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Vinegar beverage consumption improves defecation status in constipation-prone healthy adults: a randomized, double-blind, placebo-controlled trial

Yuto Aoki^{1,*}, Joto Yoshimoto¹, Akihiro Tokushige², Shin Kawasoe³, Takuro Kubozono³, Mikiya Kishi¹, Mitsuru Ohishi³, and Tsuyoshi Takara⁴

¹Group Innovation Center, Mizkan Holdings Co., Ltd., Handa-shi, Aichi, Japan

²Department of Clinical Pharmacology and Therapeutics, University of the Ryukyus, Okinawa, Japan

³Department of Cardiovascular Medicine and Hypertension, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima-shi, Kagoshima, Japan

⁴Medical Corporation Seishinkai Takara Clinic, Tokyo, Japan

*Corresponding author: yuto_aoki@mizkan.co.jp

Abstract

Constipation is a common gastrointestinal condition, and dietary modifications play a crucial role in its management. This randomized, double-blind, placebo-controlled trial evaluated the effects of a vinegar beverage on defecation status in healthy adults with mild constipation (defecation frequency of 3–5 times per week). A total of 44 participants were randomly assigned to the vinegar or placebo groups and consumed the test beverage daily at breakfast for 4 weeks; the sample size was determined based on feasibility considerations. The primary outcome, defecation frequency at Week 4, was significantly higher in the vinegar group than in the placebo group ($p = 0.042$). Exploratory analyses of secondary outcomes without adjustment for multiplicity showed no consistent differences across all time points. However, at Week 1, we observed changes suggestive of potential improvements in defecation frequency ($p = 0.007$), number of defecation days ($p = 0.021$), and stool volume ($p = 0.043$). No significant changes in fecal microbial diversity were observed following the intervention, and no adverse events related to the intervention occurred. In conclusion, these findings suggest that daily consumption of vinegar beverages may improve defecation status in healthy adults with mild constipation, supporting a potential dietary approach for maintaining bowel health.

Keywords

Constipation, Vinegar beverage, Defecation improvement, Defecation frequency, Stool volume

Introduction

Constipation, characterized by infrequent defecation and difficulty or incomplete defecation, reduces quality of life [1,2] and increases the risk of chronic diseases [3–5]. With a global prevalence of

approximately 10%–20% [6–8], it is common yet remains a significant public health concern. Although treatment approaches may vary slightly depending on the causes and symptoms of constipation, the first-line treatment for most patients is lifestyle modification, particularly dietary improvements, such as increased dietary fiber intake [9]. Meta-analytical studies have demonstrated that dietary fiber intake increases defecation frequency and can be a useful therapeutic approach for constipation [10,11]. However, dietary fiber intake has been declining annually in Japan [12]. In the United States, only approximately 5% of the population consumes the recommended daily fiber intake [13], indicating the challenges of incorporating high-fiber foods into modern dietary habits. Given this background, providing a simple and sustainable dietary habit that can improve defecation is valuable.

Based on a cross-sectional analysis of cohort data, we previously reported that the frequency of vinegar-based dish consumption was positively associated with defecation status [14]. This association remained statistically significant even after adjusting for factors that could affect defecation status, such as age, sex, gastrointestinal medication history, and total dietary fiber intake, suggesting that vinegar consumption may improve defecation. However, owing to study limitations, including the inability to determine exact intake amounts of vinegar and its potentially active constituent, acetic acid, lack of time-series data analysis, and the potential presence of unknown confounding factors, the target intake amount of vinegar and the causal relationship remain unclear. Therefore, further detailed randomized controlled trials are required to verify these findings. In this study, given that acetic acid may play a key role in the effects of vinegar and that its intake can be strictly controlled, we conducted a randomized controlled trial in which healthy adults with mild constipation (defecation frequency of 3–5 times per week) consumed a vinegar beverage instead of vinegar-based dishes to investigate the effects of vinegar on defecation.

Methods

Study design

This was a randomized, double-blind, placebo-controlled, parallel-group trial conducted between August and December 2024 at Medical Corporation Seishinkai Takara Clinic and Nerima Medical Association Minami-machi Clinic in Japan. As no previous randomized trials have evaluated the effects of vinegar beverages on defecation outcomes, no reliable estimates of effect size were available for a formal power calculation. Therefore, this trial was designed as an exploratory study, and the target sample size of 40 participants (20 per group) was determined based on feasibility, including resource availability and funding. To account for an estimated dropout rate of 10%, we aimed to enroll 44 participants. The study participants, investigators, and clinical staff remained blinded to the group allocation until all primary data analyses were completed.

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and approved by Medical Corporation Seishinkai Takara Clinic (approval number: 2408-04086-0074-

12-TC). Participants were informed that their participation was voluntary and that no disadvantage would result from refusing consent. All participants provided online informed consent. Participants received modest financial compensation for completing the study visits, as burden alleviation for time and travel costs. This study was registered with the UMIN Clinical Trials Registry (UMIN trial ID: UMIN000055288, registered on August 20, 2024, URL: https://center6.umin.ac.jp/cgi-open-bin/ctr/ctr_view.cgi?recptno=R000063172) and was conducted in accordance with the Consolidated Standards of Reporting Trials (CONSORT 2025) reporting guidelines [15]. The CONSORT 2025 checklist was used to prepare the report [16].

Participants

Participants were recruited via the online platform Go106 (<https://www.go106.jp/>), operated by ORTHOMEDICO Inc. (Tokyo, Japan), a contract research organization (CRO), from its pool of registered monitors. Individuals potentially meeting the eligibility criteria were invited to participate and provided informed consent. At the time of informed consent, an initial eligibility check based on self-reported information was conducted for all inclusion and exclusion criteria. A total of 83 candidates who met these preliminary criteria underwent a screening examination, which included evaluation of defecation frequency as well as urine and blood analyses and medical interviews to confirm overall eligibility. Of these, 44 participants who met all eligibility criteria were enrolled in the study. The detailed inclusion and exclusion criteria are described below.

The inclusion criteria were as follows: (1) Japanese men and women, (2) age 20–69 years, and (3) defecation frequency of 3–5 times per week during the 1-week period prior to the screening examination.

Individuals meeting any of the following exclusion criteria were excluded from this study: individuals who are undergoing medical treatment or have a medical history of malignant tumor, heart failure, or myocardial infarction; individuals who have a pacemaker or an implantable cardioverter defibrillator; individuals who are currently undergoing treatment for any of the following chronic diseases: cardiac arrhythmia, liver disorder, chronic kidney disease, cerebrovascular disorder, rheumatic disease, diabetes mellitus, dyslipidemia, hypertension, or any other chronic diseases; individuals who are taking "Foods for Specified Health Uses" or "Foods with Functional Claims"; individuals who are taking or using medications (including herbal medicines) and supplements; individuals who do not take three meals daily: breakfast, lunch, and dinner; individuals who are allergic to medicines and foods related to the test product; individuals who are pregnant, lactating, or planning to become pregnant during this study; individuals who have been enrolled in other clinical studies within the last 28 days before the agreement to participate in this study or plan to participate in another study during this study; individuals who are judged as ineligible to participate in this study by the physician.

Intervention

The participants were randomly assigned to one of two groups ($n = 22$ per group): citric acid-based placebo beverage group or vinegar beverage group. The random allocation sequence was generated by the allocator in a 1:1 ratio using block randomization without stratification. To ensure allocation concealment, block sizes were varied at random. The sequence was generated in R (version 4.3.2) using the blockrand package (version 1.5), resulting in block sizes of 4, 6, 6, 6, 6, 8, and 8. The investigators responsible for participant enrollment and assignment had no access to the random allocation sequence. The participants received 28 bottles (over a period of 4 weeks) of either a placebo or a vinegar beverage under blinded conditions and consumed one test beverage bottle daily with breakfast. The vinegar beverage was prepared by dissolving brewed vinegar in water, resulting in a final concentration of 750 mg of acetic acid per 200 mL. The placebo beverage contained 30 mg of anhydrous citric acid per 200 mL instead of brewed vinegar to provide acidity and facilitate blinding. Both test beverages were flavored with sucralose and apple flavoring. Participants were instructed to maintain their usual diet and lifestyle, and no additional concomitant care was provided during the trial. To support participants, a contact system was established whereby participants could reach the CRO on weekdays for study-related inquiries. On weekends, contact was restricted to same-day cancellations of scheduled visits, for which participants could directly call the study clinic. The detailed examination schedule and nutritional composition of the test beverages are shown in Tables 1 and S1, respectively.

Data measurements

The participants maintained daily bowel diaries during two periods: for 1 week prior to Visit 1 (screening examination) and for 4 weeks from the start to the end of the intervention. During the intervention period, they also recorded whether they consumed the test beverage each day. Comprehensive evaluations, including fecal examinations, body measurements, urinalysis, blood tests, and medical interviews, were conducted at Visit 1 and Visit 2 (day after the 4-week intervention ended). The detailed examination schedule is shown in Table 1.

▪ Body measurements

Height was measured in the standing position using a standard stadiometer at Visit 1. Body weight was measured using a digital scale (HN-300T2; Omron Healthcare Co., Ltd., Kyoto, Japan), and systolic and diastolic blood pressures were assessed using an automated digital sphygmomanometer (HEM-6022; Omron Healthcare Co., Ltd.) at both visits.

▪ Urine and blood analyses

Urine and blood samples were collected during Visit 1 and Visit 2 to assess eligibility and safety of the participants. Urinary protein, glucose, pH, and occult blood levels were measured by LSI Medience Corporation (Tokyo, Japan). Hematological parameters included white blood cell count, red blood cell count, hemoglobin level, hematocrit level, and platelet count. Biochemical parameters included levels of hepatic enzymes (aspartate aminotransferase, alanine aminotransferase, and gamma-glutamyl transpeptidase), total bilirubin, total protein, renal function markers (blood urea nitrogen, creatinine, and uric acid), electrolytes (sodium, potassium, and chloride), serum amylase, lipid profile (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides), glucose, and hemoglobin A1c (National Glycohemoglobin Standardization Program). All urinary and blood parameters were measured using standard methods established by LSI Medience Corporation.

▪ Bowel diary

The participants recorded their defecation frequency, stool volume, stool form, stool color, stool odor, and sensation of defecation completeness in a daily bowel diary [17]. Stool volume was recorded by converting it to the number of film canisters (5.5 cm) corresponding to the visually estimated volume. Stool form was assessed using the seven-point Bristol Stool Form Scale [18], which was translated into Japanese for participant use, ranging from small, hard lumps resembling rabbit droppings (pellet-like stool) to watery stool without solid matter. Specifically, the scale included (1) small, hard lumps; (2) hard, sausage-shaped stool; (3) sausage-shaped stool with surface cracks; (4) smooth, soft sausage-shaped or coiled stool; (5) soft stool with distinct wrinkles; (6) mushy, shapeless stool with broken boundaries; and (7) watery stool lacking any solid content. Stool color was categorized into six groups: (1) yellow; (2) yellow ochre; (3) brown; (4) dark brown; (5) deep brown; and (6) blackish brown. Stool odor was evaluated on a five-point scale relative to usual: (1) markedly stronger; (2) slightly stronger; (3) usual; (4) slightly weaker; and (5) markedly weaker. The sensation of defecation completeness was recorded on a three-point scale, (1) comprising complete; (2) normal; and (3) incomplete defecation. These assessments were conducted through the participants' self-reports during the study.

▪ Fecal sampling, DNA extraction, and sequencing

Fecal samples were self-collected at home using Mykinso fecal collection kits containing guanidine thiocyanate solution (Cykinso, Inc., Tokyo, Japan). Participants were instructed to collect a small stool sample according to the manufacturer's instructions within 3 days prior to each visit (Visits 1 and 2) and bring it to the clinic. If sample collection was not possible before the visit, participants collected the sample at home afterward and mailed it to the CRO, ORTHOMEDICO Inc. All fecal samples were subsequently sent to Cykinso, Inc., where DNA extraction and sequencing of fecal samples were

performed. DNA was extracted from the fecal samples using an automated DNA extraction machine (GENE PREP STAR PI-1200A; Kurabo Industries Ltd., Osaka, Japan), according to the manufacturer's protocol. The V1–V2 region of the 16S rRNA gene was amplified using forward primer (16S_27Fmod: TCGTCG GCA GCG TCA GAT GTG TAT AAG AGA CAG AGR GTT TGA TYM TGG CTC AG) and reverse primer (16S_338R: GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GTG CTG CCT CCC GTA GGA GT) using the KAPA HiFi Hot Start Ready Mix (Roche, Basel, Switzerland). To sequence the 16S rRNA amplicons using the Illumina MiSeq platform, dual-index adapters were attached using the Nextera XT Index kit. Each library was diluted to 5 ng/μL, and equal volumes of the libraries were mixed at 4 nM. The DNA concentration of the mixed libraries was quantified by quantitative polymerase chain reaction (qPCR) with the KAPA SYBR FAST qPCR Master Mix (KK4601; KAPA Biosystems, Wilmington, MA, United States) using primers 1 (AAT GAT ACG GCG ACC ACC) and 2 (CAA GCA GAA GAC GGC ATA CGA). Libraries were prepared according to the Illumina 16S library preparation protocol (Illumina, San Diego, CA, United States). Libraries were sequenced using the MiSeq Reagent Kit version 2 (500 Cycles), 250 bp paired-ends.

• Taxonomy assignment based on 16S rRNA gene sequences

Illumina MiSeq FASTQ reads were subjected to Basic Local Alignment Search Tool (BLAST) homology searching against the NCBI 16S microbial database using a Homology Analysis tool (World Fusion Co., Ltd., Tokyo, Japan), and taxonomy was classified using Metagenome@KIN (World Fusion). The sequences with an E-value > 0.001 were classified as No BLAST hit, and those with an E-value > 10E–30 and sequence identity < 98% were classified as Unclassified hit.

Outcome measures

The 1-week pre-intervention period (Pre), defined as the week prior to the screening examination, served as the baseline. The intervention period was divided into four weekly segments. Daily bowel diary results were summarized weekly for Weeks 1–4. Based on the defecation frequency recorded in the daily bowel diary, the number of days with defecation per week was defined as the “defecation days.” The primary outcome measure was defecation frequency at Week 4. The secondary outcomes included defecation frequency at Weeks 1–3, defecation days at Weeks 1–4, stool volume at Weeks 1–4, the number of participants who showed at least one level of improvement in stool characteristics (form, color, and odor) from the pre-intervention period baseline, the sense of relief after defecation at Weeks 1–4, and alpha diversities of the fecal microbiota before and after the intervention. To assess whether a participant achieved at least a one-level improvement in subjective bowel symptoms, the median score for each period was calculated and rounded to the nearest integer to establish the measured value; cases with missing data were conservatively treated as having no improvement. Based on the numerical scales defined above, a one-level or greater improvement was defined as follows: (1)

for stool appearance, an evaluation value greater than the baseline value and ≤ 5 (when the baseline value was ≤ 4), or an evaluation value less than the baseline value and ≥ 5 (when the baseline value was ≥ 6); (2) for stool color, an evaluation value less than the baseline value, indicating a shift toward the yellow spectrum; (3) for stool odor, an evaluation value greater than the baseline value; and (4) for the sensation of defecation completeness, an evaluation value less than the baseline value. Beta diversity of the fecal microbiota before and after the intervention was not pre-specified in the trial protocol and was performed to further explore potential changes in overall microbial community structure. Physical measurements, urinalysis, and blood tests were performed to assess safety.

Statistical analyses

• Mixed model for repeated measures

The mixed model for repeated measures (MMRM) was used to evaluate the effects of the intervention groups and time points, because defecation outcomes were measured repeatedly over four weeks and adjustment for baseline values and inter-individual variability was required. All scheduled measurements were obtained, and no missing data occurred for the primary and secondary defecation outcomes. The model included the following fixed effects: baseline (“defecation frequency” or “defecation days” or “defecation volume”), time points (Weeks 1, 2, 3, and 4), group (placebo and vinegar groups), interaction between group and time points, and interaction between baseline and time points. To account for within-subject variability, an unstructured covariance structure was specified for the time points nested within each participant. Estimated marginal means (EMMs) were calculated using the emmeans package in R to evaluate the differences between the groups at each time point. *P*-values for Weeks 1–4 were calculated from the MMRM using *t*-statistics with Satterthwaite-adjusted degrees of freedom. All statistical analyses were performed using R (version 4.5.0).

No adjustments for multiplicity were applied to any of the analyses. The significance level for the primary outcome, defecation frequency at Week 4, was set at $p < 0.05$. Analyses of secondary outcomes were considered exploratory and presented as reference values.

• Fecal microbial diversity

Alpha diversity was assessed using the Shannon index after excluding no-BLAST hits and unclassified reads. Following dataset integration and alignment of Visit 1 and Visit 2 measurements for each participant, paired comparisons were conducted within each group (placebo and vinegar) using the Wilcoxon signed-rank test. Beta diversity, representing differences in microbial community composition between visits, was assessed using permutational multivariate analysis of variance (PERMANOVA) with repeated measures (adonis2, vegan R package), based on generalized UniFrac distance matrices calculated using weight = 0.5. Participant identifiers were included as a stratification factor to account for the paired design. Principal coordinates analysis was performed for visualization,

with 95% confidence ellipses plotted for each visit. Statistical significance was determined using 999 permutations.

Results

Participant characteristics

Table 2 presents the baseline characteristics of the participants at screening by group, and Figure 1 shows the participant flow diagram. Of the 83 candidates who provided informed consent, 44 who satisfied the eligibility criteria were enrolled and randomly assigned to the two groups ($n = 22$ per group). All participants received the allocated intervention and completed the study; therefore, all 44 participants were included in the final analysis. No significant differences were observed between the groups in age, sex, body mass index, blood pressure, or defecation status (defecation frequency, defecation days, and stool volume) at baseline. Adherence to the test beverage was $99.8\% \pm 2.6\%$ (range, 92.9%–100%) in the placebo group and $99.5\% \pm 1.7\%$ (range, 92.9%–100%) in the vinegar group. Safety was assessed comprehensively, primarily through medical interviews, supplemented by body measurements, urinalysis, and blood tests. As no adverse events attributable to the interventions were observed, detailed safety data are not shown.

Defecation status

To account for time point and inter-individual variability, the EMMs of the defecation status (defecation frequency, defecation days, and stool volume) were calculated for the placebo and vinegar groups using an MMRM, and between-group comparisons were performed. The detailed analytical results are presented in Tables 3 and 4. Regarding defecation frequency, the vinegar group showed significantly higher values than the placebo group at Week 4 ($p = 0.042$), which was the prespecified primary outcome. In exploratory analyses of secondary outcomes, a tendency toward an increase in defecation frequency at Week 1 was also observed in the vinegar group compared with the placebo group ($p = 0.007$) (Fig. 2a). A tendency toward an increase in defecation days was observed in the vinegar group compared with the placebo group at Week 1 ($p = 0.021$) (Fig. 2b). A tendency toward an increase in stool volume was observed in the vinegar group compared with the placebo group at Weeks 1 ($p = 0.043$) and 2 ($p = 0.048$) (Fig. 2c). Results regarding improvements in stool characteristics and defecation sensation parameters during the intervention compared with baseline are provided in Supplementary Table S2. A tendency toward a higher proportion of participants showing improvement in the sensation of defecation completeness at Week 1 was observed in the vinegar group compared with the placebo group ($p = 0.042$). The p -values for all outcomes were not adjusted for multiple comparisons, and the analyses of secondary outcomes were prespecified as exploratory.

Fecal microbial diversity

To investigate the effects of vinegar beverage consumption on the fecal microbiota, we examined whether there were changes in alpha diversity of the fecal microbiota before and after the intervention. Alpha diversity showed no significant differences before and after the intervention in either group (placebo, $p = 0.679$; vinegar, $p = 0.371$) (Fig. 3). An exploratory analysis of fecal microbial beta diversity before and after the intervention was conducted. Beta diversity also showed no significant differences in either group (placebo, $p = 0.208$; vinegar, $p = 0.399$) (Fig. 4).

Discussion

In the present study, we demonstrated that continuous consumption of the vinegar beverage significantly increased defecation frequency at Week 4 of the intervention in healthy adults with a tendency toward constipation (defecation frequency of 3–5 times per week at baseline). Furthermore, exploratory analyses suggested that defecation status (defecation frequency, defecation days, and stool volume) may improve as early as 1 week after the initiation of the intervention. To our knowledge, this is the first human clinical trial to suggest that consumption of vinegar beverages may improve defecation status.

The Rome IV criteria provide internationally accepted diagnostic standards for functional constipation [19], incorporating not only objective indicators such as defecation frequency, but also subjective bowel symptoms such as straining and the sensation of incomplete evacuation. In the present study, the vinegar beverage intervention showed a tendency to improve not only defecation status but also the sensation of defecation completeness. In Week 1, the vinegar beverage intervention appeared to improve the sensation of defecation completeness; however, this effect did not persist in the subsequent weeks. This may suggest that participants were more sensitive to perceived changes between the pre-intervention and early intervention periods. Taken together, these exploratory findings may suggest that vinegar beverage consumption could be a dietary approach for healthy adults with relatively infrequent or unsatisfactory defecation, although these results should be interpreted with caution due to the lack of adjustment for multiple comparisons.

In previous studies, when healthy men in their 20s were randomly assigned to alternate between weeks of adequate fluid intake (2.5 L) and restricted fluid intake (0.5 L) with a 2-week washout period between crossover interventions, defecation status decreased during the fluid-restricted week and returned to baseline during the adequate fluid intake week [20]. Based on observed tendencies in the line graphs in Figure 2, defecation status appeared to gradually improve over the course of beverage consumption, regardless of beverage type, suggesting that habitual underhydration may have contributed to constipation in the study participants. Moreover, in exploratory analyses without adjustment for multiple comparisons, we observed that during Week 1, immediately after the start of the intervention, parameters related to defecation status showed a tendency toward greater

improvement in the vinegar group than in the placebo group. These exploratory findings may indicate a potential early response to vinegar or acetic acid intake that is independent of fluid consumption; however, given their exploratory nature and the lack of adjustment for multiple comparisons, they should therefore be interpreted with caution.

Gastrointestinal system activity is essential for defecation, and colonic motility dysfunction is one of the primary pathophysiological mechanisms of constipation [21,22]. Colonic peristalsis comprises two types of contractions: repetitive nonpropulsive contractions and coordinated contractions known as high-amplitude propagated contractions (HAPCs) [22]. Although repetitive nonpropulsive contractions facilitate the mixing and absorption of contents, HAPCs, which are defined by the presence of high amplitude and propagation, transport contents over long distances from the ascending colon toward the descending colon [22] and tend to increase upon awakening and after meals [23]. Patients with slow-transit constipation tend to exhibit reduced HAPCs compared with healthy individuals [24]. Regarding the association between diet and defecation, a well-known physiological phenomenon, the gastrocolic reflex (GCR), defined as increased colonic activity following a meal, has been extensively documented [25]. GCR typically occurs within minutes of meal consumption and propagates fecal content and gas toward the rectum while allowing new intestinal content to enter the colon [25]. In this study, the participants were instructed to consume the test beverage at breakfast every morning. Therefore, vinegar may have enhanced the GCR during breakfast consumption and increased the frequency of morning HAPCs, thereby improving bowel function.

Typically, the fecal microbiota composition of patients with constipation differs from that of healthy individuals [26–29]. In the present study, no significant changes in the fecal microbiota were observed before and after the intervention in either the placebo or vinegar group. As the sample size was determined based on feasibility considerations, the possibility of insufficient statistical power to detect changes in fecal microbial diversity cannot be ruled out. Moreover, we did not perform more informative analyses, such as shotgun metagenomic sequencing or metabolomic profiling. Therefore, the findings regarding the fecal microbiota should be interpreted with caution. Furthermore, although the study participants experienced mild constipation, they did not have severe constipation, which may have resulted in little difference between their fecal microbiota and that of individuals with normal defecation frequency. The four-week intervention period may have been insufficient to induce meaningful improvements in the fecal microbiota. Therefore, fecal microbiota alterations associated with improvements in defecation following vinegar beverage intake may not have been detectable under the present study conditions. Despite these considerations, these findings suggest that vinegar beverage consumption alone may not substantially influence the fecal microbiota, which is consistent with previous reports indicating that orally ingested acetic acid is largely absorbed in the stomach and small intestine [30,31] and may not reach the large intestine. Taken together, a possible mechanism

underlying the defecation improvement induced by vinegar beverage consumption may involve enhanced peristaltic activity via the GCR pathway, rather than being primarily driven by changes in the fecal microbiota. This hypothesis is consistent with the current findings, but it should be noted that we did not evaluate defecation timing, colonic motility, or hormonal responses; thus, the proposed mechanism remains speculative and requires further investigation.

For most patients with constipation, dietary modification is a fundamental first-line intervention [32], and, in particular, increasing dietary fiber intake is a well-established dietary strategy for improving defecation frequency [10,11]. In daily life, however, substantially increasing dietary fiber intake is difficult for many individuals worldwide, including those in Japan [12,13]. Achieving the recommended dietary fiber intake may require major changes to dietary habits, which can be difficult without adequate information or access to dietary counselling by registered dietitians. Additionally, some patients may not achieve satisfactory improvement even with fiber supplementation [11]. In such situations, consumption of vinegar beverages may offer a feasible dietary option for improving defecation status. Moreover, our previous cohort study demonstrated a positive association between habitual consumption of vinegar-containing dishes and defecation frequency, independent of dietary fiber intake [14], suggesting that incorporating vinegar into regular dishes, in addition to beverages, may also be a promising approach.

Future studies are needed to clarify the mechanisms underlying these effects, such as the GCR pathway and changes in the gut microbiota and related metabolites, and to examine the impact of different forms of vinegar use, including both beverages and culinary applications. Furthermore, considering the possibility that dietary fiber and vinegar improve defecation status through distinct mechanisms, it will be important to investigate whether the addition of dietary fiber to vinegar beverages can produce additive or synergistic effects. Beyond the physicochemical properties of vinegar, a recent study indicates that subjective changes in taste and smell are closely linked to symptom burden in patients with functional constipation and irritable bowel syndrome [33]. Building on these findings, future studies could examine whether and how the characteristic aroma and flavor of vinegar-containing foods and beverages influence defecation status and related gastrointestinal symptoms.

This study has some limitations. First, as this investigation was conducted in a limited geographical region with a Japanese population, further studies are required to determine whether similar results can be obtained in other ethnic populations. Differences in dietary habits and genetic backgrounds may lead to different responses to the intervention. Second, we enrolled healthy participants with relatively infrequent defecation; therefore, the efficacy of the intervention in individuals with severe constipation was not evaluated. Pharmacological treatment should be considered the first-line approach for severe constipation [9]. Third, participants were recruited from the pool of registered online monitors, which may introduce selection bias. In addition, relatively strict

inclusion and extensive exclusion criteria were used to enhance internal validity; as a result, the external validity and generalizability of our findings to more heterogeneous populations may be limited. Fourth, only a single dosage of vinegar (750 mg acetic acid) was evaluated, and thus dose-response effects could not be elucidated. Fifth, as this study did not include a post-intervention observation period following the cessation of beverage consumption, further investigation is necessary to determine whether the observed effects are sustained over time. Sixth, the study relied on self-reported bowel diaries, which may be subject to self-report bias and inaccuracies, particularly in the evaluation of subjective sensory perceptions such as stool color, stool odor, and the sensation of defecation completeness. In addition, the real-world assessment of stool characteristics was subject to practical constraints. The evaluation of some subjective sensory perceptions may be affected by recall bias, because participants are required to judge changes relative to the perceived baseline over several weeks. Seventh, dietary intake during the intervention period was not recorded. Although participants were instructed to maintain their usual diet and lifestyle and no additional concomitant care was provided, we cannot exclude the possibility that the intervention indirectly altered their dietary habits or lifestyle, which may have influenced the outcomes.

In conclusion, continuous consumption of vinegar beverages for 4 weeks by constipation-prone healthy adults may improve defecation status, exemplified by an increase in defecation frequency at Week 4. Although these findings should be regarded as preliminary and require more confirmation in further studies, our findings suggest that vinegar beverage consumption could represent a promising dietary approach to improving defecation status in healthy adults prone to constipation.

Abbreviations

CRO: Contract research organization

BLAST: Basic local alignment search tool

Pre: Pre-intervention period

MMRM: Mixed model for repeated measures

PERMANOVA: Permutational multivariate analysis of variance

HAPCs: high-amplitude propagated contractions

GCR: gastrocolic reflex

BMI: Body mass index

SBP: Systolic blood pressure

DBP: Diastolic blood pressure

SD: Standard deviation

Med: Median

Min: Minimum value

Max: Maximum value

EMM: Estimated marginal mean

SE: Standard error

CI: Confidence interval

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Author contributions

YA was responsible for the conceptualization and design of the study, analysis and interpretation of the data, and writing of the manuscript. JY contributed to the study conceptualization and design, data analysis and interpretation, and writing of the manuscript. AT, SK, and TK contributed to the study design. MK contributed to the data interpretation and was involved in manuscript editing. MO was involved in study design and data interpretation. TT was responsible for conducting the clinical trial at the study site, including participant management, safety monitoring, and data collection. All the authors have read and approved the final version of the manuscript.

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Competing interest statement

YA, JY, and MK are employees of Mizkan Holdings Co., Ltd. None of the principal investigators involved in this study or their family members are shareholders of Mizkan Holdings Co., Ltd., company officers, directors, or advisors. AT, SK, TK, MO, and TT declare no conflict of interest.

Data availability statement

The 16S rRNA gene amplicon sequencing data generated in this study are available in the DNA Data Bank of Japan (DDBJ) under accession number DRR900481–DRR900568. Other data supporting the findings of this study are available from the corresponding author upon reasonable request but are not publicly available due to privacy and ethical restrictions.

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Table 1. Examination schedule

			Registration		Intervention		Measurement				
			Screening based on eligibility criteria	Allocation	Placebo beverage	Vinegar beverage	Bowel diary	Fecal sampling	Body measurement	Urinalysis	Blood test
Clinical trial information session			●								
Screening	Day 1	Pre					↕				
	Day 7					↕					
	Day 8	Visit 1					●	●	●	●	●
Enrollment			●	●				●	●	●	●
Main study	Day 1	Week 1			↑	↑	↑				
	Day 7										
	Day 8	Week 2									
	Day 14										
	Day 15	Week 3									
	Day 21										
	Day 22	Week 4									
	Day 28										
	Day 29	Visit 2						●	●	●	●

Pre, pre-intervention period.

Table 2. Baseline characteristics of the participants

	Placebo beverage (n = 22)	Vinegar beverage (n = 22)
Age (years), mean (SD)	49.5 (10.1)	50.1 (9.1)
Sex n (%)		
Male	8 (36.4)	8 (36.4)
Female	14 (63.6)	14 (63.6)
Weight (kg), mean (SD)	60.7 (10.9)	58.5 (13.2)
BMI, mean (SD)	23.0 (4.0)	21.6 (3.3)
SBP (mmHg), mean (SD)	110.5 (12.1)	113.4 (12.9)
DBP (mmHg), mean (SD)	70.9 (9.2)	72.2 (7.4)
Defecation days/week, mean (SD)	3.4 (0.6)	3.4 (0.6)
Defecation frequency/week, mean (SD)	3.6 (0.5)	3.6 (0.5)
Stool volume/week, mean (SD)	13.9 (7.6)	16.1 (10.9)

Data are expressed as means (SDs) or numbers (%).

SD, standard deviation; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 3. Defecation status during the intervention period

	Time point	Placebo (n = 22)										Vinegar (n =22)									
		Mean	SD	Med	Min	Max	EMM	SE	95% CI-	95% CI+	Mean	SD	Med	Min	Max	EMM	SE	95% CI-	95% CI+		
Defecation frequency (times/week)	Pre	3.64	0.49	4.0	3	4	-	-	-	-	3.64	0.49	4.0	3	4	-	-	-	-		
	Week 1	4.18	1.82	4.0	2	9	4.18	0.36	3.45	4.91	5.64	1.68	5.5	3	9	5.64	0.36	4.91	6.37		
	Week 2	4.55	1.53	4.5	2	8	4.55	0.58	3.38	5.71	6.00	3.46	5.5	2	16	6.00	0.58	4.84	7.16		
	Week 3	4.77	1.77	5.0	1	9	4.77	0.48	3.81	5.74	5.59	2.58	5.5	1	10	5.59	0.48	4.63	6.55		
	Week 4	4.73	1.78	4.5	1	8	4.73	0.40	3.92	5.53	5.91	1.97	6.0	3	10	5.91	0.40	5.10	6.71		
Defecation days (days/week)	Pre	3.41	0.59	3.0	2	4	-	-	-	-	3.41	0.59	3.0	2	4	-	-	-	-		
	Week 1	3.68	1.52	3.0	2	7	3.68	0.28	3.11	4.25	4.64	1.05	5.0	3	7	4.64	0.28	4.07	5.21		
	Week 2	4.23	1.15	4.0	2	6	4.23	0.32	3.59	4.87	4.55	1.74	5.0	2	7	4.55	0.32	3.91	5.19		
	Week 3	4.18	1.37	4.0	1	7	4.18	0.33	3.52	4.84	4.59	1.65	5.0	1	7	4.59	0.33	3.93	5.25		
	Week 4	4.36	1.59	4.0	1	7	4.36	0.32	3.72	5.01	5.05	1.40	5.0	3	7	5.05	0.32	4.40	5.69		
Stool volume (film canisters/week)	Pre	13.89	7.60	12.0	4.0	37.0	-	-	-	-	16.11	10.87	11.3	4.5	41.5	-	-	-	-		
	Week 1	17.57	14.11	11.5	3.5	48.0	18.80	3.62	11.49	26.11	30.77	23.97	26.0	10.0	105.0	29.54	3.62	22.23	36.85		
	Week 2	19.14	14.90	13.5	4.0	55.0	21.00	4.68	11.54	30.45	36.41	34.69	27.0	3.0	125.0	34.55	4.68	25.09	44.00		
	Week 3	20.04	13.59	18.0	2.0	50.0	21.47	4.96	11.45	31.49	34.27	34.01	25.0	4.0	150.0	32.84	4.96	22.82	42.86		
	Week 4	21.34	15.00	18.0	2.0	62.0	22.29	4.39	13.42	31.17	33.73	26.96	26.5	8.0	120.0	32.77	4.39	23.90	41.65		

Pre, pre-intervention period; SD, standard deviation; Med, median; Min, minimum value; Max, maximum value; EMM, estimated marginal mean; SE, standard error; CI, confidence interval.

Table 4. Comparison of defecation status between the intervention groups

	Time point	Placebo vs. vinegar				
		Between-group difference	SE	95% CI–	95% CI+	<i>p</i>
Defecation frequency (times/week)	Pre	0.00	0.15	−0.30	0.30	> 0.999
	Week 1	1.45	0.51	0.42	2.49	0.007 ^{††}
	Week 2	1.45	0.81	−0.19	3.10	0.082
	Week 3	0.82	0.67	−0.54	2.18	0.232
	Week 4	1.18	0.56	0.04	2.32	0.042 [*]
Defecation days (days/week)	Pre	0.00	0.18	−0.36	0.36	> 0.999
	Week 1	0.95	0.40	0.15	1.76	0.021 [†]
	Week 2	0.32	0.45	−0.59	1.22	0.482
	Week 3	0.41	0.46	−0.52	1.34	0.381
	Week 4	0.68	0.45	−0.23	1.59	0.137
Stool volume (film canisters/week)	Pre	2.23	2.83	−3.50	7.95	0.436
	Week 1	10.74	5.14	0.36	21.12	0.043 [†]
	Week 2	13.55	6.64	0.13	26.97	0.048 [†]
	Week 3	11.37	7.04	−2.86	25.59	0.114
	Week 4	10.48	6.24	−2.11	23.08	0.100

Between-group differences are shown, with values for Pre representing the mean and values for weeks 1–4 representing EMMs. *P*-values for Pre were calculated using Welch's t-test, and those for weeks 1–4 were calculated from the MMRM using t-statistics with Satterthwaite-adjusted degrees of freedom. Pre, pre-intervention period; SE, standard error; CI, confidence interval. ^{*}*p* < 0.05, [†]*p* < 0.05 (exploratory, not adjusted for multiplicity), ^{††}*p* < 0.01 (exploratory, not adjusted for multiplicity).

Fig. 1 Participant flow diagram.

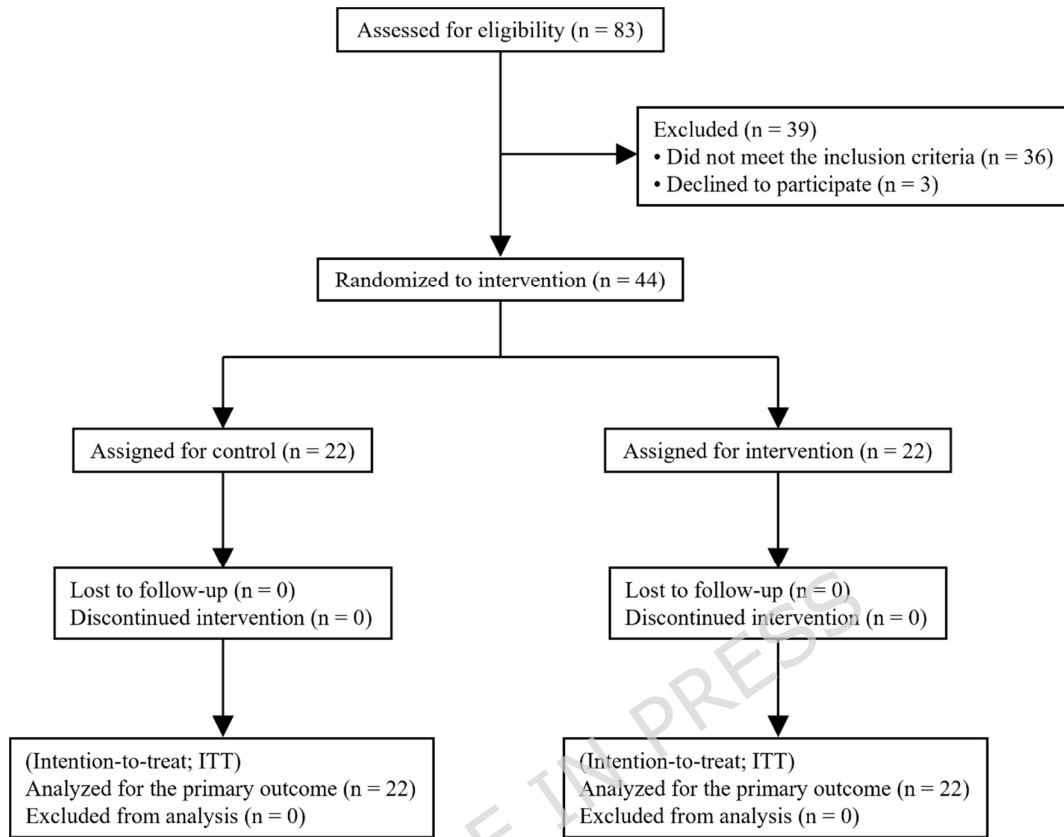
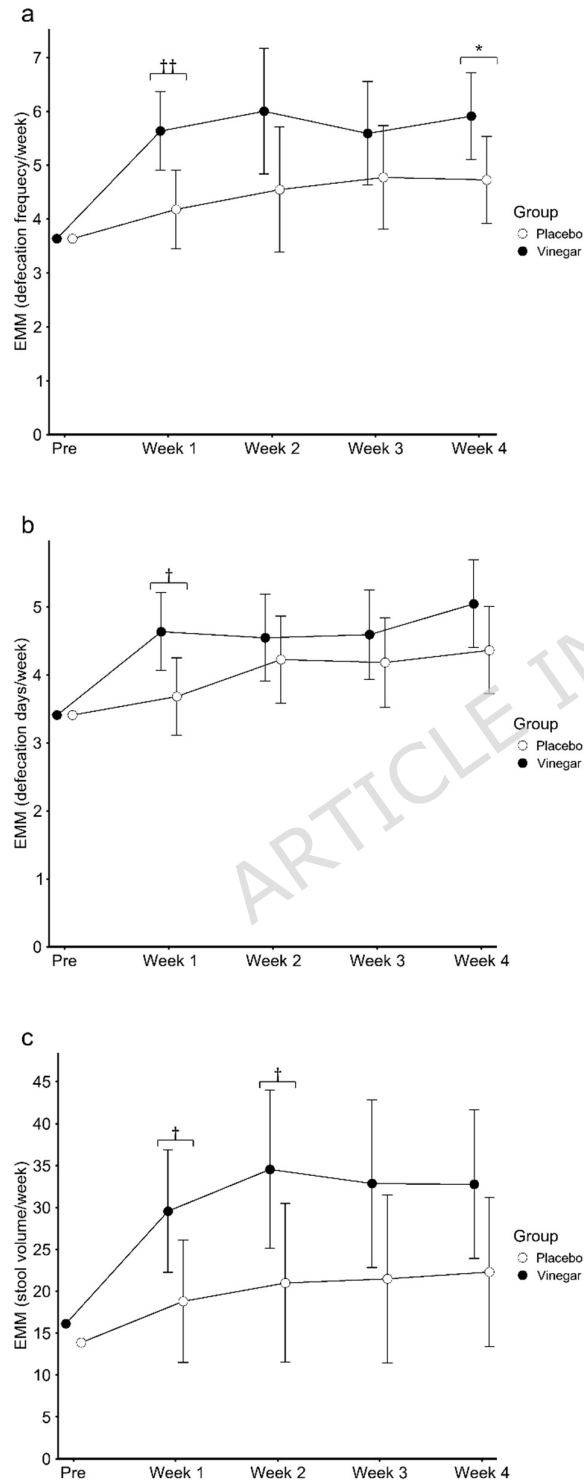


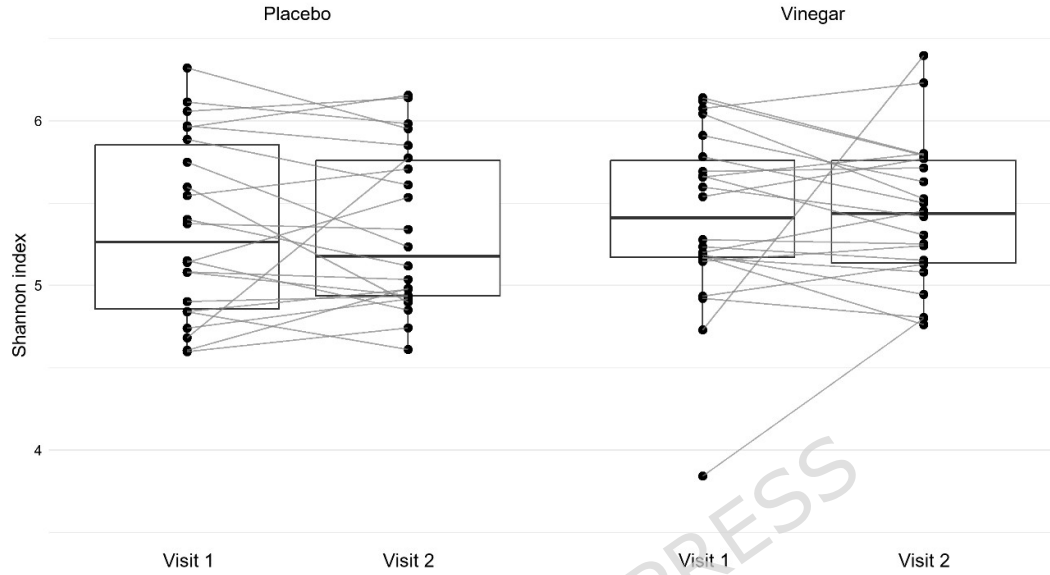
Fig. 2 Estimated marginal means (EMMs) of defecation frequency, defecation days, and stool volume over 4 weeks in the placebo and vinegar groups.



The line graphs show EMMs with 95% CIs for (a) defecation frequency, (b) defecation days, and (c) stool volume over 4 weeks. The placebo and vinegar groups are indicated by open (○) and filled (●) circles, respectively. Statistical significance was assessed using MMRM. Symbols indicate significant differences: * $p < 0.05$; † $p < 0.05$ (exploratory, not adjusted for multiplicity); †† $p < 0.01$ (exploratory, not adjusted for multiplicity). Pre, pre-intervention period.

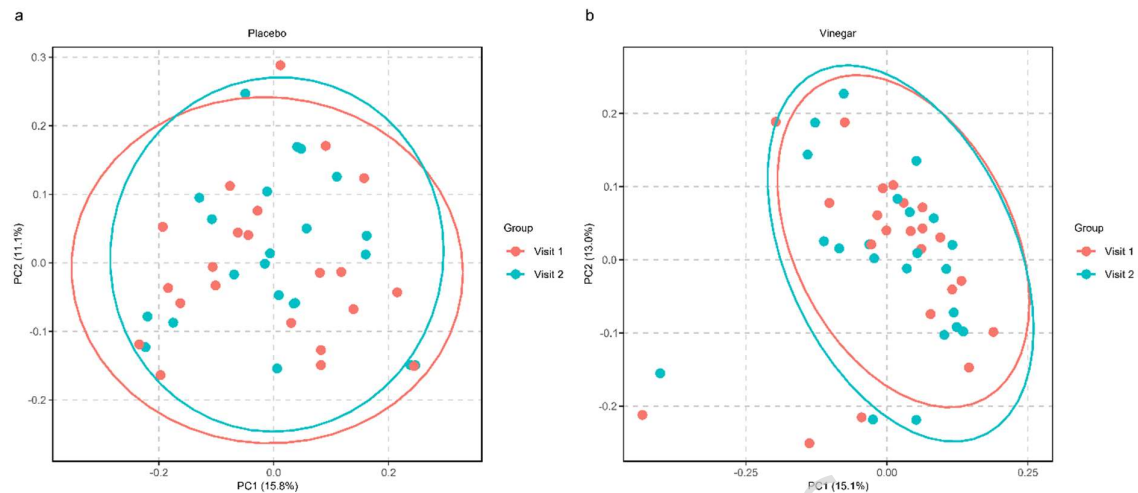
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Fig. 3 Comparison of Shannon index between Visit 1 and Visit 2 in the placebo and vinegar groups.



Shannon index of the fecal microbiota in the placebo ($p = 0.679$) and vinegar ($p = 0.371$) groups at Visit 1 and Visit 2. Box plots represent the median and interquartile range, with individual participant values overlaid, and lines connect measurements from the same participant across visits. P -values were obtained using the Wilcoxon signed-rank test.

Fig. 4 Principal coordinates analysis of weighted UniFrac distances showing within-group changes from Visit 1 to Visit 2 in the placebo and vinegar groups.



(a) Placebo group ($p = 0.208$) and (b) vinegar group ($p = 0.399$). Beta diversity between Visit 1 and Visit 2 was assessed using permutational multivariate analysis of variance (PERMANOVA) with 999 permutations, with participant identifiers included as a stratification factor to account for the paired design. Principal coordinates analysis was performed for visualization using weighted UniFrac distance matrices (weight = 0.5) calculated between paired samples from Visit 1 and Visit 2. Each point represents an individual sample, and 95% confidence ellipses are shown for each visit.