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MAJOR ARTICLE

A Randomized, Placebo-Controlled Trial Assessing the Effect of VISBIOME ES Probiotic in People With HIV on Antiretroviral Therapy

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Background. A5350, a phase II, randomized, double-blind study, evaluated the safety and tolerability of the probiotic Visbiome Extra Strength (ES) over 24 weeks and measured effects on inflammation and intestinal barrier function.

Methods. The primary outcome was change in soluble CD14 (sCD14) levels; secondary outcomes included safety and tolerability, markers of inflammation and cellular activation, and microbiome. In a substudy, gut permeability was assessed by paired colonic biopsies measuring the area of lamina propria occupied by CD4+ cells, interleukin (IL)-17+ cells, and myeloperoxidase (MPO). Changes between arms were compared with the 2-sample *t* test with equal variance or the Wilcoxon rank-sum test. For safety, the highest graded adverse events (AEs) were compared between arms using the Fisher exact test.

Results. Overall, 93 participants enrolled: 86% male, median age 51 years, median CD4 count 712 cells/mm3. Visbiome ES was safe and well tolerated. There was no difference in mean change in sCD14 from baseline to week 25/26 between placebo (mean change, 92.3 μg/L; 95% CI, −48.5 to 233 μg/L) and Visbiome ES (mean change, 41.0 μg/L; 95% CI, −94.1 to 176.2 μg/L; $P = .60$). Similarly, no statistically significant differences between arms in inflammatory marker changes were identified. In substudy participants, no statistical differences between arms for change in cellular marker expression or gut permeability were observed ($P > .05$ for all). The microbiome demonstrated increased probiotic species and a significant decrease in *Gammaproteobacteria* ($P = .044$) in the Visbiome ES arm.

Conclusions. Visbiome ES was safe and altered the microbiome but demonstrated no effect on systemic inflammatory markers, pathology, or gut permeability in antiretroviral therapy–treated people with HIV.

Keywords. HIV; human microbiome; inflammation; probiotics.

HIV infection confers a chronic inflammatory state, impairing immune function and exacerbating chronic disease risk. HIV-related alterations in the intestinal microbiome are associated with CD4+ T-cell depletion and chronic inflammation [1–4]. Previous studies have found that gut microbiota and related metabolites, including tryptophan metabolism, are altered in people with HIV (PWH) [1, 2, 5, 6]. HIV infection of intestinal CD4+ T cells results in intestinal epithelial damage, with decreased colonic epithelial tight junction proteins and increased colonic permeability, and facilitates microbial translocation despite suppressive antiretroviral therapy (ART) [7]. As systemic inflammation has been linked with long-term morbidity and mortality [8], adjunctive interventions are needed to improve gut integrity.

Probiotics are organisms such as yeast or bacteria available in foods and supplements that may improve overall gut health and reduce excess intestinal permeability [9, 10]. Various probiotics have been studied in disease states associated with gastrointestinal dysbiosis, including inflammatory bowel disease (IBD) and infectious diarrhea. In addition to intestinal health, probiotic bacteria may have effects on immune function and response to infection or vaccination [11]. This has been most clearly demonstrated in the case of diarrheal illness, such as *Clostridioides difficile* disease [12–14].

The promising effects of probiotics on gut dysbiosis and inflammation have been described in simian immunodeficiency

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virus (SIV)–infected macaques [15]. Colonic CD4+ T cells were reconstituted to near normal levels in the animals that received ART and Visbiome compared with ART alone, and a significantly greater number of antigen presenting cells could be measured. Probiotic products have also been studied in the setting of HIV infection, but with mixed results (reviewed in [16]). Given its promise in animal models, in AIDS Clinical Trials Group study A5350, we evaluated whether the probiotic Visbiome Extra Strength (ES) reduced measures of systemic inflammation in persons with well-controlled HIV on ART. In a substudy (A5352s), we obtained colonic biopsies and performed immunohistochemistry to evaluate gut pathology. We additionally performed lactulose mannitol testing to evaluate functional gut permeability before and after Visbiome ES treatment. We hypothesized that Visbiome ES would be safe and repair intestinal pathology and reduce gut microbial translocation and inflammation in PWH.

METHODS

Study Participants and Design

PWH at AIDS Clinical Trials Group sites in the United States, age >18 years, on stable ART, with CD4+ T-cell count >200 cells/mm^3 were eligible. History of inflammatory bowel disease, total colectomy, or chronic liver disease; recent or current use of antimicrobials, immunomodulatory or probiotic treatment (including probiotic yogurt), or active substance abuse interfering with study procedures were exclusionary. Participants were randomized 1:1 to Visbiome ES or placebo for 24 weeks starting at week 2 and followed for an additional 12 weeks off study product.

Patient Consent

Written informed consent was obtained from all participants before participation, and the human experimentation guidelines of the US Department of Health and Human Services were followed. The study was approved by institutional review boards at all participating sites (NCT02706717).

Collections

Blood samples were collected to measure markers of cellular activation, inflammation, gut damage, and bacterial translocation. Plasma concentrations of sCD14 were quantified using the human sCD14 enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems) per the manufacturer's instructions. Commercially available ELISA kits were used to determine plasma levels of interferon-inducible protein 10 (IP-10 or CXCL10) and D-dimer (Sekisui Diagnostics) and I-FABP (Hycult Biotech) according to the manufacturer's instructions. Duplicates of 20% of the samples were included in each ELISA plate. The plasma kynurenine-to-tryptophan (KT) ratio was determined using published techniques [17]. Glucose and insulin were batch-analyzed on stored plasma. Fasting lipid profiles were batch-analyzed on stored serum. Insulin resistance was estimated by the homeostasis model assessment–insulin resistance (HOMA-IR) [18]. Participants were provided with stool collection kits. In the substudy, colonic biopsies were collected by flexible sigmoidoscopy at baseline and week 24 to assess tissue-specific effects related to immunologic outcomes, inflammation, bacterial translocation, and gut integrity. A lactulose mannitol (LM) test for gut permeability was performed at baseline and week 26 [19, 20]. The methods used for blood testing for inflammatory and metabolic biomarkers, immunohistochemistry staining of the colonic biopsies, microbiome analysis, symptom and dietary questionnaires, and lactulose mannitol testing are provided in the Supplementary Methods. Safety assessments were performed at weeks 6, 14, 26, and 38.

Study Product

DuPont/Danisco manufactured Visbiome ES and matched placebo for Visbiome ES for Exegi Pharma (Rockville, MD, USA), who supplied the product. Visbiome ES contains 1 strain of *Streptococcus thermophiles*, 3 strains of *Bifidobacteria,* and 4 strains of *Lactobacilli* in defined ratios. Each sachet contains at least 900 billion lyophilized lactic acid bacteria. For weeks 2–4, participants were instructed to take 1 sachet orally daily. For weeks 4–26, participants were instructed to take 1 sachet orally twice daily.

Statistical Analysis

The primary outcome was change in sCD14 levels from baseline (average of entry and week 2) to week 26 (average of week 25 and week 26). Based on a prior study, a 0.07 $\log_{10}$ µg/Ll sCD14 difference was associated with a 23% decreased odds of a non-AIDS event or nonaccidental death at the pre-event time point [21], suggesting it would be a clinically significant reduction, and this guided the effect size for the study. With 45 participants per study arm, there was 90% power to detect this 0.07 $\log_{10}$ µg/L between-arm difference assuming an SD of 0.09 $\log_{10}$ µg/L, a 5% type 1 error, and 20% of participants with missing end points. Loss to follow-up in this study was higher in the placebo arm, but despite this, with 42 Visbiome ES participants and 31 placebo participants, we still had 89.97% power to detect this difference. The continuous secondary outcomes assessed changes over the 24-week treatment period and the 12-week post-treatment period. Unlike the primary sCD14 outcome, secondary outcomes did not utilize averaging at baseline and week 26.

For the subset of participants who completed paired colonic biopsies, the primary outcome was change in CD4+ T cells (median % positive staining) in colonic tissue over 24 weeks of treatment. With 20 participants per study arm, there was 90% power to detect a 20.1% between-arm difference in CD4+ T cells assuming an SD of 16.4%, a 5% type 1 error, and 20% of participants with missing end points.

To examine the biologic effects of Visbiome ES, we used per-protocol analyses, limited to participants on treatment through week 26, without confirmed virologic failure (2 consecutive ≥200 copies/mL of HIV-1 RNA) at or before week 26, who had primary outcome data (sCD14 for main study outcomes and quantifiable intestinal CD4+ T cells for substudy outcomes). Mean changes in main study outcomes were compared between arms using a 1-sample *t* test with equal variance. If data were highly skewed and $\log_{10}$ transformed, means were exponentiated to estimate geometric mean fold changes within arms and the percent difference in geometric mean fold changes between arms. Due to the sample size in the substudy, the Wilcoxon rank-sum test was used to compare treatment arms. Participants who received any study product were included in the safety analysis, which compared the proportion with adverse events between arms using the Fisher exact test. Absolute change was used for all continuous outcomes except for CD4+/CD8+ ratio and lactulose mannitol ratio (LMR), which used fold change. All statistical tests were 2-sided with a nominal alpha level of .05 and no adjustment for multiple testing.

RESULTS

Cohort Characteristics

Overall, 93 participants enrolled between April and December 2016 and completed follow-up in September 2017 per protocol: 46 placebo, 47 Visbiome ES; 86% natal male sex; 55% White, 42% Black or African American, 20% Hispanic/Latino ethinicity; median (Q1, Q3) age was 51 (45, 56) years, BMI (Q1, Q3) was 27.1 (24.2, 30.7) kg/m^2, CD4 count (Q1, Q3) was 712 (542, 893) cells/mm^3, and 99% had HIV-1 RNA <40 copies/mL; 1 participant had 48 copies/mL (Figure 1). Excluding 19 participants who did not complete study treatment and 1 virologic failure, 73 participants (31 placebo, 42 Visbiome ES) remained in the population. Of 42 participants

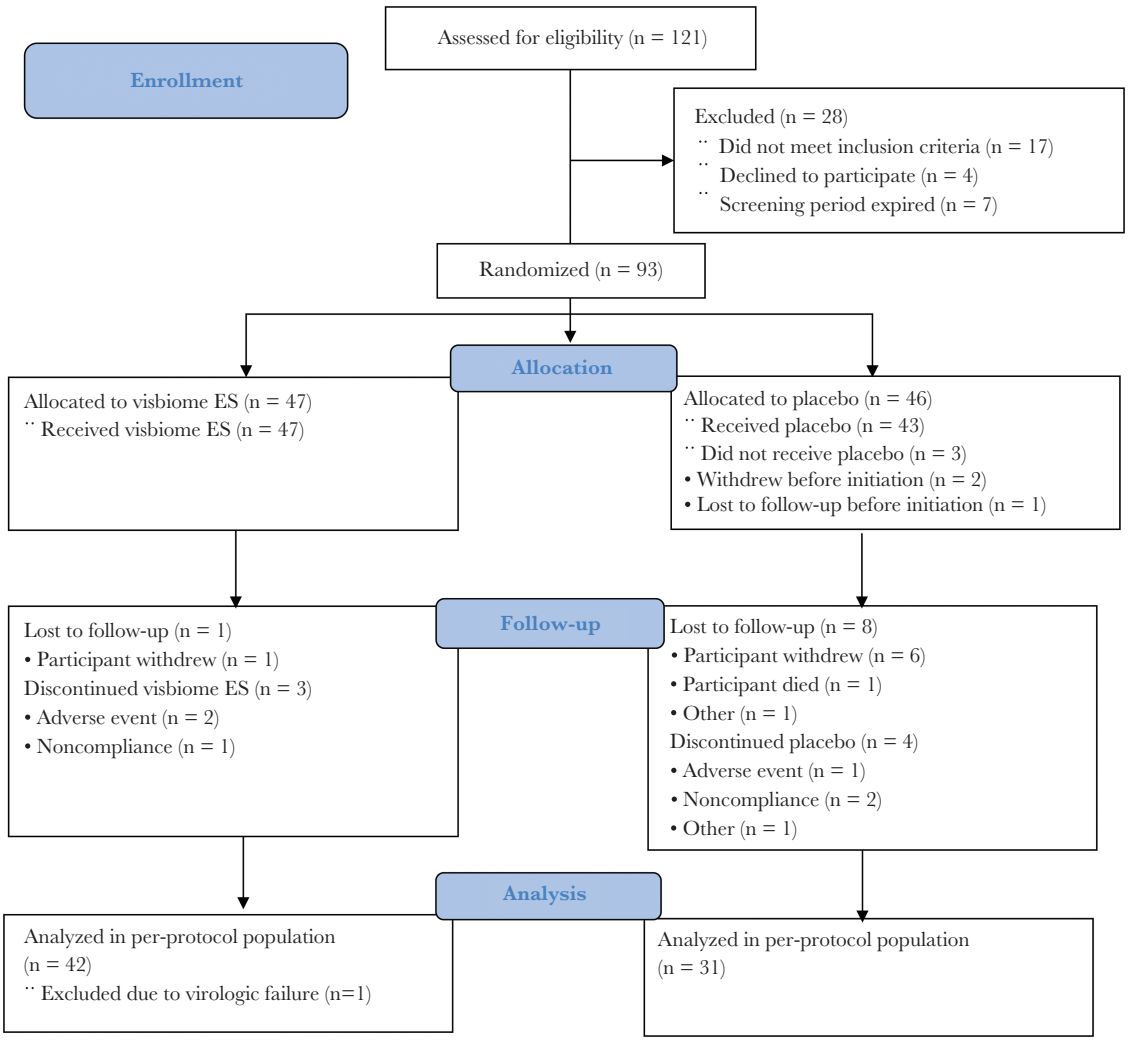

Figure 1. Trial flowchart. Abbreviation: ES, Extra Strength.

enrolled into A5352s, 29 had paired biopsy specimens for analysis. The median (Q1, Q3) age for the substudy per-protocol population was 50 (44, 56) years; 26 (90%) natal male sex; baseline CD4 count (Q1, Q3) was 718 (601, 925) cells/mm^3 (Table 1).

Effect on Biomarkers

After 24 weeks of treatment, Visbiome ES did not significantly reduce sCD14 compared with placebo; Δ = –51.3 (95% CI, –246 to 143.9) μg/L (P = .60), after $\log_{10}$-transforming Δ = –0.009 (95% CI, –0.043 to 0.025) $\log_{10}$ μg/L (P = .59) (Figure 2A). After $\log_{10}$-transforming d-dimer values, the geometric mean fold change from baseline to week 26 was 1.20 (95% CI, 0.97 to 1.50) in the Visbiome ES arm, indicating a 20% relative increase to the baseline level, and 28.4% (95% CI, –3.6% to 71.0%) greater than the placebo (P = .09) (Figure 2B). Mean fold changes in kynurenine-to-tryptophan (KT) ratio from baseline to week 26 were 1.04 (95% CI, 0.94 to 1.14) in the Visbiome ES arm and 1.0 (95% CI, 0.94 to 1.05) in the placebo arm. There was no evidence of a difference between the arms (Δ = 0.04; 95% CI, –0.09 to 0.17; P = .51). Similar results were seen for IP-10 (Supplementary Table 1).

The mean changes in circulating CD4 cell counts from baseline to week 26 were 10 (95% CI, –32 to 52) cells/mm^3 in the Visbiome ES arm and 43 (95% CI, –3 to 88) cells/mm^3 in the placebo arm, with a difference in mean changes of –33 (95% CI, –94 to 28) cells/mm^3 between arms (P = .29) (Figure 2C). Similarly, changes in CD4+/CD8+ ratio demonstrated no difference between arms (P = .41), with mean fold changes from baseline to week 26 of 1.03 (95% CI, 0.99 to 1.07) in the Visbiome ES arm and 1.05 (95% CI, 1.01 to 1.09) in the placebo arm. The difference in mean fold changes was –0.02 (95% CI, –0.08 to 0.03) (Figure 2D).

We did not identify differences in changes for most peripheral blood mononuclear cell flow cellular markers analyzed, except for the difference in percent expression of ($CD8^+$) $CD28^-CD57^+$ between arms in changes over 26 weeks, with a mean increase over placebo of 2.28% (95% CI, 0.07% to 4.48%; P = .043) (Supplementary Tables 2–4). Over the post-treatment follow-up period (weeks 26–38), decreases in percent expression of ($CD4^+$) $CD28^-CD57^+$ and ($CD20^-$) $CD27^+CD38^+$ in the Visbiome ES arm were statistically significantly greater from placebo (P = .042 and P = .012, respectively); no other markers were found to be statistically different in their changes over this period.

Effect on the Microbiome

No differences between groups or changes over the course of the study were seen in the microbial diversity as measured by Shannon diversity index nor richness as measured by the Chao1 Richness Index. Although not statistically significant, we detected an increase in both *Lactobacillus* and *Bifidobacterium* in participants on Visbiome ES, which decreased to baseline values after discontinuation of study product (Figure 3A, C). Geometric mean fold differences from baseline to week 26 between the Visbiome ES and placebo groups were demonstrated for *Lactobacillus* of +109.0% (95% CI, –62.6% to 1068.2%; P = .39) and *Bifidobacterium* of +199.5% (95% CI, –63.7% to 2373.4%; P = .30), with variability among participants (Figure 3B, D). Of other microbial communities, *Gammaproteobacteria*

Table 1. Baseline Characteristics of Main Study and Substudy Population

	Main Study			Substudy		
Characteristic	Overall (n = 93)	Visbiome ES (n = 47)	Placebo (n = 46)	Overall (n = 29)	Visbiome ES (n = 14)	Placebo (n = 15)
Age, y	51 (45, 56)	51 (47, 56)	52 (42, 56)	50 (44, 56)	50 (45, 56)	50 (42, 56)
Female sex	13 (14)	7 (15)	6 (13)	3 (10)	3 (21)	0 (0)
Race						
White Black/African American	50 (55) 38 (42)	24 (53) 21 (47)	26 (57) 17 (37)	13 (45) 15 (52)	6 (43) 8 (57)	7 (47) 7 (47)
Hispanic ethnicity	19 (20)	10 (21)	9 (20)	3 (10)	2 (14)	1 (7)
Body mass index, kg/m^2	27 (24, 31)	27 (25, 31)	26 (24, 30)	27 (24, 29)	27 (26, 29)	26 (24, 28)
Current smoker	19 (21)	9 (19)	10 (23)	8 (28)	5 (36)	3 (20)
Current ethanol use	60 (66)	29 (62)	31 (70)	20 (69)	10 (71)	10 (67)
CD4 count, c/mm^3	712 (542, 893)	702 (483, 866)	715 (546, 897)	718 (601, 925)	790 (601, 951)	712 (583, 897)
HIV RNA <40 copies/mL	92 (99)	47 (100)	45 (98)	29 (100)	14 (100)	15 (100)

Data are presented as median value (Q1, Q3) or No. (%).

Abbreviation: ES, Extra Strength.

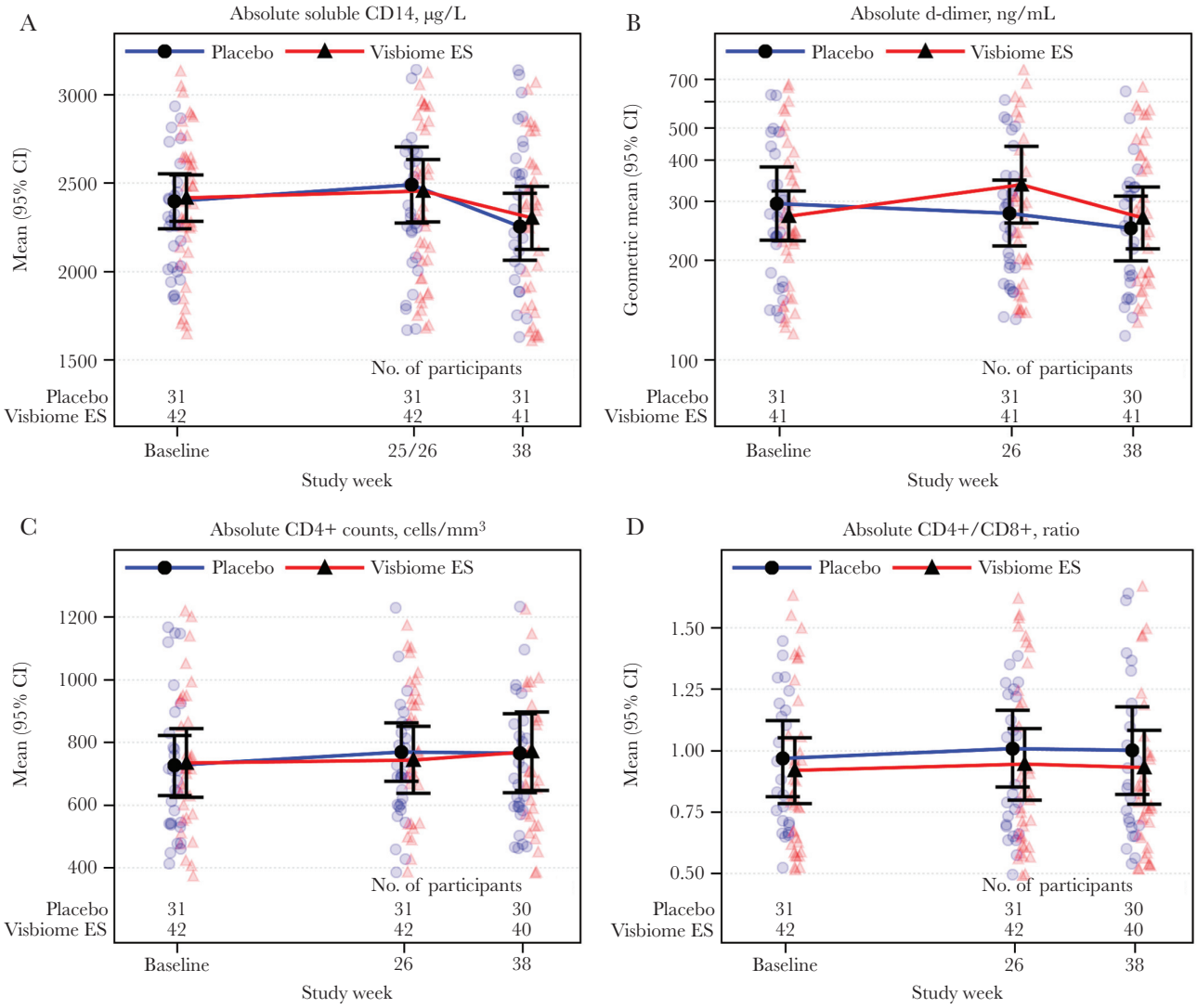

Figure 2. Effects of Visbiome ES on biomarkers and cell counts. Mean values with 95% CIs shown in black bars, individual participants in blue circles (placebo) or red triangles (Visbiome ES). Effects are shown at baseline, week 25/26, or week 38 for soluble CD14 (A), D-dimer (B), CD4+ T-cell count (C), or CD4+ T-cell/CD8+ T-cell ratio (D). Abbreviation: ES, Extra Strength.

demonstrated statistically significantly different (P = .044) geometric mean fold changes from baseline to week 26 between the Visbiome ES and placebo groups, with a percent difference of –76.9% (95% CI, –94.4% to –4.0%) (Figure 3E). As seen in Figure 3F, a distinguishable portion of individuals with a fold change of *Gammaproteobacteria* <1 were in the Visbiome ES group. No other changes or differences were seen in the rest of the microbial communities analyzed.

Diet

Participants completed 24-hour recall [22] at baseline and weeks 14, 26, and 38 using ASA24. We extracted the following Healthy Eating Index (HEI) measures [23]: added sugar, sodium, dairy, fatty acid ratio, saturated fats, whole fruit, total fruit, refined grains, whole grains, dark green vegetables and beans, total vegetables, protein foods, seafood and plant protein, and total score. Of all HEI measures, we saw no differences between arms over the 24 weeks of active study treatment. During the post-treatment follow-up period (weeks 26–38), participants reported differences in saturated fat intake, with a mean increase of 2.51 (95% CI, 0.02 to 5.00; P = .049). No other notable changes or differences were seen in the rest of the HEI measures analyzed. However, the diet of the participants was low for whole fruit, whole grains, and dark green vegetables and beans, with a total HEI score across the groups of 47, significantly lower than the average American score of 59, and far from the ideal score of 100 [23].

Effects on Metabolism

We did not observe any notable changes or differences between arms in fasting lipids (cholesterol, LDL, HDL, non-HDL

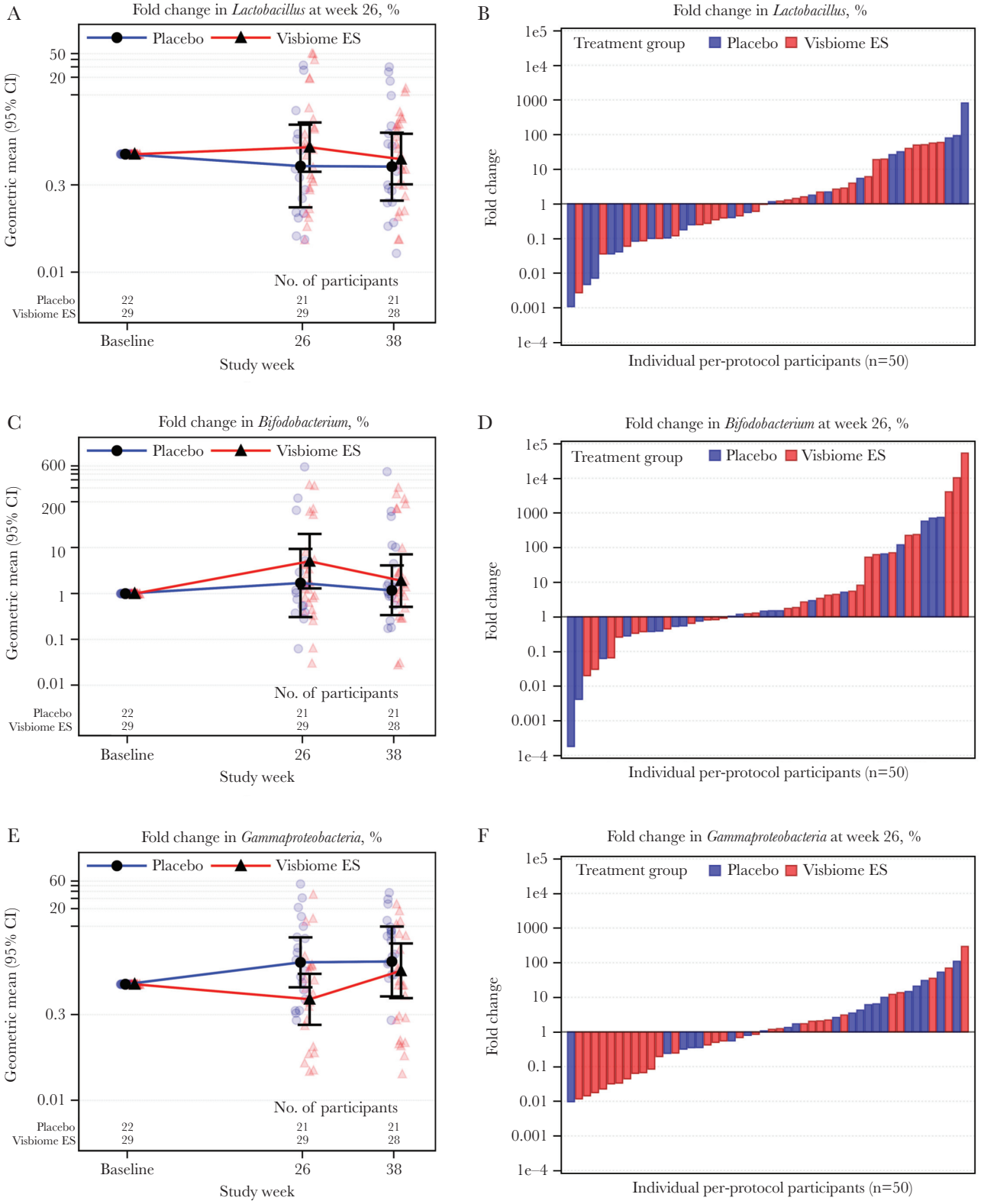

Figure 3. Effects on the microbiome. Geometric means with 95% CIs shown in black bars, individual participants in blue circles (placebo) or red triangles (Visbiome ES). Effects are shown for *Lactobacillus* (A), *Bifidobacterium* (C), and *Gammaproteobacteria* (E). The fold change in each genus is shown for each participant for *Lactobacillus* (B), *Bifidobacterium* (D), and *Gammaproteobacteria* (F). Participants in the placebo arm are shown in blue, and participants in the Visbiome ES arm are shown in red. Abbreviation: ES, Extra Strength.

cholesterol or triglycerides), which were measured at baseline and weeks 14 and 26. However, in exploratory analyses, we identified a statistically significant treatment group difference in fasting insulin and HOMA-IR fold changes from baseline to week 26, with a geometric mean HOMA-IR difference of –39.7% (95% CI, –59.0% to –11.3%; $P = .011$), although this was driven by an increase in the placebo arm (Supplementary Table 5).

Safety of Study Product

Overall, 25 participants (28%) reported at least 1 AE (8 [19%] placebo and 17 [36%] Visbiome ES; $P = .098$). Examining system organ classes where the difference in treatment arm proportions was >5%, 5 (11%) in Visbiome ES and 2 (5%) in placebo reported any gastrointestinal disorder; 4 (9%) in Visbiome ES and 0 (0%) in placebo reported a musculoskeletal and connective tissue disorder; and 4 (9%) in Visbiome ES and 1 (2%) in placebo reported a vascular disorder. Table 2 summarizes the adverse events and grades. One participant in the placebo arm discontinued study product due to an AE, while 2 discontinued due to an AE in the Visbiome ES arm. Three participants assigned to placebo never initiated study product. There was 1 death in the placebo arm due to herpes encephalitis that was not attributed to study drug.

Effect on Gastrointestinal Symptoms

Although gastrointestinal adverse events were more common in the Visbiome ES arm, gastrointestinal symptom scores did not reveal significant differences. At each visit, we performed a symptom questionnaire assessing 5 symptoms (passing gas, soft stools, excessive gas, hard stools, and watery stools) on a scale of 0 (not present) to 10 (very severe). The mean changes in the Visbiome ES arm ranged from –0.74 to –0.27, while in the placebo arm they ranged from –0.29 to 1.10. For all 5 symptom scores, the mean change in Visbiome ES was less than in the placebo arm. Notably, the largest and only statistically significant ($P = .002$) difference was for passing gas, which was the measure with the largest mean increase in placebo (1.10) and the largest mean decrease in Visbiome ES (–0.74).

Effect on the Gastrointestinal Tract

In the substudy with 42 participants enrolled, 29 with paired biopsy samples, there were no significant changes seen in CD4+, IL-17, or MPO staining, measures of CD4+ T cells, Th17 cells, and neutrophils. At baseline, the median % positive staining for CD4+ T cells was 2.0% in the Visbiome ES arm and 2.1% in the placebo arm. The median % CD4 decreased to 1.74% in the Visbiome ES arm and 1.65% in the placebo arm, with a median change of –0.21 for placebo and –0.03 for Visbiome ES ($P = .089$) (Supplementary Figure 1A). IL-17 staining was highly variable but demonstrated no significant change over 24 weeks ($P = .65$) (Supplementary Figure 1B). MPO minimally decreased in placebo from 0.18 to 0.11, with a median change of –0.04, while it increased in Visbiome ES from 0.14 to 0.18 for a median change of 0.05 over 24 weeks ($P = .081$) (Supplementary Figure 1C). Consistent with these findings, we demonstrated no significant changes in gut permeability as measured by LMR, although this may not have been the best measure of gut permeability [24]. At baseline, the overall median (Q1, Q3) ratio was 0.03 (0.02, 0.06). The median (Q1, Q3) fold change from baseline to week 26 was 0.66 (0.42, 1.35) in the Visbiome ES arm and 0.86 (0.74, 1.55) in the placebo arm. There was no evidence of a difference between the arms ($P = .35$) (Supplementary Figure 1D). In addition, we saw no significant change in circulating intestinal fatty acid binding protein (I-FABP), a marker of intestinal barrier dysfunction, which demonstrated a baseline mean of 311 (95% CI, 186.7 to 518) pg/mL. I-FABP decreased in both arms similarly over the course of the study to a mean of 211 (95% CI, 115 to 385) pg/mL [25].

Table 2. Adverse Events

	Visbiome ES (n = 47), No. (%)			Placebo (n = 43), No. (%)		
	Overall	Grade 1–2	Grade ≥3	Overall	Grade 1–2	Grade ≥3
Total[a]	17 (36)	10 (22)	7 (15)	8 (19)	4 (9)	4(9)
GI disorder	5 (11)	4 (8)	1 (2)	2 (5)	1 (2)	1 (2)
Infections & infestations	4 (9)	3 (6)	1 (2)	2 (5)	1 (2)	1 (2)
Musculoskeletal	4 (9)	3 (6)	1 (2)	0	0	0
Metabolic/nutritional	1 (2)	1 (2)	0	1 (2)	1 (2)	0
Neoplasm	1 (2)	0	1 (2)	1 (2)	1 (2)	0
Renal/urinary	1 (2)	1 (2)	0	0	0	0
Psychiatric	0	0	0	1 (2)	1 (2)	0
Respiratory/thoracic	2 (4)	1 (2)	1(2)	0	0	0
Skin/soft tissue	0	0	0	1 (2)	1 (2)	0
General disorders	1 (2)	1 (2)	0	1 (2)	1 (2)	0
Injury/poisoning/procedural	2 (4)	1 (2)	1(2)	2 (5)	0	2 (5)

Abbreviations: AE, adverse event; ES, Extra Strength; GI, gastrointestinal.

[a]Any AE: 17 (36%) vs 8 (19%); $P = .098$ by Fisher exact test.

DISCUSSION

Persistent microbial translocation and increased gastrointestinal permeability have been hypothesized to contribute to chronic inflammation, morbidity, and mortality in PWH [8]. We performed a prospective, randomized, placebo-controlled clinical trial to measure the effects of the probiotic Visbiome ES on markers of inflammation, coagulation, the microbiome, gastrointestinal structure and function, and metabolism. In a cohort of virologically suppressed PWH with high CD4 counts and minimal symptoms, we were unable to demonstrate a significant benefit of Visbiome ES administration.

Changes in several markers of systemic inflammation and coagulation, including sCD14, d-dimer, KT ratio, and IP-10, or in relevant measures of immune activation of lymphocytes and monocytes were not different between arms (Supplementary Data). There were also no significant effects on CD4+ T-cell count or CD4/CD8 ratio in this cohort. Baseline sCD14 levels were relatively low, and CD4+ T-cell counts and CD4/CD8 ratios (721 c/mm^3 and 0.93, respectively) near normal. These surrogate measures of immune function and inflammation indicated preserved or reconstituted immune function and presumably low systemic inflammation upon which the Visbiome ES could render a meaningful benefit. Data from the START trial suggest that PWH who initiate ART at CD4 counts >500 c/mm^3 and suppress HIV viremia have low measures of inflammation and very low incidence of non-AIDS comorbidities that are historically linked to excess inflammation [26].

We considered whether variable engraftment of the probiotic might have affected results, but when comparing participants within the Visbiome ES arm with (n = 16) and without (n = 13) increased *Lactobacillus* from baseline to week 26, no apparent difference in sCD14 changes was identified. One potential explanation for this finding is the high baseline CD4+ T-cell counts with consistent virologic suppression and generally immunologically healthy population studied. A second possibility may be poor dietary quality that did not facilitate engraftment of the probiotic over time. Alternatively, the probiotic used may not have been sufficient or appropriate to affect a meaningful change in the microbiome composition of these individuals [27]. Finally, there is also a possibility that gut dysbiosis and inflammation are consequences rather than causes of systemic inflammation. Despite this, a recent study in SIV-infected nonhuman primates suggested that altering the composition of the GI tract microbiome does not accelerate untreated SIV disease [28], suggesting that the influence of the composition of the microbiome may be more complex in its effects on HIV disease course.

As mentioned, dietary factors may have influenced the study outcomes. Our study population had a persistently low-fiber diet. The population of PWH living in the United States generally has a diet containing <20 g of fiber per day [29]. Recent studies highlight that dietary fiber intake strongly influences successful engraftment of probiotic bacteria, the duration of engraftment, and the effect on functional and clinical parameters [30–32]. The nonhuman primate study of Visbiome that demonstrated colonic CD4+ cell restoration also provided the soluble prebiotic fiber inulin [15]. An unsuitable dietary nutrient composition may prohibit engraftment or the ability of microbes to produce metabolites, such as short-chain fatty acids, that improve health and anti-inflammatory outcomes [33–35]. We did not identify any apparent association within participants in the Visbiome ES arm between baseline total HEI and fold changes in *Lactobacillus* or *Bifidobacterium* communities.

In conclusion, we present data from a well-powered, randomized, placebo-controlled intervention of probiotics in healthy PWH on ART. The study product was generally safe and well tolerated and did appear to shift the microbiome, but the study did not demonstrate any significant benefit on inflammation or gut permeability or translocation in this population, although there may have been a benefit of preserved insulin sensitivity. In an era in which PWH are aging and current ART is associated with an increase in weight gain and potential loss of insulin sensitivity [35, 36], dietary interventions may be useful to ameliorate these consequences. A dietary intervention or combined probiotic/prebiotic intervention, such as the prebiotic/probiotic combination used in animal models [15], might result in better engraftment and demonstrate biologic efficacy if utilized in a population with more significant gastrointestinal pathology.

Supplementary Data

Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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