),⁵³ which may be responsible for the development and persistence of intestinal inflammation in IBD.¹⁵

Evidence for the hypothesis that dysbiosis, or an imbalance of the normal intestinal microbiota, is the means by which intestinal flora lead to IBD is growing.⁵⁴ First, patients with IBD have an abundance of Enterobacteriaceae and a paucity of *Faecalibacterium prausnitzii*.¹¹ A reduction in the anti-inflammatory commensal *F prausnitzii* has been shown to be associated with increased Crohn's disease activity.⁵⁵ Second, patients with IBD also have a 30% to 50% reduction in the biodiversity of their intestinal microbiota, which is attributable to decreased Firmicutes (specifically Lachnospiraceae) and Bacteroidetes.^{11,34-38,40-56} Third, patients with IBD are more likely than control cohorts to have been prescribed antibiotics in the 2 to 5 years preceding their diagnosis.⁵⁷ Finally, colitis is absent in germ-free, genetically susceptible mice yet develops in the presence of intestinal microbiota.⁵⁴ Thus, it seems reasonable that restoration of a healthy balanced intestinal microbiota by FMT could be therapeutic for IBD.

FMT for refractory UC has been described in 3 publications, comprising 9 patients, all of whom had severe, active, long-standing UC (mean, 8.6 years) refractory to treatment with glucocorticoids, 5-aminosalicylates, and azathioprine.^{2,58,59} FMT was administered as retention enemas and resulted in the complete resolution of all symptoms with cessation of UC medications within 6 weeks without relapse.² Remission was maintained for up to 13 years, and follow-up colonoscopy in 8 of the 9 patients showed no evidence of UC (n=6) or only mild chronic inflammation (n=2).⁵⁸⁻⁶⁰ In one case report on FMT in Crohn's disease, a patient who was refractory to prednisone and sulfasalazine responded to FMT within 3 days, allowing discontinuation of medications.⁶⁰ Disease relapsed within 18 months.²

The use of colonoscopic FMT followed by self-administered fecal enemas in a tapered fashion and as maintenance therapy for IBD has been described in an additional 16 patients, 14 with UC and 2 with Crohn's disease.⁶¹ After FMT, 14 (87.5%) of these 16 patients reported improvement in stool frequency and abdominal pain; however, the degree of benefit varied widely and was maximal in those with concomitant CDI (n=4) and in patients who were able to retain the enemas. In this series, FMT was effective in managing refractory UC; however, multiple infusions on a tapering daily to weekly to monthly schedule were given to maintain remission. Additionally, FMT provided greater therapeutic benefit in patients whose onset of UC was associated with an alteration in the fecal microbiota from antibiotic use or concomitant colonic infection. Experience with FMT for UC is just beginning, and controlled trials are needed to establish its safety, administration regimen, and therapeutic role, if any.

Irritable Bowel Syndrome

The pathogenesis of irritable bowel syndrome (IBS) is multifactorial and now believed to involve a complex interplay among the brain-gut axis, immune system, and intestinal microbiota.⁶² Perturbation of the intestinal microbiota has been shown to result in altered GI motility and visceral hypersensitivity, which have been observed in patients with IBS and are thought to play a role in disease pathophysiology.⁶³⁻⁶⁵ Additionally, observations have been made that link preceding gastroenteritis, small bacterial overgrowth (SIBO), and IBS, further implicating intestinal microbiota in the development of IBS.⁶² In fact, postinfection IBS ensues in 10% to 30% of patients who experience a bout of acute gastroenteritis,^{57,61,62,66-71} which translates to a 6- to 7-fold increase in the development of IBS.⁶⁹⁻⁷¹

In a recent study, it was proposed that pathogens causing acute gastroenteritis release cytolethal distending

toxin and, through molecular mimicry and auto-antibodies to vinculin (a native cytoskeletal protein), normal gut motility is disturbed, leading to SIBO.⁷² SIBO can lead to similar symptoms and altered intestinal motility as seen in IBS, and eradication of bacterial overgrowth can result in some normalization of motility.⁷³

Differences in the intestinal microbiota have been demonstrated between healthy patients and those with IBS in early studies.^{74,75} Patients with constipation-predominant IBS have been shown to have increased populations of sulphate-reducing bacteria compared with healthy controls.⁷⁶ Additionally, *Methanobrevibacter smithii* has been isolated as the predominant methanogen in patients with constipation-predominant IBS and methane-positive breath tests.⁷⁷ Probiotics can restore the intestinal microbiota of patients with IBS^{68,78} and result in improvement of postinfection IBS in animal models.¹¹ FMT, however, may prove more beneficial, as donated feces, in a sense, are the ultimate human probiotic.

In a series of 55 patients with IBS and IBD treated with FMT, cure was reported in 20 (36%) patients, decreased symptoms in 9 (16%) patients, and no response in 26 (47%) patients.⁶⁰ In another series, 45 patients with chronic constipation were treated with colonoscopic FMT and subsequent fecal enema infusions, 89% of whom (40 of 45 patients) reported relief in defecation, bloating, and abdominal pain immediately after the procedure.⁷⁹ Normal defecation, without laxative use, persisted in 18 (60%) of 30 patients who were contacted 9 to 19 months later.⁷⁹ In a recent study of 13 patients who underwent FMT for refractory IBS (9 IBS-diarrheal, 3 IBS-constipated, 1 IBS-mixed), 70% of patients reported improvement or resolution of symptoms, including abdominal pain (72%), bowel habit (69%), dyspepsia (67%), bloating (50%), and flatus (42%).⁸⁰ FMT resulted in improved quality of life in 46%.

US Food and Drug Administration Regulations

In September 2013, the US Food and Drug Administration (FDA) announced that fecal microbiota met the agency's definition of a drug/biologic substance and that, thereafter, an investigational drug application (IND) would be required to perform FMT for any indication. This decision to apply IND requirements made FMT largely unavailable to the community physician. Permission to perform FMT for the treatment of CDI was granted in emergent cases after discussion with the FDA; however, submission of an IND was still required within 2 weeks of the procedure. In July 2013, after much dialogue and a *C difficile* fecal transplant public forum, the FDA decided to liberalize the restriction on FMT while maintaining discretionary regulation.

Currently, FDA regulations permit a treating physician to perform FMT for CDI in patients who are unresponsive to standard therapy, without an IND, provided that the physician obtains adequate informed consent. At a minimum, such consent should include a statement that the use of FMT for the treatment of CDI is investigational and a discussion of the potential risks of FMT. The FMT product must be obtained from a donor known to either the patient or the treating licensed healthcare provider. Finally, the donor and the donor's stool must be qualified by screening and testing performed under the direction of the licensed healthcare provider. The FDA still requires an IND for the use of FMT to treat all other GI and non-GI diseases.

Safety of Fecal Microbiota Transplantation

In the only long-term follow-up study of FMT to date, which was a combined effort from 5 medical centers, 77 patients who had had FMT and were followed for more than 3 months experienced and maintained a 91% primary cure rate and a 98% secondary cure rate, the latter defined as cure enabled by use of antibiotics to which the patient had not responded before the FMT or by a second FMT.⁵¹ It is not unusual for some transient GI complaints or altered bowel habits to occur for several days after FMT, including absence of bowel movements, abdominal cramping, gurgling bowel sounds, or increased feelings of gaseousness and bloating. Autoimmune disease (rheumatoid arthritis, Sjogren syndrome, idiopathic thrombocytopenic purpura, and peripheral neuropathy) developed in 4 of the 77 patients studied after FMT, although a clear relationship between the onset of autoimmune disease and FMT was not evident,⁵¹ and detailed information on these diseases is lacking.

The safety of FMT in immunocompromised patients was reported in a retrospective, multicenter study of 61 adult and 5 pediatric immunocompromised patients treated with FMT for refractory, recurrent, or severe CDI.⁸¹ Patients were immunocompromised due to HIV infection, solid organ transplantation, oncologic conditions, immunosuppressive therapy for IBD, or other immunosuppressive medications or conditions. The overall CDI cure rate in this population was 89%, with an average follow-up period of 12 months. Ten (15%) patients experienced an adverse event within 12 weeks of FMT. Eight of these patients were hospitalized for various indications. Two deaths occurred within 12 weeks of FMT, 1 of which was the result of aspiration during sedation administered for colonoscopic FMT, while the other was unrelated to FMT. No patients experienced new infections or other diseases related to FMT. Three (9%) patients with IBD experienced a flare post-FMT.

In another study of 12 patients with IBD who were on immunosuppressive therapy (eg, infliximab [Remicade, Janssen], azathioprine, 6-mercaptopurine, or oral glucocorticoids) and underwent FMT for treatment of IBD, transient abdominal bloating and distention in 2 (17%) patients were the only adverse events encountered.⁸² Thus, few adverse events and no infectious complications were reported in all 78 patients in the 2 series described above.^{81,82} Nonetheless, safety remains the prime consideration, and larger numbers of observations in controlled circumstances are needed.

The Future

Emerging data have shown that ingestion or infusion of a defined bacterial mixture can cure CDI, obviating the need to use donor feces. Lawley and colleagues showed that CDI resulted in intestinal dysbiosis in a mouse model and that infusion of donor feces from healthy mice into mice with CDI resulted in resolution of disease.⁸³ Moreover, the authors of this study isolated bacteria from healthy mice and created a mixture of 6 phylogenetically diverse bacteria that also were able to disrupt intestinal dysbiosis when given to mice with CDI and, as a result, resolve disease and contagion.⁸³ In another study, a stool substitute preparation consisting of 33 isolates obtained from purified intestinal bacterial cultures derived from a single healthy donor was used to treat recurrent CDI in 2 patients in whom repeated standard antibiotics had failed. The fecal substitute was infused colonoscopically in both patients, and each patient reverted to their baseline bowel habits in 2 to 3 days and remained symptom-free at 6 months after infusion.⁸⁴ More recently, Graham and colleagues used 3 species of *Bacteroides* (*Bacteroides ovatus*, *Bacteroides vulgatus*, and *B. thetaiotaomicron*) to cure 1 patient of RCDI.⁸⁵ These studies set the stage for a time in the not-too-distant future when a “designer” capsule of selected microorganisms, either alone or as part of a microbiotic community and with or without a possible microbiotic metabolic product, will be given to restore a balanced microbiota or correct an abnormality of commensal organisms, thereby curing recalcitrant CDI and reversing or perhaps even preventing a wide variety of GI and non-GI diseases. Such a pill already has had success in Canada, although it has not been approved by the country’s Therapeutic Products Directorate.⁸⁶

In conclusion, while FMT and future modifications of microbiotic therapy are very exciting and likely to change the way physicians think about disease causation and treatment, safety must remain paramount, and randomized controlled trials must be performed to establish efficacy and safety.

The authors have no relevant conflicts of interest to disclose.

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