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Research Article

The Combination of Radix Codonopsis, Semen Raphani and Resistant Dextrin Relieves Constipation and Regulates Gut Microbiota: A Randomized, Double-blind, Placebo-Controlled Clinical Trial

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Abstract

Objectives: Constipation has high prevalence in all age groups, notably in the elderly population. Traditional treatment with laxatives may cause unexpected adverse effects. Therefore, alternative nutritional supplements are necessary to alleviate constipation and improve gut health. The aim of the present study was to evaluate the effects of Relief Drink formula (RDF) composed of *Radix Codonopsis*, *Semen Raphani* and resistant dextrin on adults with functional constipation in a randomized, double-blind and placebo-controlled 2-week clinical trial.

Methods: The participants (N=117) were randomized to receive RDF (N=58) or placebo (N=59) for 2 weeks. The daily self-reported defecation index, including defecation frequency, defecation condition and fecal character were collected. In addition, the fecal samples were collected at the 0-week and 2-week time points for metagenomic sequencing using the shot-gun method.

Results: The RDF intervention significantly increased defecation frequency (P<0.0001), improved defecation condition (P<0.0001) and feces character (P<0.0001), whereas such alterations were not observed in the placebo group. Moreover, the

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microbiota profile of RDF group following intervention was significantly increased with regard to the *Streptococcus salivarius* (*P*=0.008), *Ruminococcus obeum* (*P*=0.013), *Ruminococcus gnavus* (*P*=0.021), *Lactobacillus salivarius* (*P*=0.033) and *Collinsella aerofaciens* (*P*=0.016) strains. Significant differences of functional metabolites-producing potential, such as short-chain fatty acids (SCFAs) and L-glutamine (*P*<0.05) were observed between the RDF and the placebo groups.

Conclusions: The RDF could alleviate constipation possibly by regulating the growth of specific gut microbiota, including *Ruminococcus*, *Streptococcus*, *Collinsella* and *Lactobacillus* genera. Our current results indicated RDF could be a nutritional supplement solution to alleviate constipation.

Keywords

Radix Codonopsis, Semen Raphani, Resistant Dextrin, Constipation, Gut Microbiota, Clinical Trial

Introduction

Constipation is defined by National Institutes of Health (NIH) as a bowel movement less than three times per week. This condition is more like a symptom rather than a disease [1]. The prevalence of constipation ranges with median prevalent rate of 16% epidemiologically [2]. In general, female adults, elderly population, high BMI and low socioeconomic status are associated with higher prevalence of constipation [2,3]. In elderly, constipation is more likely caused by the increased illness and multiple medications rather than the colonic transit time slows down with aging [4,5]. Current treatments for constipation are categorized into bulking agents, stool softeners, stimulants, lubricants, chloride channel activators, 5-HT_4 receptor agonists and guanylate cyclase-c receptor agonists. However, these treatments may cause adverse effects, including flatulence, nausea, or diarrhea. Stimulants treatments with anthraquinones post a potential risk of colonic carcinoma if used for a long-term period [6]. Alternative treatments with particular probiotic supplementation have been reported to relieve constipation [7,8], though their underlying mechanism is not well understood.

The human intestinal tract contains a large number of microorganisms, with a concentration of up to 10^{11}-10^{12} cells/g, which aid the food digestion and metabolism, and support the normal immune defense function. Multiples studies have revealed the association between constipation and the composition and stability of the gut microbiota. The decrease of *Lactobacillus* and *Bifidobacterium* and the increase of the pathogenic microorganisms have been identified in chronic constipation patients [9]. The physiologically active substances and gut environment can influence the intestinal motility and secretory functions [10]. Produced by gut microbe, short-chain fatty acids (SCFAs) including butyric acid, propionic acid and acetic acid, facilitate defecation by stimulating ileal propulsive contractions [10]. SCFAs also regulate 5-hydroxytryptamine (5-HT) release from enterochromaffin (EC) cells to promote gut motility [11], and stimulate water and electrolyte absorption in the colon to reduce the stool volume [12]. Thus, the proper alteration in gut microbiota composition may be an effective way to relieve constipation.

From Traditional Chinese medicine (TCM) point of view, constipation in elderly is mainly caused by gastrointestinal *Qi* (vital energy) stagnation and spleen Qi deficiency by aging and improper dietary habits [13]. *Radix Codonopsis* and *Semen Raphani* are herbs traditionally used for defecation, since *Radix Codonopsis* improves Qi and fortifies spleen [14], whereas *Semen Raphani* benefits Qi regulation and promotes digestion [15]. The health benefits of *Radix Codonopsis* on immunity [16] [17], digestion [18], blood sugar [19], antioxidation [20] and anti-inflammatory [21] have been described in modern pharmacological studies. Water-soluble polysaccharides in *Radix Codonopsis* are considered as the active fraction contributing to bowel movement [22-24]. Similarly, the

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defecation function of *Semen raphani* has been well described in elsewhere [21,26,27].

Previously, we demonstrated the potential synergistic defecation effects of herbal ingredients (*Radix Codonopsis* and *Semen raphani*) and resistant dextrin (water-soluble dietary fiber) in a diphenoxylate-induced mice constipation model [28]. In this randomized, double-blind, placebo-controlled clinical trial, the primary objective is to evaluate the effect of the combination of *Radix Codonopsis*, *Semen Raphani* and resistant dextrin (relief drink formula, RDF) on constipation relieving. Moreover, metagenomics shotgun sequencing is applied to analyze the differences in the relative abundances of taxonomic units of gut microbiota before and after intervention, to understand whether the combination regulates gut-microbiome and related functional metabolites production to relieve constipation.

Material and Methods

Study design

The present study was a two-arm randomized, double-blind and placebo-controlled trial that aimed to determine the efficacy of a two-week supplementation of the combination of two traditional herbal ingredients and the resistant dextrin agent on bowel movement improvement and gut microbiome regulation. The present study was in agreement with the Declaration of Helsinki (*WMA, 2013*) and adhered to the method described in the following guideline: Assessment of Facilitating Feces Excretion Function, China Technical Standards for Testing & Assessment of Health Food, Ministry of Public Health of the People's Republic of China, 2003 Edition. All participants in this study were given informed consent. The Tianjin Third Central Hospital (TTCH) institutional review board approved the conduct of this study. The present trial consisted of two phases, including a baseline period of 1 week (from the screening visit to week 0) as a run-in observation period and a treatment period of 2 weeks (weeks 1–2) as the intervention period. A total of 117 participants were finally enrolled, and randomly divided into the RDF group (n=58) and the placebo group (n=59) according to the defecation frequency. The participants received RDF or placebo once daily for 14 days, respectively. The subjects were instructed to continue their normal dietary habit throughout the trial.

Inclusion/exclusion criteria

Both male and female volunteers, 18-65 years old, were recruited according to the following criteria: decrease in frequency of defecation and increase in hardness of feces, frequency of defecation less than 3 times/week, habitual constipation and absence of organic constipation. The subjects were excluded according to the following criteria: inability to receive food by mouth, inability for sample testing according to the stipulation protocol, the presence of unclear chief complaint, severe debilitation rendering the subject unable to undergo the trial, constipation symptom induced by surgical operation within 30 days or severe organic pathological conditions (carcinoma of colon, severe enteritis, intestinal obstruction, inflammatory diseases of intestine, etc.), defecation accompanied by pain, pregnancy and menstruation, acute gastrointestinal diseases within 30 days or severe conditions, such as cardiovascular diseases and diseases of liver, kidney and hematopoietic system, the administration of treatment for concomitant diseases, or the recent administration of drugs related to the test function, which could influence the assessment of the results.

Study Products

The RDF is a powder drink that include 2.20 g *Radix Codonopsis* aqueous extract, 0.88 g *Semen Raphani* 50% ethanol extract and 3.30 g resistant dextrin per daily serving (10g). A mixture contains maltodextrin (6.0 g), caramel pigment (0.20 g) and yellow pigment (0.18 g) was utilized in the placebo. Both the RDF and the placebo were manufactured in a GMP pilot plant (Amway, Guangzhou, China) following specific quality assurance instructions for the active compounds, microorganisms, heavy metals and pesticide residues.

Sample collection

All participants were routinely examined, blood, urine and stool specimens were collected. Moreover, liver and kidney function tests were conducted prior to and following intervention for safety checks. The frequency of defecation was inquired and recorded

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every day, the defecation condition and fecal character were assessed according to the scoring system presented in Table 1 and subsequently recorded. In addition, fecal samples were collected at the beginning of the trial (0 week) and at 2-week intervention for metagenomic sequencing, respectively. A fecal sampler (QuantiHealth Technology Co., Ltd., Beijing, China) was used to collect fecal specimens. The fecal samples were stored in sealed tubes filled with protective fluid and transported to the lab within a week for total gut microbe DNA extraction. The protective fluid contains amine sulfate, EDTA and sodium citrate, which has been validated to maintain the abundance of bacteria and the stability of genetic substance in the fecal samples at room temperature for 2 weeks.

Table 1: Scoring system of defecation condition and fecal character

Defecation condition			Feces character		
Classification	Score	Description	Classification	Score	Description
Class I	0	Normal bowel movement	Class I	0	like sausage or snake, smooth and soft; Like sausage, but with crack in surface; Soft lump with distinct edges (easily expelled)
Class II	1	Only Straining and discomfort feelings	Class II	1	Sausage shape but has lumps; loose lumps, rough edges, like muddy stool
Class III	2	Obvious Straining and discomfort feelings, or frequent but difficult bowel movement with low amount, few abdominal pain or anal burning	Class III	2	Separated hard masses like fruit pit (not easily expelled)
Class IV	3	Frequent abdominal pain or anal burning, defecation is affected			

Metagenomic Sequencing and Analysis

In this study, we only randomly selected 64 fecal samples from 16 subjects in the RDF group and 16 subjects in the placebo group for further sequencing. Fecal DNA was extract using specialized kits (ZD Biotech. Ningbo, China) according to the manufacturer's instructions and subsequently sequenced using the Illumina HiSeq X platform (Illumina, Inc., San Diego, CA) operated by QuantiHealth Technology Co., Ltd. (Beijing, China). The DNA library preparation was performed according to the Illumina's instructions. Cluster generation, template hybridization, isothermal amplification, linearization, blocking, denaturing and hybridization of the sequencing primers were performed according to the workflow indicated by Illumina. The libraries were constructed with an insert size of approximately 350 bp, followed by high-throughput sequencing to obtain paired-end reads with 150 bp in the forward and afterward directions.

The detail method of data quality control, *De novo* assembly and non-redundant metagenomic gene catalogue construction could be found in our pervious study [29].

Taxonomic profiling

The microbial community composition from each level of phylum, class, order, family, genus and species was analyzed using the Metaphlan2 software according to the literature report [30]. R software was applied to paint the gut microbiota profile of both individual participants and groups at phylum, family, genus, and species level, respectively.

The network of the metabolites and involved human gut microbiota species has been constructed by Sung, et al [31]. This is an extensive data resource composed of ~570 microbial species and 3 human cell types metabolically interacting through >4,400 small-molecule transport and macromolecule degradation events. QuantiHealth integrated this data resource and estimated the metabolite producing-capability by the relative abundance of its involved species via adding or subtracting according to the species ability of production or consumption, respectively.

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Statistical analysis

JMP14 (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis of defecation frequency, defecation condition and feces character. The Student's *t* test was applied to compare the means of the two groups.

For the metagenomics data analysis, the paired Wilcoxon test was performed to examine differences of data measured at baseline and post-intervention. The Wilcoxon test was performed to compare differences between the RDF and placebo groups. These statistical analyses were performed using the R statistical package.

Results

Characteristics of subjects and procedures

A total of 117 participants were initially enrolled in the present study. During the 14-day intervention, 2 subjects in the RDF group and 3 subjects in the placebo group dropped out and were excluded (Figure 1). The demographic data from the compliant 112 subjects, including gender, age, and constipation duration (year) were presented in Table 2. No significant differences were observed between the two groups in terms of psychological health, sleep status, dietary status and urinary status before and after the intervention (Supplementary Table 1). No difference were noted regarding to blood pressure, heart rate, routine blood tests and serum biochemical tests (Supplementary Table 2-4).

Clinical Outcomes

In the RDF group, the frequency of defecation was significantly increased, while defecation condition and feces character score were significantly decreased post-intervention compared the baseline (Table 3), but such alterations were not observed in the placebo group. Significant between-group differences were observed for defecation frequency, defecation condition and feces character. These results indicated that 14-days RDF supplementation could apparently improve constipation according to the criteria defined in China Technical Standards for Testing & Assessment of Health Food.

Table 2: Demographic data of constipation subjects

Outcome	RDF (n=56)	Placebo (n=56)	P-value
Male (Number(Percentage))	7 (12.5)	7 (12.5)	1.000
Mean ±SD	59.59±4.17	59.32±4.44	0.743
Constipation Duration (y)	2.07±0.68	2.00±0.74	0.596

Metagenomic sequencing

A total of 32 fecal DNA samples from 16 subjects in the RDF group (before and after intervention) and 28 samples from 14 subjects in the placebo group (before and after intervention) were obtained (The fecal DNA of 2 subjects in placebo group were failed to be extracted). An average of 31,157,264 clean reads from above 60 available samples were obtained following metagenomic sequencing and quality control of the raw data. These reads were subsequently used for *de novo* assembly and gene prediction to construct a non-redundant gene catalogue. Based on these data, the following actions were performed: analysis of non-redundant gene counts, alpha-diversity, species accumulation, microbiota composition and metabolite production prediction.

The gene counts between the two groups at baseline and post-

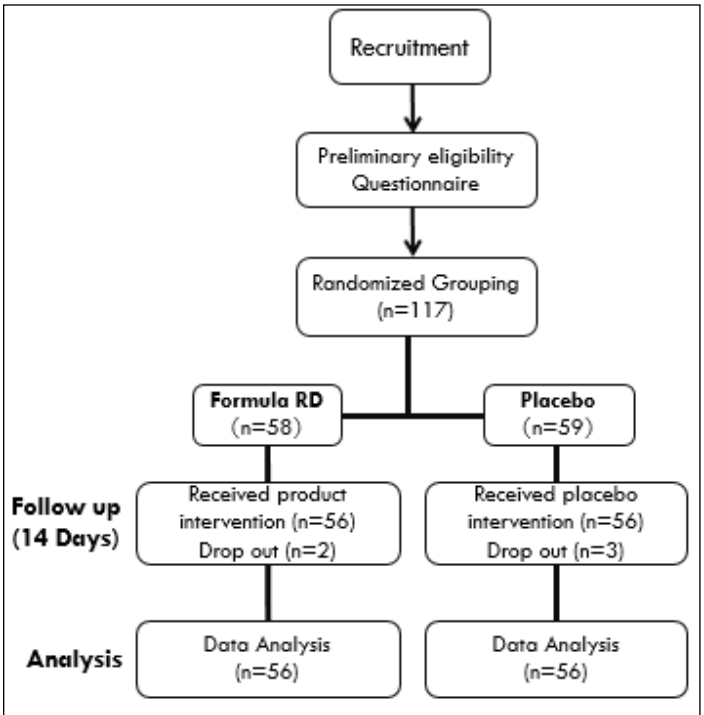

Figure 1: Flowchart of the clinical trial

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Table 3: Evaluation of group difference on constipation status.

Outcomes	RDF (n=56) (mean ± SD)	Placebo (n=56) (mean ± SD)	Difference (RDF vs. Placebo)	95% Confidence interval	P-value
Baseline					
Defecation frequency	1.36±0.48	1.32±0.47	0.035	(-0.143, 0.214)	0.693
Defecation condition	1.68±0.66	1.46±0.69	0.214	(-0.038, 0.467)	0.095
Feces character	1.39±0.59	1.38±0.56	0.018	(-0.198, 0.234)	0.870
Post-intervention					
Defecation frequency	2.93±0.85 *	1.45±0.50	1.482	(1.220, 1.744)	<0.0001
Defecation condition	0.95±0.77 *	1.46±0.60	-0.518	(-0.777, -0.258)	<0.0001
Feces character	0.80±0.55 *	1.38±0.56	-0.571	(-0.779, -0.363)	<0.0001

The Student's t test was applied for statistics analysis. * P<0.0001, post-intervention vs. baseline; Bold p values P<0.0001, RDF group vs. placebo group.

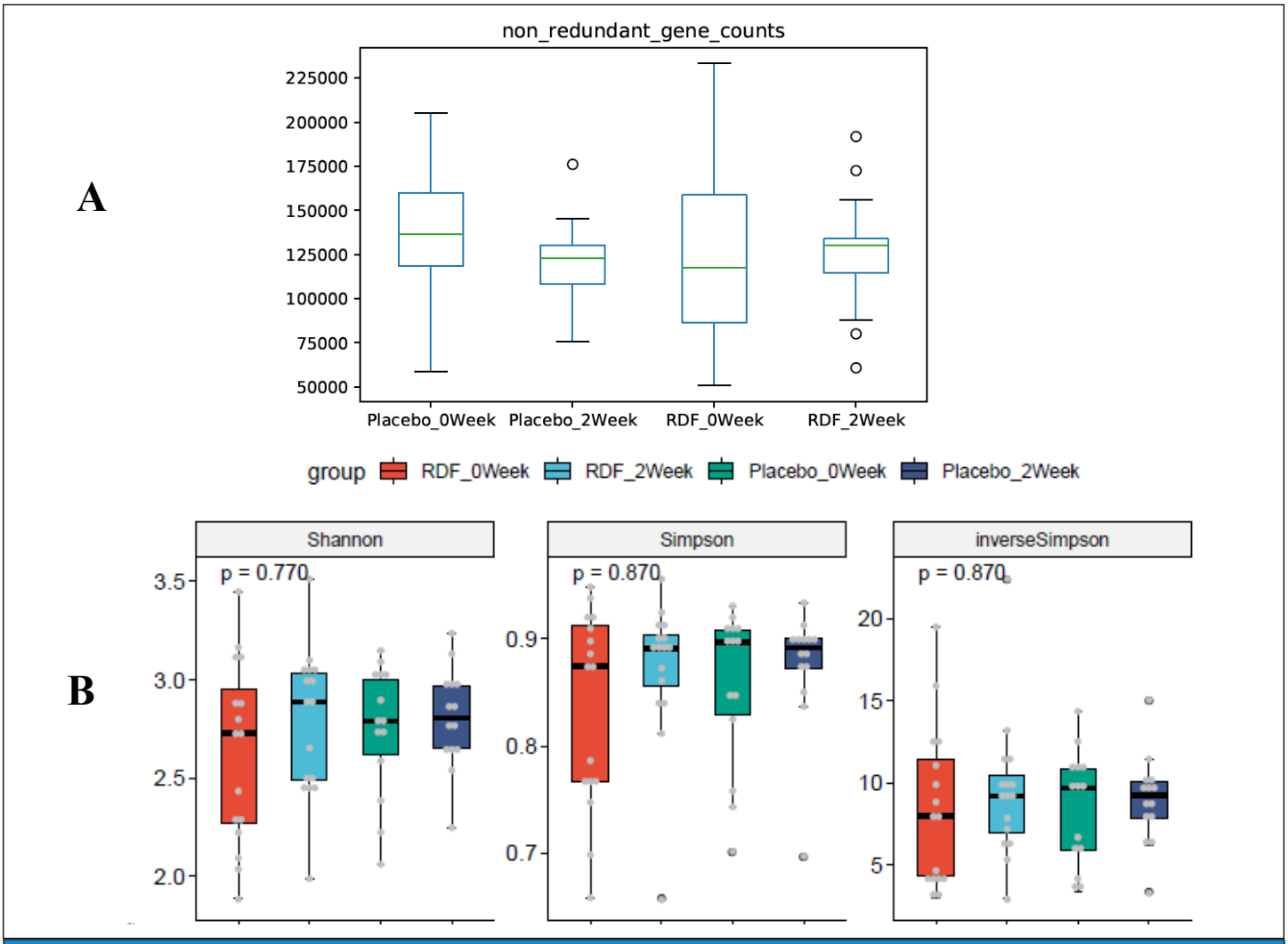

Figure 2: Diversity analysis of RDF and Placebo groups at 0-week (baseline) and 2-week (post-intervention). Comparison analysis of non-redundant gene counts (A). Alpha-diversity analysis including Shannon, Simpson and inverseSimpson index (B).

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intervention were similar (Figure 2A). No apparent difference regarding to α-diversity was observed, between the baseline and post-intervention in either the RDF or the placebo groups (Figure 2B). These results indicated that the RDF imposed limited influence on gut microbiota gene counts and species diversity. The species accumulation curves of the two groups at baseline (0-week) and post-intervention (2-week) are shown in Supplementary Figure 1. in which sufficient species enrichment of each group was observed.

Shift of gut microbiota profile

Gut microbiota profile of individual participant and group at species level was shown in Figure 3. More taxonomic results including phylum, family and genus level were presented in Supplementary Figure 2 and 3. Significantly changed gut microbiota at genus and species level between the baseline and post-intervention in RDF group was shown in Table 4. The relative abundance of genera *Sterptococcus*, *Collinsella* and *Ruminococcus* were significantly increased after 2-week RDF supplementation in comparison with that at baseline. No similar alterations were observed in the placebo group. Accordingly, the relative abundances of *Streptococcus salivarius*, *Collinsella aerofaciens*, *Ruminococcus obeum* and *Ruminococcus gnavus* were significantly increased in the RDF group following intervention, whereas such alteration was not seen in the placebo group. In addition, the increase of *Lactobacillus salivarius* was found in RDF group but not in placebo group following intervention.

Analysis of metabolite production potential

Previous study [24] indicated the potential defecation effects of RDF via regulating 5-HT and vasoactive intestinal peptide (VIP) level in colon. Due to the 5-HT and VIP are respectively associated with SCFAs and amino acid production (more information could be found in latter discussion section), we firstly evaluated the alteration of SCFA or AA producing capability caused by RDF/placebo intervention. In this study, the SCFAs and amino acid production capability was estimated by relative abundance of involved species but do not measure the content of SCFAs or amino acid in fecal samples. Total SCFAs producing capacity is expressed as the sum of relative species abundance of acetic acid, propionic acid and butyric acid. An increase of total SCFAs-production potential was observed in the RDF group and a decrease of that was noted in the placebo group. The difference in the potential of total SCFAs producing was significant (P=0.048) (Figure 4A). To be specific, the level of acetic acid-production increased in the RDF group (P=0.083) but not in placebo group; the level of propionic acid-productioin decreased in the placebo group (P= 0.013) and remained unchanged in the RDF

Table 4: Significant alterations of gut microbiota as determined by genus and species classification of the RDF and placebo groups at baseline and post-intervention.

Genus/Species	RDF group (n=16)			Placebo group (n=14)		
	Baseline	post-intervention	*P*-value	Baseline	post-intervention	*P*-value
Collinsella	0.4578 (0.5466)	1.1327(1.7229)	0.016*	0.5997 (0.5635)	1.1542 (1.3211)	0.294
Collinsella aerofaciens	0.4578 (0.5466)	1.1327 (1.7229)	0.016*	0.5997 (0.5634)	1.1542 (1.3186)	0.294
Lactobacillus	0.0248 (0.0957)	0.0114 (0.8323)	0.083	0.0000 (0.0014)	0.0010 (0.0184)	0.192
Lactobacillus salivarius	0.0000 (0.0126)	0.0013 (0.0694)	0.033*	0.0000 (0.0000)	0.0000 (0.0000)	1.000
Ruminococcus	1.8407 (3.7242)	4.5359 (7.2037)	0.016*	3.3605 (3.5527)	4.8661 (5.6457)	0.096
Ruminococcus gnavus	0.0000 (0.0201)	0.0091 (0.3702)	0.021*	0.0062 (0.0249)	0.0010 (0.0402)	0.722
Ruminococcus obeum	0.3282 (0.2281)	0.5137 (0.9434)	0.013*	0.7044 (0.5264)	0.7209 (0.8468)	0.626
Streptococcus	0.1221 (0.1465)	0.3229 (1.2952)	0.011*	0.2715(0.4994)	0.2052(0.5982)	0.715
Streptococcus salivarius	0.0928 (0.1338)	0.2682 (1.1072)	0.008**	0.2451 (0.4408)	0.0923 (0.2667)	0.583

The data of relative taxonomic abundance were presented as median (interquartile range, IQR). Paired Wilcoxon test was performed to examine differences of data. *P<0.05, **P<0.01, baseline vs. post-intervention.

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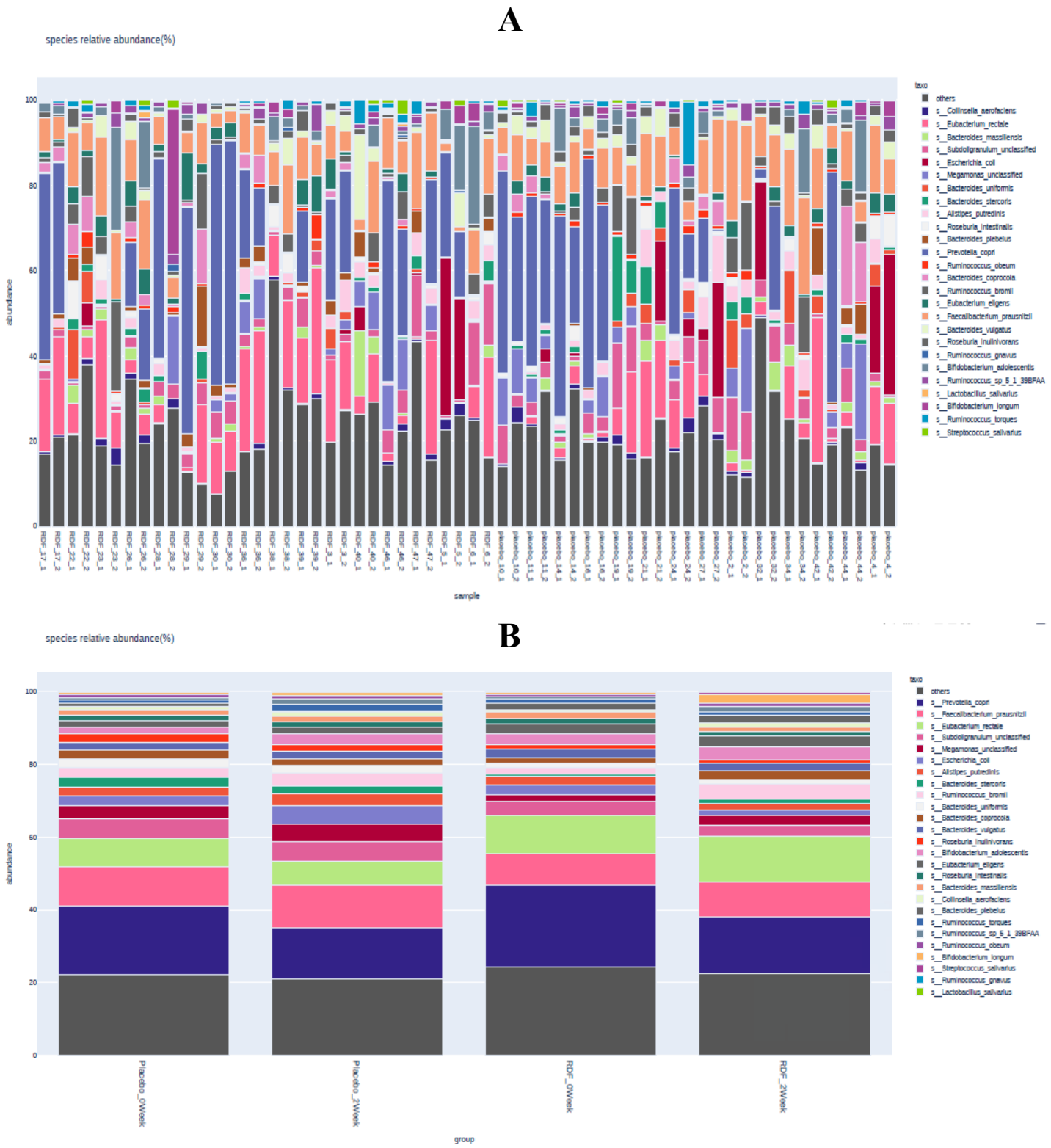

Figure 3: Gut microbiota profile of each participant (A) and group (B) at species level. The participants were identified by random numerical codes.

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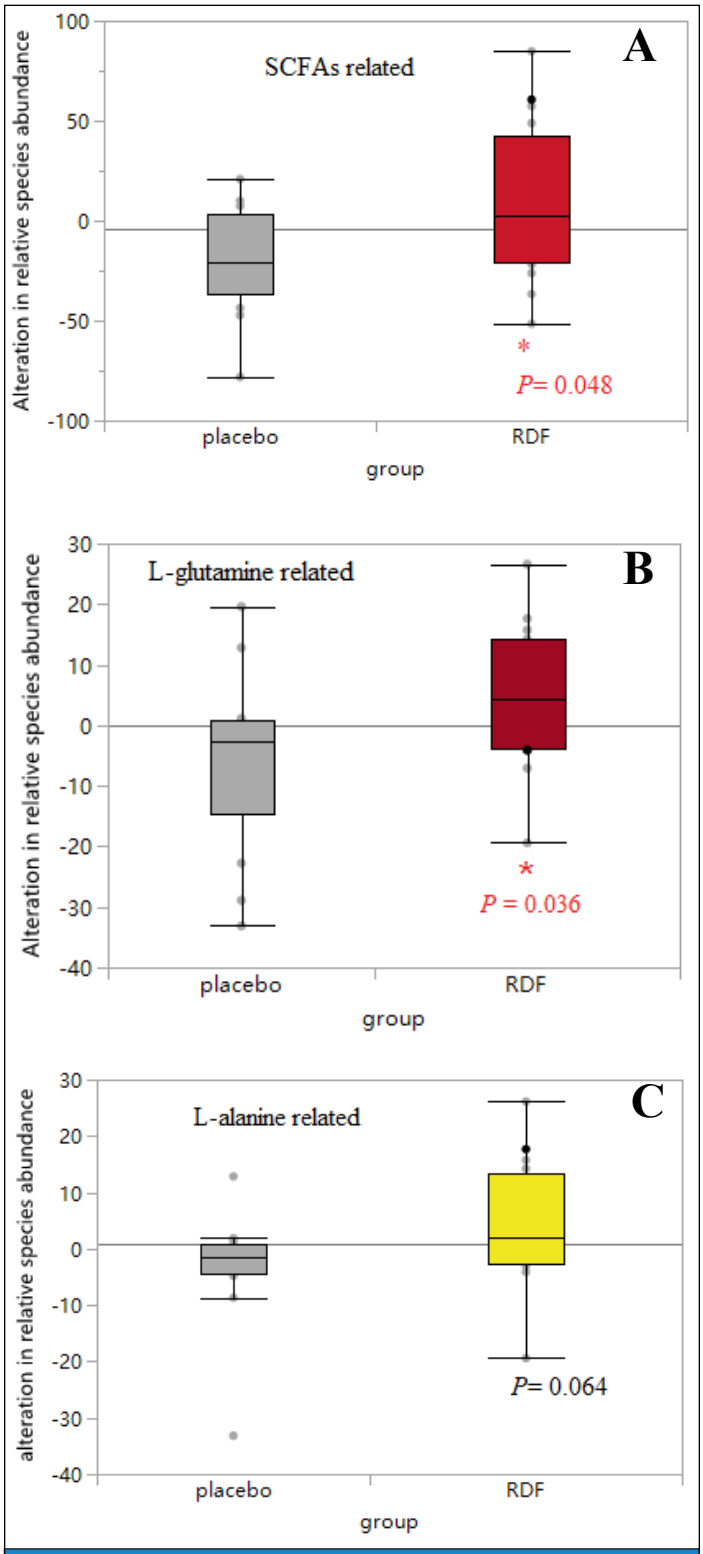

Figure 4: Alterations of metabolite production- relative species abundance. The difference of alteration in relative species abundance between RDF and placebo, involved in total SCFAs production (A), L-glutamine (B) and L-alanine (C). Wilcoxon test was performed to evaluate difference of two groups, * P <0.05, RDF vs. placebo.

group following 2-week intervention; the abundance of butyric acid-producing exhibited an uptrend in the RDF group and a downtrend in the placebo group, but no significant difference was found. The results of acetic acid, propionic acid and butyric acid were showed in Supplementary Figure 4. Furthermore, we observed that producing-capability of certain amino acids was significantly different between two groups. For instance, the levels of L-glutamine and L-alanine increased in the RDF group but decreased in the placebo group following intervention, the between-group difference of L-glutamine was significant (P=0.034) (Figure 4B), and the between -group difference of L-alanine was slight (P=0.061) (Figure 4C).

Discussion

In the present clinical trial, the efficacy of RDF in improving chronic constipation and provided the first clinical evidence supported the benefits of the combination of *Radix Codonopsis* and *Semen Raphani* with dietary resistant dextrin in relieving constipation. The results of our current clinical trial and previous pre-clinical study provided sufficient evidence that RDF could alleviate constipation condition.

The present clinical trial was the first one to investigate the regulatory effects of the combination of traditional food ingredients and resistant dextrin on gut microbiota, in which we found RDF could regulate genus level of *Ruminococcus*, *Streptococcus*, *Collinsella* and *Lactobacillus*. This result is supported by the studies of individual ingredients. *Lactobacillus* level in mice increased after *Radix Codonopsis* administration, which was attributed to the presence of polysaccharides and saponin in *Radix Codonopsis* [32,33]. Similar results were also observed in *Semen Raphani*. The main active component of *Semen Raphani* is known as sinapine that could improve *Lactobacillaceae* level in mice [34]. The effects of resistant dextrin on gut microbiome have been widely studied, and is known to increase the level of *Ruminococcus* and *Lachnospiraceae* [35,36]. In addition, dietary fiber is associated with the increase of *Collinsella* colonies in *in vitro* fermented experiment using human fecal microbiota samples [37]. The lower level of *Lactobacillus* associated with constipation occurrence [9], while *lactobacillus*-probiotics supplement showed positive defecation effects [38,39]. Notably, *Streptococcus*

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salivarius, is well known for its anti-inflammatory properties [40] [41,42]. The relative abundance of this bacterium improved after RDF intervention (P<0.01), suggesting that RDF may play a role in the regulation of microbe abundance by inflammatory signals. Currently, no direct evidence support the defecation function of *Ruminococcus*, *Streptococcus* and *Collinsella*, their beneficial effects on constipation is warranted for future study.

The present study demonstrated that the levels of SCFAs-producing potential, notably the level of the acetic acid, was enhanced by RDF supplementation. Indeed, *Ruminococcus* is an important genus of Firmicutes bacteria within the colonic microbial communities which enable to utilize complex carbohydrates to produce acetic acid [43,44]. Similarly, the ability of SCFAs production (e.g, propionic acid and butyric acid) of *Lactobacillus* [45] and *Collinsella* [46] has been reported, respectively. It has been well documented that the SCFAs levels are strongly associated with enteric 5-HT production and homeostasis [47,48], which play an important role in gastrointestinal motility and secretion [49]. By activating the 5-HT_4 receptor, 5-HT can promote the contraction of intestinal smooth muscle leading to the improvement of constipation [11]. The *Radix Codonopsis* water-extract increased 5-HT levels in the colon tissues of constipated mice was observed in our previous study [24]. Taken together, these results suggested that the underlying mechanism of RDF on improving constipation may involve regulating specific gut microbiome to induce SCFAs and 5-HT production.

Moreover, the L-glutamine producing potential increased after RDF supplementation in this study. The function of L-glutamine in promoting defecation has been already described in literature, as well as maintaining intestinal structure and function [50]. Besides, L-glutamine could activate peroxisome proliferator-activated receptor gamma (PPARγ) and diminishes oxidative stress leading to the reduction of VIP [51,52]. The elevated VIP levels have been considered as a compensatory response of early constipation [53], and associated with the pathogenesis of idiopathic chronic constipation [54] and irritable bowel syndrome [55]. Interestingly, *Radix Codonopsis* water-extract decreased VIP levels in the colon tissues of constipated mice has been observed in our previous study [24]. Therefore, RDF may also alleviate constipation by promoting L-glutamine production, which can reduce enteric oxidative stress, improve intestinal environment and reduce VIP excretion.

Conclusion

In conclusion, the present double-blind, placebo-controlled study highlighted the ameliorative effect of RDF on chronic constipation and confirmed the beneficial effects of this regimen in the defecation frequency, defecation condition and feces character. The defecation role of RDF may be associated with the increase of the *Ruminococcus*, *Streptococcus*, *Collinsella* and *Lactobacillus* genera, as well as the production increasement potential of functional metabolites (i.e., SCFAs and L-glutamine). These results provide additional insight in the combined action of dextrin and the traditional food ingredients for mitigating constipation. In addition, the data provided clinical and theoretical evidence to support the combination of above ingredients in developing functional food supplements.

Conflict of interest

The authors declare that there are no conflicts of interest.

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