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Jacobs Journal of Gastroenterology and Hepatology

Research Article

Colon Hydrotherapy and Probiotic Intervention Improves Gut Convenience Due To Gut Microbiota Amendment

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Received: 01-14-2016

Accepted: 03-16-2016

Published: 03-30-2016

Abstract

An impaired gut microbiota together with affected host-microbial interactions is known to be an important element in the pathogenesis of digestive problems. Based on this knowledge we suggested a colon hydrotherapy in combination with a probiotic intervention as effective therapy.

Individuals suffering from digestive problems, namely I BS, received an intervention of a colon hydrotherapy and a probiotic formula, or as control a vitamin B supplement for six weeks. Gut microbiota of fecal samples was analyzed on the basis of 16S rDNA with RT-PCR and PCR-DGGE.

Gut microbial diversity and abundance of *Bifidobacteria* showed a significant increase due to colon hydrotherapy with a probiotic intervention between the time points. *Lactobacilli* and *Faecalibacterium prausnitzii* showed an increase, but no effects were shown in *Clostridium* Cluster IV or *Clostridium Cluster XIVa*. *Akkermanisa, Prevotella*, and *Enterobacteria* showed no changes on average. No effects on microbial composition due to intervention with Vitamin B complex supplement could be shown. The number of individuals harboring Archaea decreased in the probiotic group over study period.

Our results suggest no adverse effects of colon hydrotherapy on gut microbiota. On the contrary, a combined intervention of colon hydrotherapy with probiotics changed gut microbiota composition and might improve reported symptoms.

(ClincialTiral.gov ID NCT02372201, declaration of the Viennese Human Ethics committee (EK 14-092-VK_NZ))

Keywords: Digestive Problems; Colonic Lavage; Colonic Irrigation; PCR-DGGE

Abbreviations:

BMI: Body Mass Index;

IBS: Irritable Bowel Syndrome;

FFQ: Food Frequency Questionnaire;

PCR-DGGE: Polymerase Chain Reaction – Denaturing Gradient Gel Electrophoresis;

RT-PCR: Quantitative Real Time Polymerase Chain Reaction;

T: Time Point

Introduction

Three phyla (*Firmicutes, Bacteroidetes, Actinobacteria*) are predominately represented in the human gut microbiota [1-3], although varying in abundance [4]. In recent years it has become increasingly evident that the intestinal microbiota is not only important for metabolic and nutritional processes but also plays a crucial role in the health and immunity of their hosts. Thus, a large number of disorders have been associated with changes in the microbiota as well as with host-microbiota interactions, ranging from metabolic disorders such as obesity or diabetes [2,5,6] to food intolerances [7] and digestive disorders ranging from bloating, diarrhea, and constipation to IBS (irritable bowel syndrome) [8,9]. The pathophysiologic mechanisms of digestive disorders are still incompletely understood, but factors including abnormal gastrointestinal motility, visceral hypersensitivity, altered regulation of the brain-gut axis, low-grade inflammation, psychosocial disturbance, and dysbiosis of intestinal microbes are considered as contributing factors. Pathogenic microorganisms do not always play a role in these conditions; rather, components of the normal microbiota have an influence on these diseases [10]. A recent study of patients suffering from IBS indicated decreased fecal *Lactobacilli* and *Bifidobacteria* [8], *Bacteroidetes*, a decline in diversity [9], increased *Streptococci*, and *Clostridia* [11,12]. *Enterobacteria* were also shown to have a higher abundance [13].

Conventional therapies primarily target mucosal inflammatory responses, but the cause often remains untreated, although the contribution of the gut microbiota in certain clinical manifestations justifies the use of probiotics. Recent research addresses not only quantitative and qualitative changes in mucosal and fecal microbiota, but also their impact on mucosal innate immune responses through increased epithelial permeability, activation of nociceptive sensory pathways, and dysregulation of the enteric nervous system. Moreover, treatment with probiotics seems promising: several studies show an improvement after probiotic intake [14]. The most promising results have been shown for *Bifidobacterium infantis* 35624 at a dose of 13108 cfu/day taken for at least 4 weeks [15].

In addition, dietary composition is known to profoundly alter gut microbiota. A reduction in fiber intake can improve bloating and diarrhea by altering the intestinal microbiota. Prebiotic-administered oligosaccharides, e.g. inulin, increase fecal concentration of *Bifidobacterium* spp., but IBS patients suffer from increased flatulence due to fermentation [14]. Thus, an additional treatment with colon hydrotherapy (colonic irrigation or colon cleansing) might improve the probiotic impact by depletion of persistent gut microbiota and clearing space for mucosal adherence of probiotic administered strains. According to Gail Naas (I-ACT President), "Colon hydrotherapy is a safe method of removing waste from the large intestine, without the use of drugs." For the implementation of a colon-hydrotherapy about 60 liters of warm, filtered water (often with additional compounds, for example herbs or coffee) are pumped via a tube through the patient's rectum in several cycles. Additionally the patients are given abdominal massage. In this way the bowel is stimulated to eliminate long-term depositional fluids and waste. The duration of one treatment is about 40 minutes [16].

In the early 1900´s colon hydrotherapy was used mainly to avoid autointoxication, a poisoned body by toxins having their origin in the intestine [17]. The reasons why people use it nowadays are different: mainly for relief of gastrointestinal symptoms like bloating, constipation and diarrhea, but it is also used as a treatment for allergies, food intolerances, or skin problems, or simply to enhance general well-being [18].

Thus, we investigated patients undergoing a colon hydrotherapy with a subsequent intervention with probiotics or with B-vitamins as a control. We examined the gut microbial diversity using DGGE and the relative abundances of microorganisms in the gastrointestinal tract using qPCR of the 16S rDNA.

Methods

Study participants and study design

In accordance with the declaration of the Viennese Human Ethics committee (EK 14-092-VK_NZ), all study participants gave written consent for use of stool samples and food frequency questionnaire (FFQ). Individuals (n=55, aged 45±13 years, BMI 25.31±6.91) suffering from digestive problems, namely IBS, meeting the inclusion/ exclusion criteria were recruited for this study. Inclusion criteria implicated patients under consultation at doctors or nutritionists for Inflammatory Bowel Syndrome were included in the study. Exclusion criteria included: pregnancy, antibiotic therapy 0.5 years before start,

hormone therapies, malignant diseases. In addition, patients were asked to avoid dietary supplements 4 weeks before and during the study. All participants received an implementation of colon hydrotherapy (typically 3 colon therapy treatments) to relieve symptoms. Afterwards the participants were divided into two subgroups, one receiving a probiotic intervention and the other a vitamin B supplement. The probiotic group was given Progutic® LactoVitamin BALANCE, which contains 7 different DUOLAC® bacterial strains per capsule: *Lactobacillus plantarum*, *Streptococus thermophiles*, *Lactobacillus acidophilus*, Lactobacillus rhamnosus, *Bifidobacterium lactis*, *Bifidobacterium longum*, and *Bifidobacterium breve*. In addition, each capsule contained fructooligosaccharides, 200 µg folic acid, 2.50 µg vitamin B_{12} and 55 µg selenium (all amounts corresponding to 100% of daily requirement).The vitamin group ingested a vitamin B complex capsule containing 10µg cobalamin, 450µg folic acid, and 55 µg selenium. Both supplements were continued for six weeks. Stool samples were taken at three different time points: T1, before colon hydrotherapy; T2, immediately after colon hydrotherapy (first hard stool); and T3, after six weeks of probiotic or vitamin intervention. Furthermore a FFQ () was administered at time point one and three. The FFQ reported frequencies of consumption and portion sizes, and also included questions about lifestyle, medically relevant influences, stool frequency, kind of gastrointestinal pain, BMI (body mass index), and age to ensure comparable data.

Fecal sample collection, processing, and analysis

Stool samples were stored at -70°C after collection. Bacterial DNA was extracted from fecal samples using the QIAamp® DNA Stool mini kit (Qiagen, Germany) according to the manufacturer's protocol. Additionally, samples were treated in FastPrep™ Lysing Matrix E tubes (MP Biomedicals, USA) twice for 45 sec in a bead-beater (Mini-Beadbeater 8 Bio-Spec Products, USA) with an intervening minute on ice. DNA concentration and quality was determined with a Pico100 (Picodrop, UK) and agarose gel electrophoresis.

The total bacterial diversity was measured by DGGE (denaturing gradient gel electrophoresis) using the primer set 341f-GC 5'-CCT ACG GGA GGC AGC AG-3' [19] and 518r 5'-ATT ACC GCG GCT GCT GG-3' [20] according to Remely et al. (2013) [5].

Bacterial abundance using 16S rDNA group-specific primers and archaeal primers was quantified with TaqMan qPCR and SYBR Green qPCR in a Rotorgene 3000 (Corbett Life Science, Australia). The specificity of primer and probes was checked with the ProbeMatch function of the ribosomal database project 10 (http://rdp.cme.msu.edu/). The PCR reactions mixture and serial DNA dilution of typically strains were prepared according to Pirker et al. 2012 [21].

Results

Analyses of the retrospective FFQ

Evaluation of the FFQ showed that the most of the study participants (T1:31%, T3:33%) consumed fruits and vegetables 5-10 times per week. At both dates about one fourth consumed this food group even more than 10 times weekly. In comparison, DACH guidelines recommend five portions of fruits and vegetables per day. The recommendation of the DACH guidelines eating meat and sausage only 2-3 times per week was met by about 50% of the individuals. At time point one 36% of them ate meat nearly daily and this number increased at time point three to 38%.

The question about the fish intake showed that the participating persons (46% at T1 and 48% at T3) mainly ate fish 1-3 times per week which is consistent with the DACH guidelines. In the first FFQ 38% (34% in the second) stated that their intake of this food group was lower. At the beginning about one fourth of the study participants consumed dairy products every day. In the second questionnaire only about 16% reported a daily intake of this food group. The majority of the individuals (T1:46%, T3:52%) consumed milk and milk products no more than 1-3 times weekly. Wheat and whole-grain products are recommended daily, and approximately one half of the participants at every time point complied with this recommendation. When asked how often they eat sweets, the most common response was between 1 and 5 times per week (T1:62%, T3:60%). 18% of the participating individuals at T1 and at T3 20% consumed sweets less than once per week and at both time points; only about 15% ate them every day. Questions about physical activity showed that only 32% at T1 and 40% at T3 practice daily movement. But 42% stated that they exercised 2-3 times per week. Questions about stool behavior showed that 42% documented no conscious problems with defecation at the first time point. At time point three 50% reported a stable and untroubled digestion and elimination.

Compositional evaluation of gut microbiota

Gut microbial diversity showed a significant difference in the probiotic intervention group between T1 and T3 ($p=0.003$) with a mean at T1 of 12+/-5.5 bands and at T3 of 17+/- 4.6 bands showing a correlation between the time points ($R=0.65$, $p=0.02$).

There was no significant increase in total bacterial abundance in the probiotic group ($p=0.83$), nor in the vitamin group ($p=0.91$) at all three time points. Comparing the two groups at time point three also showed no significant difference ($p=0.94$). The ratio of *Firmicutes/Bacteroidetes* showed no significant change between the groups nor between the time points ($p_{(prob)}=0.59$, $p_{(vit)}=0.45$). Furthermore, no significant

changes between the three time points were observed in the abundance of *Clostridium* Cluster IV ($p_{(prob)}$=0.63; $p_{(vit)}$=0.93) or of *Clostridium* Cluster XIVa ($p_{(prob)}$=0.85; $p_{(vit)}$=.0.43) in either group. The abundance of *Faecalibacterium prausnitzii* increased between T1 and T2 (p=0.28) in the probiotic group, whereas between T2 and T3 there was no change (p=0.95). In the vitamin intervention group no clear differences could be observed between all three time points (p=0.67). *Lactobacilli* showed an increase in the probiotic group from the first to the second time point (p=0.44) but a decline from T2 to T3 (p=0.35). There were no significant differences between the three time points in the vitamin group (p=0.74). Comparing T3 of the two groups we could also find no significant difference (p=0.43). However, the mean values of the probiotic group were higher in comparison to the vitamin group. There were no significant alterations in the abundance of *Bacteroidetes* ($p_{(prob)}$=0.64; $p_{(vit)}$=0.87) or in the abundance of *Prevotella* ($p_{(prob)}$=0.73; $p_{(vit)}$=0.49) over the study period for either group.

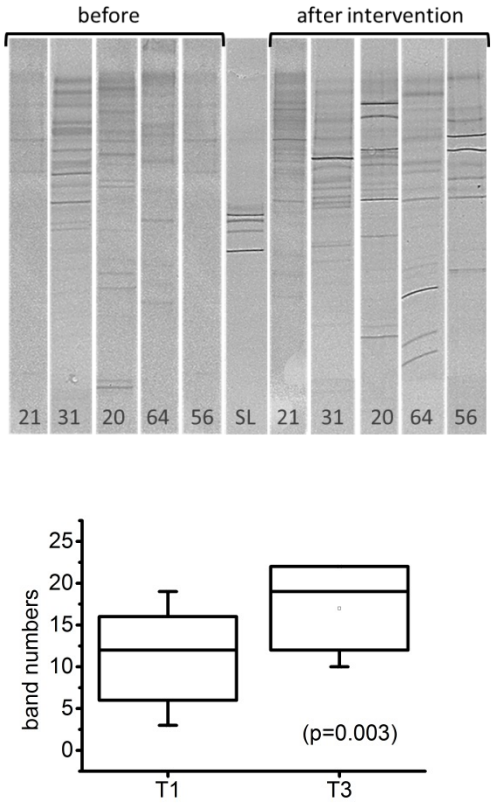

Figure 1. Diversity analysis. A PCR-DGGE fingerprinting of 16S rDNA coding regions of dominant bacteria over time indicating a lower number of bands in the probiotic intervention group at T1 in comparison to T3 B Quantification of number of bands showing an increase of diversity in the probiotic intervention group between T1 and T3 (p=0.003) Box range 25, 75 Perc; Whiskers indicate outliers; α indicates mean; x indicates maximum and minimum data range(T1: before colon-hydrotherapy, T3: after six weeks of probiotic or vitamin intervention, SL: standard lane).

In the probiotic group an increase of *Bifidobacteria* between the individual time points was observed (T1-T2: p=0.21, T2-T3: p=0.11). This increase was significant between T1 and T3 (p<0.05). The levels of the vitamin group showed no remarkable differences over the three time points (p=0.79). As we compared the third time point of the two various intervention groups, a possible difference could be detected (p=0.13).

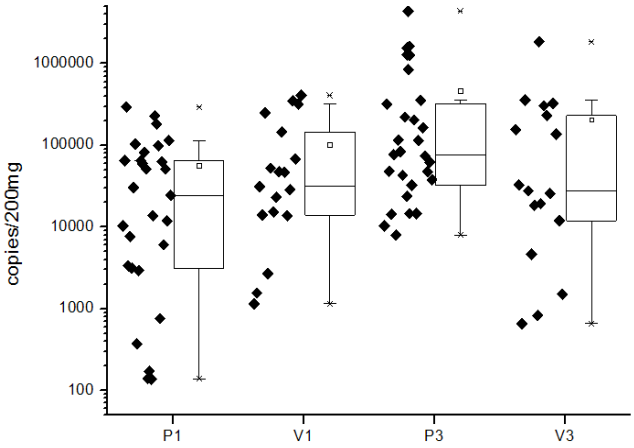

Figure 2. Quantification of *Bifidobacteria*: Showing a clear difference at T3 between the probiotic (P) and vitamin group (V; p=0.13) as well as a significant increase in the probiotic intervention group between T1 and T3 (p<0.05). Box range 25, 75 Perc; Whiskers indicate outliers; α indicates mean; x indicates maximum and minimum data range. (T1: before colon hydrotherapy, T3: after six weeks of probiotic or vitamin intervention)

Enterobacteria showed no remarkable changes: the level remained unaffected in the probiotic group as well as in the vitamin group over study period ($p_{(prob)}$=0.37; $p_{(vit)}$=0.81). *Akkermansia* levels showed an increase in the probiotic intervention group between T1 and T2 (p=0.57) and between T2 and T3 (p=0.37). Whereas in the vitamin intervention group we observed a trend of a decrease (T1-T2: p=0.58, T2-T3: p=0.95). The number of individuals with Archaea decreased in the probiotic group from 27.6% at T1 to 17% at T3. In the vitamin group 42% of the participants harbor Archaea at T1 and only 28.9% at T3.

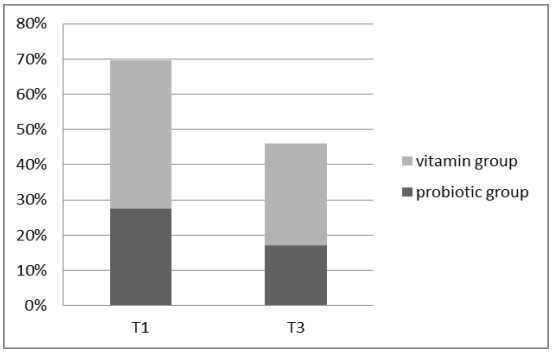

Figure 3. Percentage of individuals harboring Archaea: decreased with probiotic and vitamin B intervention about 10.6% and 13.1%

respectively. (T1: before colon-hydrotherapy, T3: after six weeks of probiotic or vitamin intervention).

Discussion

We have focused on patients suffering from digestive problems treated with a colon-hydrotherapy followed by an intervention divided into the two subgroups receiving a probiotic intervention or a vitamin B complex afterwards.

Gastrointestinal pain and changed bowel habits are often mentioned, although the exact cause is not clear and ranges from previous antibiotic use to travelers' diarrhea etc. The pathophysiology is incompletely understood, and an interaction of various mechanisms has been proposed: abnormal gastrointestinal motility, visceral hypersensitivity, altered brain-gut barrier, low-grade inflammation, and psychosocial disturbance have been suggested. The importance of gut microbiota populations in disease development is generally accepted [22]. Digestive disorders are associated with decreased abundance of *Lactobacilli, Bifidobacteria* [8], *Bacteroidetes*, and microbial diversity [9], whereas the incidence of *Streptococci*, *Clostridia* [11, 12], and *Enterobacteria* is enhanced [13]. We observed no abnormalities in gut microbiota in diseased participants compared to common gut microbiota composition; even the previously mentioned decline in *Bacteroidetes* [9] was not shown in phylum analysis itself nor in the *Firmicutes/Bacteroidetes* ratio.

We could not observe adverse effects (e.g. depletion of gut microbiota) after colon hydrotherapy on gut microbiota composition, although evidence regarding detrimental effects have been discussed before [18]. Despite a long history and current popularity, there is no scientific literature available which supports the benefits of cleansing. In contrast, a variety of adverse effects ranging from mild (e.g. cramping, abdominal pain, fullness, bloating, nausea, vomiting, perianal irritation, and soreness) to severe (e.g. electrolyte imbalance and renal failure) are mentioned [18]. In addition, Harrell et al. (2012) mentioned obvious effects of colonic lavage in some individuals although in general they can be unpredictable [18]. However, a colon hydrotherapy with an additional probiotic intervention significantly increases diversity and abundance of *Bifidobacteria*, but did not affect residual gut microbiota. Even a correlation of diversity between the time points can be shown; thus a balanced, diverse microbiota improves the impact of intervention.

A generally important ability of probiotics that affects various digestive disorders consists in improving the gut's microbial composition and preserving its stability. The absence of an additional improvement of dietary intake by the whole study population might affect these results. Although a diet rich in vegetables, salads, and fruits has been proven to be beneficial to digestive health under normal circumstances, polysaccharides might affect digestive problems depending on their application [14]. Depending on diet, different fermentation end-products and vitamins can be generated [23], although a vitamin B intervention did not show an influence on human gut microbiota composition.

Table 1. Characterization of study participants.

Group	Probiotic	Vitamin
Number	29	20
Sex	♀ 22 ♂ 7	♀ 13 ♂ 7
Age ± SD (years)	43,72±11,55	48,85±14,47
BMI ± SD (kg/m^2)	25,91±8,08	24,21±5,32

In addition, probiotics strengthen the immune system by stimulating immune mechanisms, they help to regulate the gut motility, and they act as anti-inflammatory compounds. The effects include immunostimulatory and immunomodulatory effects [24] including not only the recruitment of immune cells to the mucosa, generation and maturation of organized gut-associated lymphoid tissues and stimulation of protective epithelial cell functions, but also reversible changes in differentiation or effector function of host immune cells [24]. Unfortunately, data on specific probiotic implications are rare. *Bifidobacteria* are considered to protect against gut barrier dysfunction, metabolic endotoxemia, insulin resistance, and obesity, to reduce gastrointestinal disorders, and to correlate with inflammatory markers [25, 26]. Prebiotic inulin has been shown to significantly increase the levels of *Bifidobacteria* and also of *F. prausnitzii* [27, 28]. An increased abundance of *Lactobacilli* is mentioned to induce the expression of the immune suppressive cytokine IL-10 in Treg cells [24, 29]. Although the results are not consistent yet, *Lactobacilli* are suggested to have a role in "low-grade" inflammation [30] with a potential species dependence. Thus, the trend of an increase of Lactobacilli between the first two time points might indicate the disturbance of mucosa and induced endotoxemia due to colon-hydrotherapy even though *Enterobacteria* showed no remarkable changes.

Archaea have been reported to be more abundant in obese and anorexic patients and have been reported to adapt towards optimal exploitation of a low-caloric diet by increasing caloric intake through removal of H_2 from bacteria [31-34]. This symbiotic relationship of Archaea together with bacteria might maximize the microbiota's ability to generate energy from otherwise non-digestible food components. An enrichment of gut Archaea has been shown to guarantee an adequate caloric intake of the host through caloric restriction [2], but colon hydrotherapy together with probiotic intervention decreased their abundance (Tables 1-3).

Table 2. Primers and TaqMan® probes targeting 16rRNA coding regions of bacteria and archaea.

Target organism	Primer/ Probe	Sequence (5' - 3')	Size (bp)	Conc. [pmol/ µL]	Reference
All *Bacteria*	Fwd primer	ACT CCT ACG GGA GGC AG	468	10	[35]
	Rev primer	GAC TAC CAG GGT ATC TAA TCC		10	
	Probe	(6-FAM)-TGC CAG CAG CCG CGG TAA TAC-(BHQ-1)		2	
Clostridium* cluster IV *(Ruminococcaceae)	Fwd primer	GCA CAA GCA GTG GAG T	239	4	[36](Matsuki, 2004 #9;Matsuki, 2004 #2;Matsuki, 2004, 15574920;Zwielehner, 2009, 19376217)
	Rev primer	CTT CCT CCG TTT TGT CAA		4	
	Probe	(6-FAM)-AGG GTT GCG CTC GTT-(BHQ-1)		2	
Cluster XIVa *(Lachnospiraceae)*	Fwd primer	GCA GTG GGG AAT ATT GCA	477	5	[37]
	Rev primer	CTT TGA GTT TCA TTC TTG CGA A		5	
	Probe	(6-FAM)-AAA TGA CGG TAC CTG ACT AA-(BHQ-1)		1,5	
Bacteroidetes	Fwd primer	GAG AGG AAG GTC CCC CAC	106	3	[38]
	Rev primer	CGC TAC TTG GCT GGT TCA G		3	
	Probe	(6-FAM)-CCA TTG ACC AAT ATT CCT CAC TGC TGC CT-(BHQ-1)		1	
***Bifidobacterium* spp.**	Fwd primer	GCG TGC TTA ACA CAT GCA AGT C	125	3	[39]
	Rev primer	CAC CCG TTT CCA GGA GCT ATT		3	
	Probe	(6-FAM)-TCA CGC ATT ACT CAC CCG TTC GCC-(BHQ-1)		1.5	
Archaea	Fwd primer	ATT AGA TAC CCG GGT AGT CC		4	[40]
	Rev primer	GCC ATG CAC CWC CTC T	1044– 1059	4	[35]
	Probe	(6-FAM)-AGG AAT TGG CGG GGG AGC AC(BHQ-1)	915– 934	4	

Table 3. Primers (SYBR® Green) targeting 16rRNA coding regions of bacteria.

Target organism	Primer	Sequence (5' - 3')	Size (bp)	Conc. [pmol/µL]	Reference
Lactobacilli	Fwd primer	AGC AGT SGG GAA TCT TCC A	352-700	4	[41]
	Rev primer	ATT YCA CCG CTA CAC ATG		4	
Enterobacteria	Fwd primer	AGC ACC GGC TAA CTC CGT	492-509	3	[42]
	Rev primer	GAA GCC ACG CCT CAA GGG CAC AA	834 - 856	3	[43]
Prevotella	Fwd primer	CACCAAGGCGACGATCA	1458	2,5	[44]
	Rev primer	GGATAACGCCYGGACCT		2,5	
Akkermansia	Fwd primer	CAGCACGTGAAGGTGGGGAC	1505	2,5	[45]
	Rev primer	CCTTGCGGTTGGCTTCAGAT		2,5	

Conclusion

Disruption of microbial equilibrium and an impaired gut barrier are involved in many complex diseases. The resilience of gut microbiota structure is hard to maintain, thus long-term modifications are of interest. Colon hydrotherapy in combination with nutritional or probiotic intervention may be a possible way to improve abundance and diversity of gut microbiota.

Conflict Interests

The authors declare to have no actual or potential competing interests that might be perceived as influencing the results or interpretation of a reported study.

Acknowledgement

The work was funded by the Austrian Science Fund (FWF; AP2658721) and by the Abschlussstipendium 2014 of the University Vienna. The authors thank DR. Dkfm. Reinhard. Mechtler and special thanks to all participants without whom this study would not have been possible.

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