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A dietary intervention for postmenopausal hot flashes: A potential role of gut microbiome. An exploratory analysis☆

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ABSTRACT

Objective: This study examined the role of gut microbiome changes in mediating the effects of a dietary intervention on the frequency and severity of postmenopausal vasomotor symptoms

Methods: Postmenopausal women (n = 84) reporting ≥2 moderate-to-severe hot flashes daily were randomly assigned, in 2 successive cohorts, to an intervention including a low-fat, vegan diet and cooked soybeans (½ cup [86 g] daily) or to stay on their usual diet. Over a 12-week period, frequency and severity of hot flashes were recorded with a mobile application. In a subset of 11 women, gut microbiome was analyzed at baseline and after 12 weeks of the dietary intervention (low-fat vegan diet with soybeans), using deep shotgun metagenomic sequencing. Differences in the microbiome between baseline and 12 weeks were assessed by comparing alpha diversity with Wilcoxon signed rank tests, beta diversity with permanovaFL, and taxon abundance with Wilcoxon signed rank tests. Pearson correlations were used to assess the association between changes in hot flashes and gut bacteria.

Results: In the subset for which microbiome testing was done, total hot flashes decreased by 95 % during the dietary intervention (p = 0.007); severe hot flashes disappeared (from 0.6 to 0.0/day; p = 0.06); and moderate-to-severe hot flashes decreased by 96 % (p = 0.01). Daytime and nighttime hot flashes were reduced by 96 % (p = 0.01) and 94 % (p = 0.004), respectively. Alpha and beta diversity did not significantly differ in the intervention group between baseline and 12 weeks. Two families (Enterobacteriaceae and Veillonellaceae), 5 genera (*Erysipelatoclostridium, Fusicatenibacter, Holdemanella, Intestinimonas*, and *Porphyromonas*), and 6 species (*Clostridium asparagiforme, Clostridium innocuum, Bacteroides thetaiotaomicron, Fusicatenibacter saccharivorans, Intestinimonas butyriciproducens, Prevotella corporis*, and *Streptococcus sp.*) were differentially abundant, but after correction for multiple comparisons, these differences were no longer significant. Changes in the relative abundance of *Porphyromonas* and *Prevotella corporis* were associated with the reduction in severe day hot flashes both unadjusted (r = 0.61; p = 0.047; and r = 0.69; p = 0.02), respectively), and after adjustment for changes in body mass index (r = 0.63; p = 0.049; and r = 0.73; p = 0.02), respectively). Changes in relative abundance of *Clostridium asparagiforme* were associated with the reduction in total severe hot flashes (r = 0.69; p = 0.019) and severe night hot flashes (r = 0.82; p = 0.002) and the latter association remained significant after adjustment for changes in body mass index (r = 0.75; p = 0.01).

Conclusions: This exploratory analysis revealed potential associations between changes in vasomotor symptoms in response to a diet change and changes in the gut microbiome. Larger randomized clinical trials are needed to investigate these findings.

☆ Trial registration: ClinicalTrials.gov, NCT04587154, registered on Oct 14, 2020.

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1. Introduction

Postmenopausal vasomotor symptoms not only lead to discomfort and reduced quality of life but also signal an increased risk of cardiovascular disease [1] and diabetes.[2] Nutrition is an important modifying factor and may be helpful in alleviating vasomotor symptoms. The Women's Health Initiative used a low-fat diet rich in whole grains, fruits, and vegetables, which increased the chances of women to become free of hot flashes at one year by 14 % after adjustments for changes in body weight, and by 23 % in women who lost at least 10 % of body weight or more.[9]

The positive effects of a plant-based diet on postmenopausal vasomotor symptoms may be mediated in part by changes in gut microbiome composition, possibly by enhancing the growth of gut bacteria converting daidzein to equol, an estrogen receptor-β agonist, as people following a plant-based diet are more likely to produce equol compared with omnivores.[3,4]

In a previously published 12-week randomized clinical trial, a low-fat plant-based diet, including a daily consumption of soybeans, reduced the frequency of moderate-to-severe postmenopausal hot flashes by 88 %, and freed 50 % of the participants of all moderate-to-severe hot flashes.[5] The aim of this secondary analysis was to test the effects of a low-fat plant-based diet, including daily consumption of soybeans, on the gut microbiome composition, particularly the equol-producing gut bacteria, and their association with changes in postmenopausal vasomotor symptoms.

2. Methods

The methods have been described in detail previously.[5] Briefly, postmenopausal women 40–65 years old reporting at least 2 moderate-to-severe hot flashes per day were recruited through social media and screened by telephone. Volunteers who met the eligibility criteria, were assigned to the intervention group or control group using a computer-generated sequence. Assignment was done simultaneously, therefore allocation concealment was unnecessary. Informed consent was obtained from the participants prior to study commencement. The study was approved by the Advarra Institutional Review Board on September 2, 2020 (Pro00045315), and registered at ClinicalTrials.gov, NCT04587154, on October 14, 2020.

The main outcome was the frequency and intensity of moderate-to-severe hot flashes, which were monitored using a mobile application. In a subset of 11 participants, a gut microbiome analysis was performed at baseline and after 12 weeks of a dietary intervention. The intervention group was asked to follow a low-fat plant-based diet, including ½ cup (86 g) of cooked soybeans per day, and attended weekly classes with cooking demonstrations, while the control participants maintained their usual diets. Dietary adherence was assessed weekly, and a detailed dietary assessment was based on the analysis of 3-day dietary records. Conversely, participants in the control group were asked to continue their usual diets. Participants in both groups were provided with a vitamin B_{12} supplement (100 μg), were asked to keep their medications and physical activity constant, and alcoholic beverages were limited to one per day.

2.1. Gut microbiome analysis

The stool samples were collected, using home collection kits, and submitted to Diversigen (New Brighton, MN, USA), a laboratory accredited by the College of American Pathologists. Deep shotgun metagenomic sequencing was performed, which provides information on DNA from all bacteria in a sample. Sequence data for each sample was then processed and annotated to Diversigen's Venti database, and a Core Analysis report was generated. Alpha diversity metrics were calculated using the Shannon index, the Chao1 index and Observed OTUs, from data rarefied to the sample with the fewest mapped reads. To assess differential abundance of taxa between baseline and week 12, both an untargeted and a targeted approach were utilized. The targeted approach tested a specific group of equol-producing taxa at the genus, species, and strain level. The untargeted approach tested all taxa for differential abundance between time points at the family, genus, and species levels.

2.2. Statistical analysis

The power analysis and descriptive statistics have been described previously.[5] As distributions of the clinical outcomes did not substantially depart from approximate normality, t-tests were used to assess the difference from baseline to week 12. Alpha diversity was assessed in the intervention group for differences between time points using Wilcoxon signed-rank tests, which account for the paired nature of samples. Bray-Curtis dissimilarity metrics were calculated between every sample pair from rarefied data. Testing for compositional differences in the intervention group between baseline and 12 weeks was accomplished using *permanovaFL* from the R package LDM [6] on the Bray-Curtis dissimilarity matrix, accounting for repeated measures by setting *cluster.ID* to SubjectID. To assess differential abundance of taxa between baseline and week 12, both an untargeted and a targeted approach were utilized. However, tests could only be performed if the species or strains were present in the data set. The only equol-producing taxon present at the strain level was *Eggerthella* sp. YY7918_YY7918. Species present included *Bifidobacterium breve*, *Bifidobacterium longum*, *Adlercreutzia equolifaciens*, and *Enterorhabdus mucosicola*. Genera present included *Bifidobacterium, Adlercreutzia, Eggerthella, Enterorhabdus, Slackia, Lactobacillus, Lactococcus, [Clostridium],* and *Catenibacterium.* To account for the compositional nature of microbiome data, count abundances were transformed using the centered log-ratio transformation, and differential abundance testing was performed using Wilcoxon signed-rank tests in the R package *ALDEx2*.[7] The relationship between changes in hot flashes and changes in relative abundance of gut bacteria was tested using Pearson correlations, first unadjusted and then partial correlations, adjusted for changes in body mass index.

3. Results

Of 1662 women interested in the study, 361 were screened by telephone, 84 participants were randomly assigned to the 2 study groups, and 71 participants completed the study (Supplemental Fig. 1). Eleven participants submitted a stool sample at baseline and after 12 weeks of a dietary intervention for a gut microbiome analysis. In this subset of 11 participants (see their baseline characteristics in Table 1), total hot flashes decreased by 95 % during the dietary intervention ($p = 0.007$); severe hot flashes disappeared ($p = 0.06$); and moderate-to-severe hot flashes decreased by 96 % ($p = 0.01$). Daytime and nighttime hot flashes were reduced by 96 % ($p = 0.01$) and 94 % ($p = 0.004$), respectively (Table 2). On average, the participants lost 6.4 lbs (95 % CI –9.5 to –3.3; $p < 0.001$). The average body mass index decreased from 27.2 to 26.1 kg/m^2 ($p = 0.001$).

3.1. Gut microbiome

No significant change during the dietary intervention was observed in alpha ($p = 0.62$ for Observed OTUs; $p = 0.638$ for Chao1; $p = 0.966$ for Shannon) or beta ($p = 0.5$) diversity between baseline and week 12. None of the targeted taxa were found to be significantly differentially abundant between baseline and week 12, and the hypothesis was therefore rejected. The untargeted analysis identified 2 families (Enterobacteriaceae and Veillonellaceae), 5 genera (*Erysipelatoclostridium, Fusicatenibacter, Holdemanella, Intestinimonas*, and *Porphyromonas*), and 6 species (*Clostridium asparagiforme, Clostridium innocuum, Bacteroides thetaiotaomicron, Fusicatenibacter saccharivorans, Intestinimonas butyriciproducens, Prevotella corporis*, and *Streptococcus sp.*) that were significantly differentially abundant at week 12 from baseline before FDR correction:

At the family level, the relative abundance of Enterobacteriaceae and Veillonellaceae increased (p = 0.03 and p = 0.01, respectively; Fig. 1). At the genus level, the relative abundance of *Erysipelatoclostridium*, *Fusicatenibacter*, and *Holdemanella* increased (p = 0.04; p = 0.04; and p = 0.03, respectively), while *Intestinimonas* and *Porphyromonas* decreased (p = 0.02 and p = 0.01, respectively; Fig. 2). Finally, at the species level, the relative abundance of *Clostridium innocuum* and *Fusicatenibacter saccharivorans* increased (p = 0.02 and p = 0.03, respectively), while *Clostridium asparagiforme*, *Bacteroides thetaiotaomicron*, *Intestinimonas butyriciproducens*, and *Prevotella corporis* decreased (p = 0.004; p = 0.0499; p = 0.04; and p = 0.0476, respectively; Fig. 3). However, after correction for multiple comparisons, the changes were no longer statistically significant.

The reductions in the relative abundance of *Porphyromonas* and *Prevotella corporis* were associated with a decrease in severe day hot flashes both before (r = 0.61; p = 0.047; and r = 0.69; p = 0.02), respectively), and after adjustment for changes in body mass index (r = 0.63; p = 0.049; and r = 0.73; p = 0.02), respectively). Changes in *Clostridium asparagiforme* were associated with the reduction in total severe hot flashes (r = 0.69; p = 0.019) and severe night hot flashes (r = 0.82; p = 0.002). The latter association remained significant after adjustment for changes in body mass index (r = 0.75; p = 0.01). After correction for multiple comparisons, these associations were no longer significant.

Table 1
Baseline Characteristics of the Gut Microbiome Completers (n = 11). The variables are presented as mean with a standard deviation (SD), or as a count with percent values.

	Specification	Gut Microbiome Completers (n = 11)
Mean age, years (SD)		53.2 (3.3)
Age range		49–58
Body mass index in kg/m^2, mean (SD)		27.2 (4.3)
Years since menopause, mean (SD)		3.7 (2.4)
Race and ethnicity		
	Black, non-Hispanic	2 (18 %)
	Asian, Pacific Islander	0 (0 %)
	White, non-Hispanic	9 (82 %)
	White, Hispanic	0 (0 %)
	Native American, non-Hispanic	0 (0 %)
Marital status		
	Single	3 (27 %)
	Married	8 (73 %)
Education		
	High school, partial or graduate	0 (0 %)
	College, partial or graduate	8 (73 %)
	Graduate degree	3 (27 %)
Medication use		
	Antihypertensives	0 (0 %)
	Cholesterol-lowering medications	0 (0 %)
	Thyroid medications	1 (9 %)

4. Discussion

The dietary intervention led to a 95 % reduction in total hot flashes, and a 96 % decrease in moderate-to-severe hot flashes. Daytime and nighttime hot flashes were reduced by 96 % and 94 %, respectively. Changes in gut microbiome in the intervention group from baseline to 12 weeks included increases in the relative abundance of *Clostridium innocuum* and *Fusicatenibacter saccharivorans* and decreases in the relative abundance of *Clostridium asparagiforme*, *Bacteroides thetaiotaomicron*, *Intestinimonas butyriciproducens*, and *Prevotella corporis* at the species level, increases in *Erysipelatoclostridium*, *Fusicatenibacter*, and *Holdemanella* and decreases in *Intestinimonas* and *Porphyromonas* at the genus level, and increases in *Enterobacteriaceae* and Veillonellaceae at the family level. Changes in the equol-producing bacteria were not significant, and the hypothesis was therefore rejected. Changes in the relative abundance of *Porphyromonas* and *Prevotella corporis* were associated with the reduction in severe day hot flashes, and changes in *Clostridium asparagiforme* were associated with the reduction in total severe hot flashes and severe night hot flashes. All associations remained of the same magnitude and significance after adjustment for changes in body mass index.

The relative abundance of *Porphyomonas* decreased on the intervention diet, and its decrease was associated with the reduction in severe day hot flashes. To our best knowledge, this is a novel finding that has not been previously described. The relative abundance of *Prevotella corporis* decreased during the dietary intervention, and its decrease was associated with the reduction in severe day hot flashes. *Prevotella corporis* has been found in the gut of people with rheumatoid arthritis and seems to have pro-inflammatory properties.[8] It may also be isolated during dental procedures such as root canals,[9] and plays a significant role in dental cavities.[10] Notably, a 2018 study found oral microbiota significantly different in vegans when compared to omnivores, including lower abundance of *Prevotella*.[11] The reductions in the relative

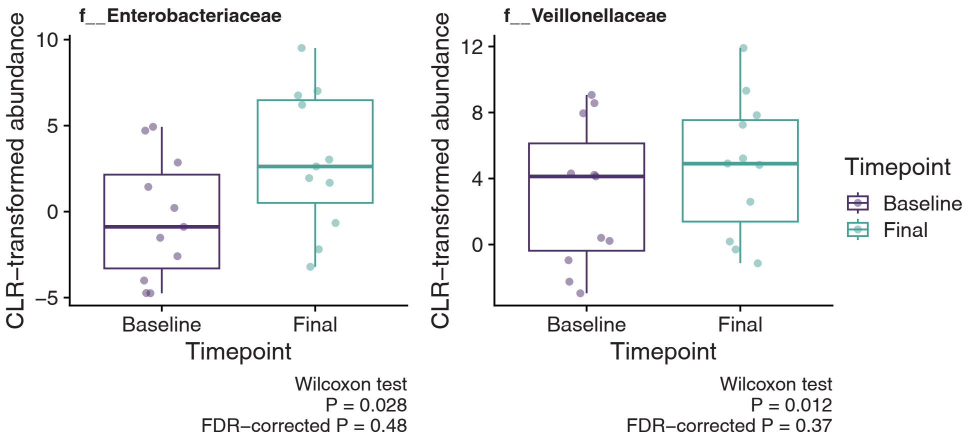

Fig. 1. Changes in gut microbiome in response to the low-fat vegan dietary intervention with soybeans at the family level. The increases in the relative abundance of *Enterobacteriaceae* (p = 0.03) and *Veillonellaceae* (p = 0.01), assessed by Wilcoxon signed-rank tests, were no longer significant after correction for multiple comparisons.

Table 2
Hot Flash Reports at Baseline and 12 Weeks of a Low-Fat Vegan Diet with Soybeans in 11 Study Completers.

Hot flashes reports	Week 0	Week 12	Change	P-value
Total Hot Flashes	6.2 (2.4–10.0)	0.3 (0.0–0.9)	–5.9 (–9.7 to –2.0)	0.007
Severe Hot Flashes	0.6 (0.0–1.2)	0.0 (0.0–0.1)	–0.6 (–1.1 to +0.0)	0.06
Mod-Severe Hot Flashes	5.4 (1.5–9.2)	0.2 (0.0–0.4)	–5.2 (–9.1 to –1.4)	0.01
Daytime Hot Flashes	4.6 (1.5–7.7)	0.3 (0.0–0.6)	–4.4 (–7.5 to –1.3)	0.01
Daytime Severe Hot Flashes	0.3 (0.0–0.6)	0.0 (0.0–0.1)	–0.3 (–0.6 to +0.0)	0.07
Daytime Hot Flashes Mod-Severe	3.9 (0.8–7.0)	0.1 (0.0–0.3)	–3.8 (–6.9 to –0.7)	0.02
Nighttime Hot Flashes	1.6 (0.7–2.5)	0.1 (0.0–0.2)	–1.5 (–2.4 to –0.6)	0.004
Nighttime Severe Hot Flashes	0.3 (0.0–0.6)	0.0 (0.0–0.0)	–0.3 (–0.6 to +0.0)	0.08
Nighttime Hot Flashes Mod-Severe	1.5 (0.5–2.4)	0.0 (0.0–0.1)	–1.4 (–2.4 to –0.5)	0.006

abundance of *Porphyomonas* and *Prevoltella corporis* and their relationships with the frequency of vasomotor symptoms are novel findings.

The relative abundance of *Clostridium asparagiforme* decreased on the low-fat vegan diet, and its decrease was associated with a reduction in total severe and severe night hot flashes.

A decrease in *Clostridium asparagiforme* may be a positive finding in light of previous studies. It has been found to produce trimethylamine-N-oxide,[12,13] a proatherogenic metabolite, which is associated with an increased risk of cardiovascular disease[14] and diabetes.[15] Although the association with hot flashes is a novel finding, decreased abundance of *Clostridium asparagiforme* may partially explain the beneficial effects of a plant-based diet on cardiovascular health [16] and provide a possible link between hot flashes and incident cardiovascular disease.

The increase in the relative abundance of *Erysipelatoclostridium, Fusicatenibacter,* and *Holdemanella* is in accordance with previous studies. *Erysipelatoclostridium* is a commensal bacterial genus that was found to have greater abundance in women using hormonal birth control during the late follicular phase of the ovarian cycle, when a sharp rise in estradiol would otherwise occur.[17] Hormonal birth control functions by maintaining a low level of estrogen, preventing fluctuations in hormones throughout the ovarian cycle. In our study, soybeans provided isoflavones that have selective affinity for the estrogen receptor-β and.

might therefore have a similar stabilization effect on estrogen levels, and thereby help alleviate postmenopausal hot flashes.

Fusicatenibacter is typically associated with gut health and has been demonstrated to induce anti-inflammatory cytokines and reduce serum C reactive protein.[18] Traumatic acid, an antioxidant found in bean plants and related to injury, is also positively correlated with *Fusicatenibacter* abundance.[18,19] *Fusicatenibacter* can also be modulated by prebiotics, specifically supplementation with guar gum,[20] which has been demonstrated to improve symptoms of menopause.[21] *Fusicatenibacter* may also result in mediation of the neurological triggers of hot flashes. Fiber-fermenting bacteria like the *Fusicatenibacter* genus are capable of producing short chain fatty acids, which have anti-inflammatory effects

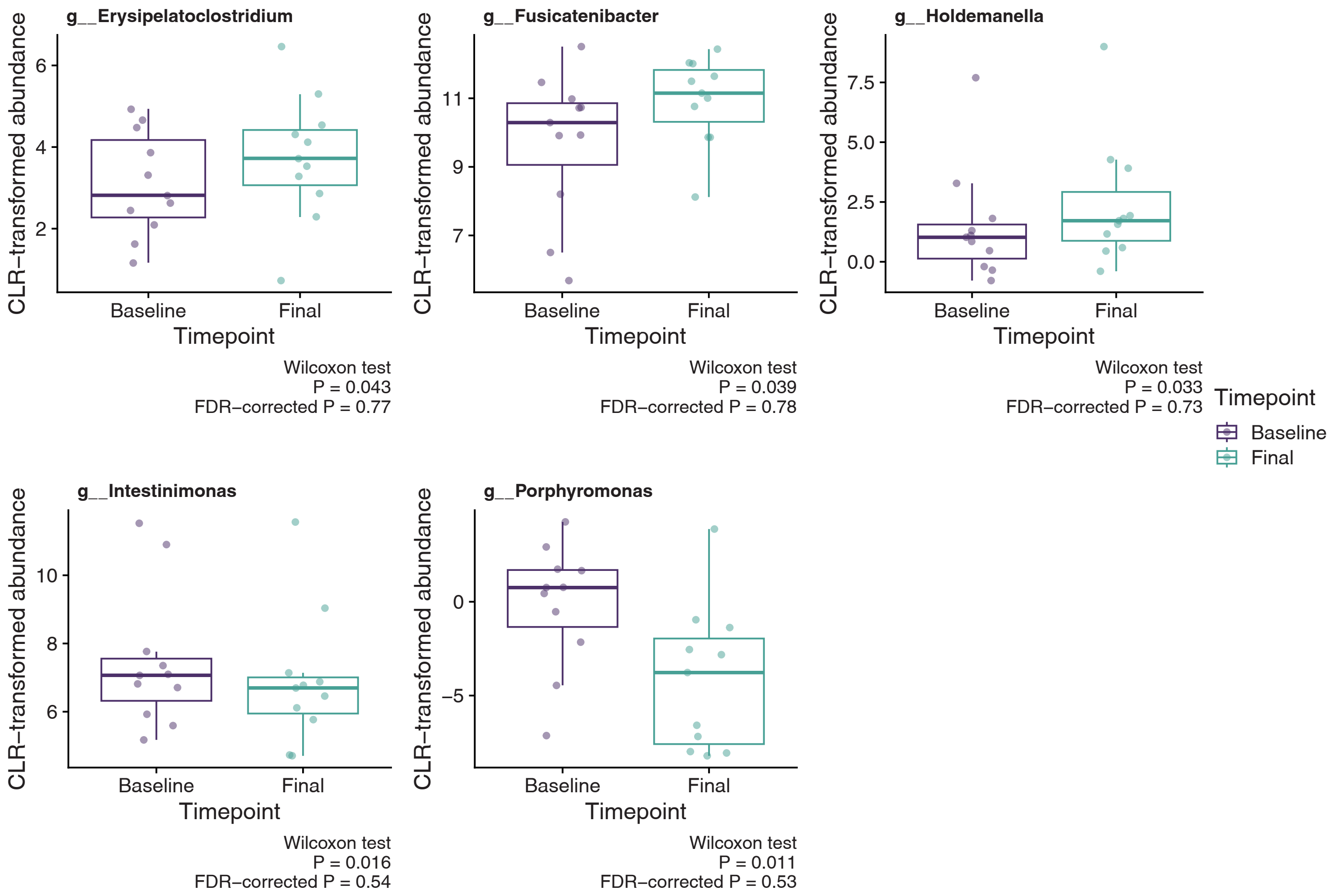

Fig. 2. Changes in gut microbiome in response to the low-fat vegan dietary intervention with soybeans at the genera level. The changes in the relative abundance of *Erysipelatoclostridium* (p = 0.04), *Fusicatenibacter* (p = 0.04), *Holdemanella* (p = 0.03), *Intestinimonas* (p = 0.02), and *Porphyromonas* (p = 0.01), assessed by Wilcoxon signed-rank tests, were no longer significant after correction for multiple comparisons.

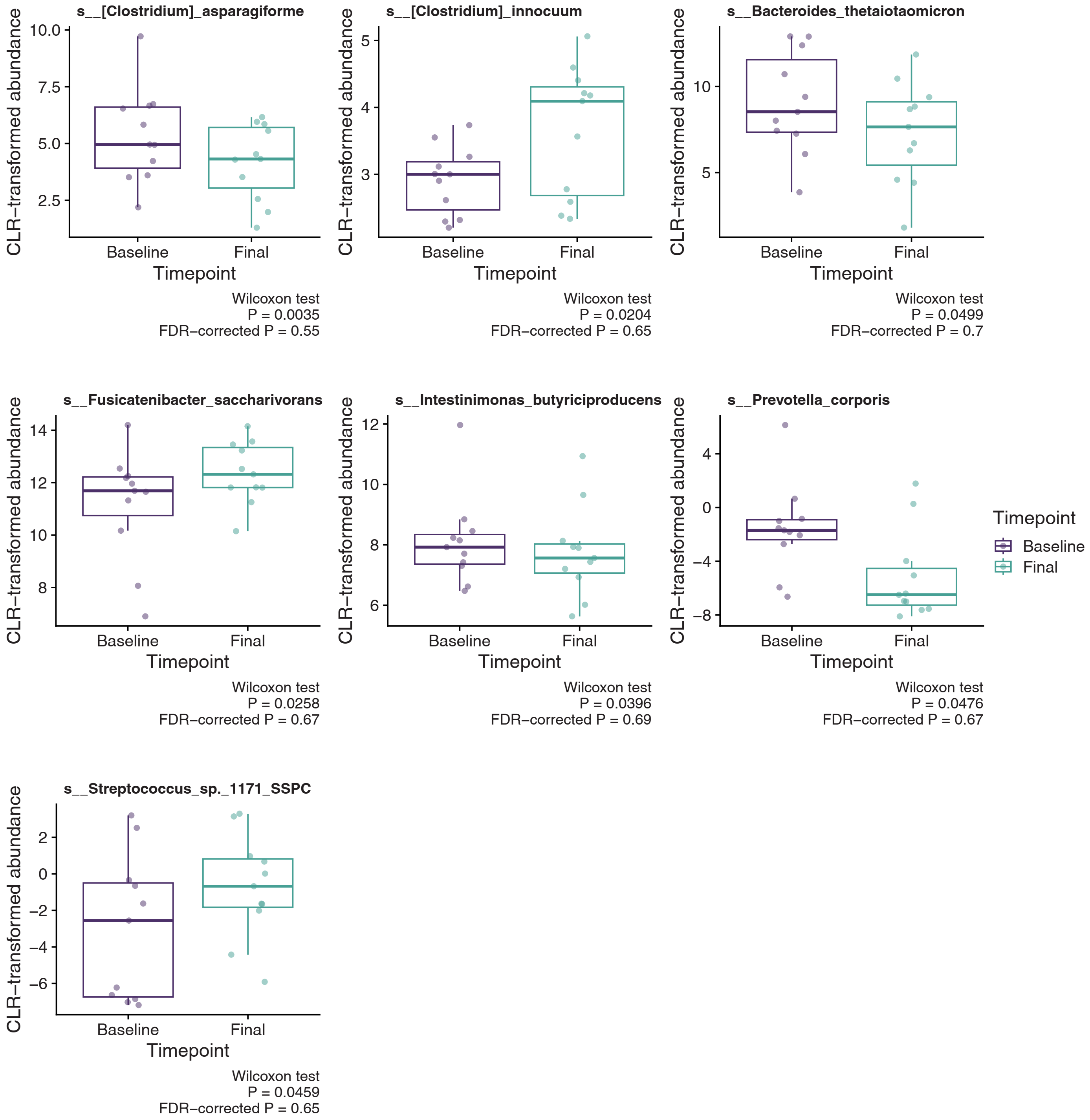

Fig. 3. Changes in gut microbiome in response to the low-fat vegan dietary intervention with soybeans at the species level. The changes in the relative abundance of *Clostridium asparagiforme* (p = 0.004), *Clostridium innocuum* (p = 0.02)*, Bacteroides thetaiotaomicron* (p = 0.0499)*, Fusicatenibacter saccharivorans* (p = 0.03)*, Intestinimonas butyriciproducens* (p = 0.04)*, Prevotella corporis* (p = 0.0476), and *Streptococcus sp.*(p = 0.0459), assessed by Wilcoxon signed-rank tests, were no longer significant after correction for multiple comparisons.

that may play a role in preventing degenerative neurological disease.[22] In fact, *Fusicatenibacter* is a predictor for Parkinson's disease, and decreased abundance correlates with disease progression.[23] Both Parkinson's disease and postmenopausal hot flashes involve dysfunction of the autonomic nervous system, implicating *Fusicatenibacter* as a possible beneficial agent in the gut-brain axis. Bacterial species *Fusicatenibacter saccharivorans,* which also increased in the present study, is an anaerobic sugar fermenter[24,25] and may be associated with increased plant polysaccharide breakdown from a plant-based diet.[25,26] Increased levels of *Fusicatenibacter saccharivorans* are found to be negatively associated with intestinal inflammation and may be a treatment option for ulcerative colitis.[27] The observed increase in *Fusicatenibacter saccharivorans* may therefore indicate a decrease in intestinal inflammation on a plant-based diet, which may have helped in reducing hot flashes in this study.

The *Holdemanella* genus increased in abundance on a low fat, plant-based diet. This is consistent with existing studies that demonstrate increased abundance with a high fiber diet.[28,29] Fibrous foods are typically associated with greater satiety which can lead to weight loss, and *Holdemanella* could play a previously unknown role in the gut-brain axis. There is some evidence that *Holdemanella* correlates with the alleviation of food-addiction behaviors.[30] Further evidence for the role of *Holdemanella* in weight loss includes an inverse association with android fat ratio in females.[31] This could explain the alleviation of hot flashes in our study, as metabolic syndrome is characterized by increased android fat and has been linked to greater frequency of vasomotor symptoms.[32]

The relative abundance of *Intestinimonas* decreased. *Intestinimonas* species are known to ferment fiber and produce butyrate[33] and to convert fructolysine, an advanced glycation end-product, to butyrate.[34,35] Recent evidence implicated the role of advanced glycation end-products in the pathogenesis of atherosclerosis. Thus, *Intestimonas* may play a protective role by clearing the advanced glycation end--products.[36] Since plant foods are generally lower in advanced glycation end-products than animal foods,[37] their lower consumption may have contributed to a decrease of *Intestinimonas*, as well as an improvement in hot flashes.[38]

The abundance of Veillonellaceae increased, which is in accordance with previous studies. Veillonellaceae is a family of gram-negative, anaerobic bacteria that produce propionate and acetate.[39] Its abundance increased in a study of healthy adult men after following a high-fiber diet.[40] As a plant-based diet used in the current study was also rich in fiber, a greater abundance of Veillonellaceae was an expected result.

On the other hand, the increase in Enterobacteriaceae is a conflicting finding. While supplementation with probiotics [41] and weight loss after gastric bypass surgery resulted in an increased abundance of Enterobacteriaceae,[42] a cross-sectional study demonstrated that people consuming a vegan diet had lower abundance of Enterobacteriaceae than omnivores,[43] partly due to the greater presence of butyrate-producing bacteria in people on a higher fiber diet Butyrate-producing bacteria lead to a lower colonic pH, which is not conducive to Enterobacteriaceae growth.[44] However, since some foods, such as arugula, bean sprouts, and spinach, contain naturally high amounts of Enterobacteriaceae,[45,46] they may have contributed to the increase in Enterobacteriaceae in the participants consuming a vegan diet in the present study.

The relative abundance of Streptococcus spp. increased during the dietary intervention. Most Streptococcus spp. are commensal species in the human gut, which means that they provide a defense against colonization of opportunistic pathogens and contribute to stimulation of the immune system.[47] A few of the species, such as *Streptococcus thermophilus*, produce lactic acid in the gut and are considered beneficial.[48]

Although this was an exploratory analysis, the 12-week period was sufficient to allow time for adaptations in gut microbiome. The targeted and untargeted gut microbiome analysis was robust and revealed important changes in the gut microbiome composition in response to a low-fat vegan diet and large correlations with symptomatic changes.

The study also has important limitations. The study sample was small, and because not everyone is an equol producer, this may be the main reason for not detecting any significant changes in equol-producing bacteria. Furthermore, the only equol-producing taxon present at the strain level was Eggerthella sp., which further limits our ability to fully test the hypothesis.

Although the study was designed as a randomized clinical trial, the gut microbiome analysis was only performed in a sub-set of participants undergoing the dietary intervention, and a control group for this analysis was absent. Any conclusions from this analysis must be viewed cautiously due to multiple comparisons. While associations of strong magnitude were noted between several gut bacteria and changes in hot flash frequency, and nominally statistically significant relative abundance changes were observed, robust statistical significance cannot be concluded for any of our reported gut microbiome assessments when the modestly large number of total comparisons is taken into account. Larger trials are necessary to further investigate the findings in this report.

In conclusion, this exploratory analysis revealed potential associations between changes in vasomotor symptoms in response to a diet change and changes in the gut microbiome.

Changes in the relative abundance of *Porphyromonas*, *Prevotella corporis,* and *Clostridium asparagiforme* were associated with the reduction in severe hot flashes. However, after the correction for multiple comparisons, these associations were no longer significant.

Larger randomized clinical trials are needed to further investigate these findings.

Funding

The study was funded by the Physicians Committee for Responsible Medicine.

Ethical statement

The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). The study was approved by the Advarra Institutional Review Board on September 2, 2020 (Pro00045315). Informed consent was obtained from the participants prior to study commencement.

Declaration of Competing Interest

The authors declare no conflict of interest related to this manuscript.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ctim.2023.103002.

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