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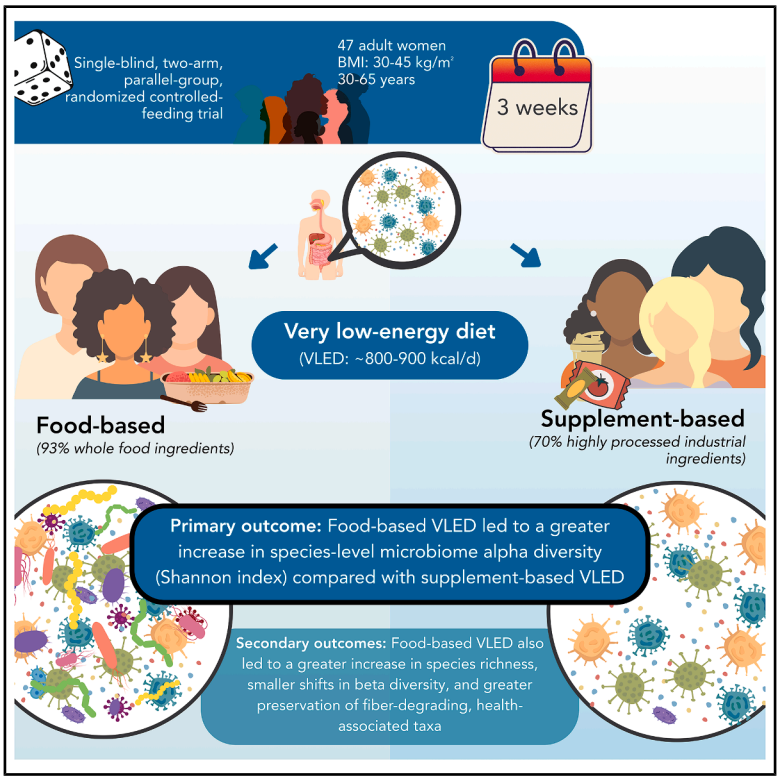
Food- vs. supplement-based very-low-energy diets and gut microbiome composition in women with high body mass index: A randomized controlled trial

Graphical abstract



Highlights

- 47 women with obesity randomized to food- or supplement-based very-low-energy diets
- Three-week diets matched at ~800–900 kcal/d, but whole vs. industrial ingredients differ
- Food vs. supplement: greater increases in species-level alpha diversity (Shannon)
- Food vs. supplement: greater richness, smaller beta diversity shifts, and preserved taxa

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In brief

Lane and McGuinness et al. show that in 47 women following 3 weeks of very-low-energy diets, a food-based approach leads to greater gains in gut microbial diversity and better preservation of beneficial taxa than a supplement-based approach, highlighting the importance of diet format in gut health during energy restriction.

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Food- vs. supplement-based very-low-energy diets and gut microbiome composition in women with high body mass index: A randomized controlled trial

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SUMMARY

In a single-blind, two-arm, randomized controlled-feeding trial (May 2021–February 2022), 47 women (30–65 years, BMI 30–45 kg/m^2) are randomized to either a food-based or a supplement-based very-low-energy diet (VLED: 800–900 kcal/d) for 3 weeks. The food-based VLED comprises pre-packaged meals (~93% whole-food ingredients), while the supplement-based VLED comprises shakes, soups, bars, and desserts (~70% industrial ingredients). The primary outcome is species-level alpha diversity (Shannon index). Secondary outcomes include species richness, beta diversity, taxonomic composition, functional potential, anthropometrics, serum biomarkers, mental health, sleep, and gastrointestinal symptoms. Modified intention-to-treat (mITT) analyses (n = 45) assess diet group × time interactions as beta coefficients (β) with 95% confidence intervals (CIs). A between-group differential change is observed for the Shannon index, with a greater increase in the food-based group (mITT β: 0.37, 95% CI: 0.15–0.60). The food-based group also shows greater species richness, smaller beta diversity shifts, and compositional changes preserving fiber-degrading, health-associated taxa.

INTRODUCTION

The gut microbiome is intricately connected to human health and disease, and understanding the influence of diet on its composition and function may inform gut-focused treatment strategies.[1] The gut microbiome is shaped by both short- and long-term dietary exposures.[2–5] Dietary interventions, including high-fiber and Mediterranean-style diets, have been shown to beneficially alter the gut microbiome.[6,7] This includes increasing bacterial diversity and the abundances of bacterial species considered beneficial for health, enhancing carbohydrate breakdown by microbiome enzymes, and reducing inflammation.[4] Conversely, more "Westernized" diets, characterized by higher intakes of sugar, fat, and protein and lower intakes of fiber, are linked to reduced gut microbiome diversity and functional capacity, higher body mass index (BMI), increased inflammatory markers, and elevated risk of diseases such as cancer.[8,9]

Although dietary interventions have been linked to alterations in gut microbiome composition and potential function,[4,6,7,10] the effects of food processing on the gut microbiome have not been thoroughly examined. Heavily processed foods comprise largely of industrially derived constituents, including colorants,

emulsifiers, flavors, microparticles, stabilizers, and thickeners, with few whole foods.[11] While human data are lacking, emerging evidence in mice suggests that these industrially derived constituents might alter the gut microbiome and increase the risk for immune-mediated diseases.[12] Additionally, the often lower nutritional quality of extensively processed foods, especially in terms of fiber diversity and quantity, may negatively affect gut bacterial metabolism of short-chain fatty acids, which are crucial for maintaining gut barrier integrity.[13] Although diets high in extensively processed foods typically have poorer nutritional profiles compared to those consisting mainly of whole foods,[14] highly processed items such as supplement-based shakes, bars, and soups used in very-low-energy diet (VLED) programs, are intended to meet nutritional needs. These products aim for an adequate ratio of macronutrients (protein, fat, and carbohydrate) and essential vitamins and minerals, despite their extensive processing. Targeted at individuals with a high BMI of 30 kg/m^2 or more, VLEDs limit energy intake to approximately 800–900 kcal per day, and are shown to effectively reduce weight and improve markers of type 2 diabetes and cardiovascular disease.[15,16] While these VLEDs demonstrate clear benefits in managing metabolic-related outcomes, the impact of highly processed supplement-based VLEDs on the gut microbiome is not well understood, especially compared to less processed, food-based VLEDs.

A systematic review examining the impact of supplement-based VLEDs on gut microbiome composition reported mixed findings for diversity and inconsistent shifts within the Firmicutes and Bacteroidetes phyla (now referred to as Bacillota and Bacteroidota, respectively), including changes in both potentially beneficial and opportunistically pathogenic taxa—likely reflecting methodological limitations in the included studies.[17] While metabolic parameters generally improved (e.g., reductions in anthropometric measures) or remained unchanged, most studies lacked randomization and used single-group designs without comparators, with further confounding introduced by concurrent lifestyle changes.[17] Only two of seven trials used a parallel group design: one compared a VLED with surgical intervention and the other against two control groups based on BMI, where neither control group received any intervention.[17] Additionally, controlled *ad libitum* feeding studies have shown that minimally processed, whole-food-based diets—whether differing in macronutrient composition[18] or designed to be matched in nutrient profiles[19] to heavily processed diets—can alter gut microbiome composition,[18] modulate immune cell activity,[18] reduce energy intake (~508 kcal/day),[19] induce spontaneous weight loss (~0.9 kg),[19] and lower inflammatory markers (~45%).19 Despite these insights, some experimental studies have directly compared how supplement- and food-based VLEDs, which are designed to be isocaloric and similar in their nutritional profiles but different in their level of food processing, affect the gut microbiome. Understanding these potential differential impacts is crucial, given the significant role of the gut microbiome in overall health,[1] and will aid in evaluating the comprehensive risk-benefit profile of these VLED formats.

This study explored the effects of a food- versus supplement-based VLED program on gut microbiome composition in women with high BMI. Our primary outcome was species-level alpha diversity, assessed using the Shannon index. Secondary gut microbiome outcomes included species richness, beta diversity, taxonomic composition, and functional potential. Secondary clinical outcomes included anthropometric measures, serum biomarkers, mental health, sleep, and gastrointestinal symptoms. We hypothesized that a food-based VLED, primarily comprising whole-food ingredients, would increase gut microbiome diversity and the relative abundances of health-associated bacteria compared to a supplement-based VLED, predominantly consisting of highly processed industrial ingredients. We also hypothesized that those randomized to the food-based VLED would experience more beneficial metabolic and mental health outcomes relative to those on the supplement-based VLED.

RESULTS

Recruitment and trial retention

We screened 102 participants for eligibility, of whom 40 were initially randomized (Figure 1). Due to participant withdrawal/loss to follow-up (n = 4) and missing fecal samples (n = 4), we aimed to recruit an additional eight participants to reach a sample size of 40 with complete data. Overall, 47 participants were randomized, including 23 in the food-based group and 24 in the supplement-based group. Of these, 45 were included in mITT analyses (food-based: n = 23, supplement-based: n = 22), and 39 in complete case analysis (food-based: n = 22, supplement-based: n = 17) of the primary outcome.

Baseline characteristics

On average, participants in the food-based group were less commonly married or employed compared to the supplement-based group (Table 1). More participants in the food-based group were taking medication, and they also had higher average BMI, body weight, physical activity levels, and waist and hip circumferences, compared to the supplement-based group.

Adherence and safety

Diet adherence

Of the complete cases (n = 39), on average, dietary intake was recorded on 20 out of the requested 21 days. The average daily energy intake of 825 kcal was within the 800–900 kcal VLED target range (Table S1). Both groups reported similar average daily intakes of carbohydrate, protein, fiber, sugar, and sodium. The food-based group reported higher average total energy intake (903 vs. 748 kcal/d) and total fat intake (40.1 vs. 22.5 g/d, equivalent to ~158 kcal) compared to the supplement-based group. The difference in average energy intake between the two groups closely matches the energy difference attributable to the variation in fat intake.

Adverse events

Participants in the supplement-based group reported more adverse events compared to the food-based group (19 vs. 8, respectively) (Table S2). The most common adverse event reported for both groups was headaches (5 vs. 3 events, respectively). No serious adverse events were reported in either group.

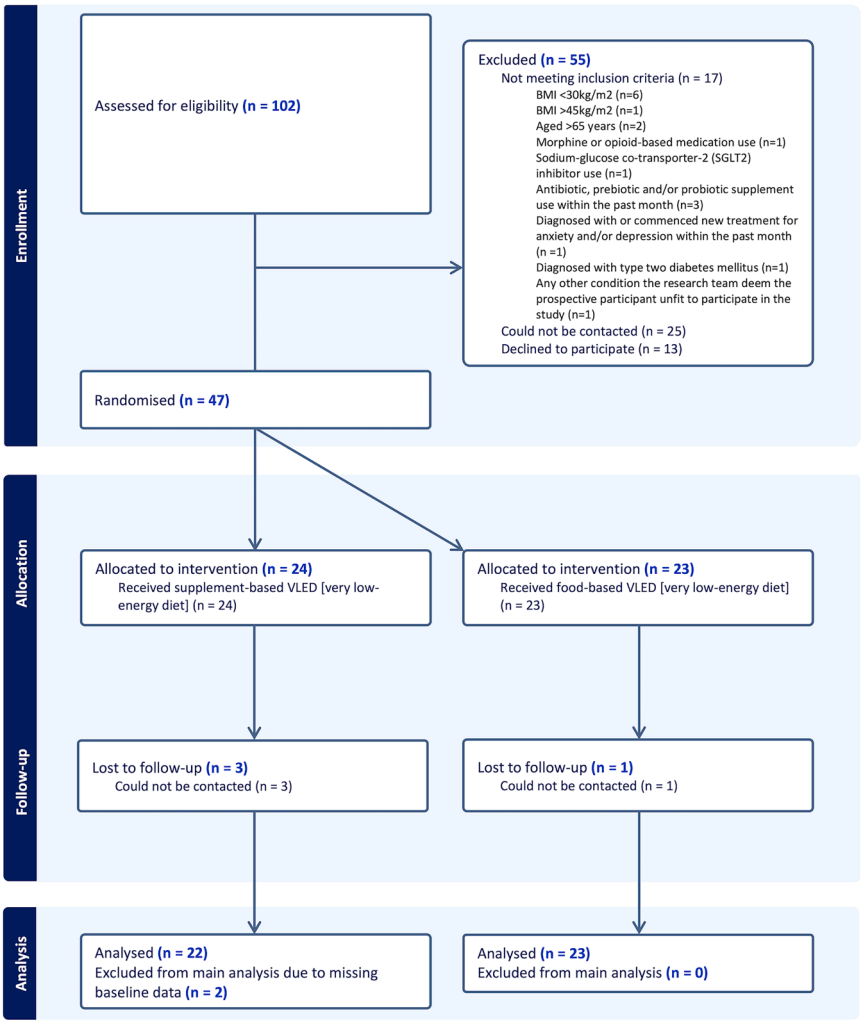

Figure 1. CONSORT flow diagram

Main analysis refers to the modified intention-to-treat (mITT) approach, which included all randomized participants who provided baseline data, regardless of follow-up completion.

Primary outcome

Species-level alpha diversity (Shannon index)

We observed a statistically significant between-group differential change over time in the Shannon index (mITT β: 0.37, 95% CI: 0.15–0.60) (Table S3), with the food-based group experiencing a greater increase (mean change: 0.27, 95% CI: 0.09–0.44) compared to the statistically non-significant decrease observed in the supplement-based group (mean change: −0.11, 95% CI: −0.27 to 0.05) (Figure 2A). Results were similar across complete case analyses (Table S3) and sensitivity analyses that adjusted for prognostic covariates and removed a sample with low read count (see quantification and statistical analysis section). Additional adjustment for age and postmenopausal status gave similar results, while adjusting for alcohol intake increased the effect size (mITT β: 0.45, 95% CI: 0.18–0.72) (Table S10).

Secondary outcomes

Species-level alpha diversity (richness)

We observed a between-group differential change in species richness (mITT β: 27.9, 95% CI: 12.1–43.7), with the food-based group experiencing a greater increase (mean change: 23.2, 95% CI: 12.7–33.7) compared to the statistically non-significant decrease exhibited in the supplement-based group (mean change: −4.59, 95% CI: −17.0 to 7.78) (Table S3; Figure 2B).

Beta diversity (Aitchison distance)

We observed a between-group differential change over time in beta diversity (complete case r^2 = 0.051, p = 0.001) (Table S4), suggesting that 5.1% of the difference in the shift in beta diversity observed between groups could be explained by diet. Visual inspection of the data using PCA suggested a greater shift in beta diversity in the supplement- versus food-based group (Figure 2C). The variance in Aitchison distances explained by time point was 1.8% in the food-based group and 4.5% in the supplement-based group (Table S4).

Taxonomic composition (species, genus, family, and phylum)

We observed between-group differential changes over time in the centered log-ratio (CLR)-transformed relative abundance of 72 bacterial species ($p < 0.05$); however, these did not survive adjustment for multiple comparisons (Table S5). There were between-group differential changes in 56 genera (Table S6), eight families (Table S7), and one phylum (Table S8); of these, eight genera and one family survived adjustment for multiple comparisons (Table 2; Figures S1 and S2); however, no phyla met the significance threshold after correction. Results of complete case analyses were similar (Tables S5, S6, S7, and S8).

For brevity, and given the large number of taxa tested and substantial within-genus variability, we report detailed results for the three most statistically significant and well-characterized genera that remained significant after correction for multiple comparisons: *Lachnospira* (mITT β: 2.17, 95% CI: 0.99–3.34), *Anaerostipes* (mITT β: −1.73, 95% CI: −2.67 to −0.80), and *Ruminococcus_A* (mITT β: −1.05, 95% CI: −1.68 to −0.43) (Figures 2D–2F). For *Lachnospira*, the food-based group experienced a greater, but non-significant, increase (mean change: 0.55, 95% CI: −0.08 to 1.19) compared to a statistically significant decrease in the supplement-based group (mean change: −1.67, 95% CI: −2.68 to −0.66). For *Anaerostipes*, the food-based group experienced a non-significant decrease (mean change: −0.41, 95% CI: −1.01 to 0.20) compared to a statistically significant increase

Table 1. Baseline characteristics of participants randomized to food- vs. supplement-based very-low-energy diets

	Food-based VLED (*n* = 23)	Supplement-based VLED (*n* = 24)	Total (*n* = 47)
Sociodemographic factors			
Age, years—*M* (SD)	46.5 (10.3)	48.3 (9.91)	47.4 (10.0)
Born in Australia, *n* (%)	22 (96%)	19 (79%)	41 (87%)
Married, *n* (%)	15 (65%)	19 (79%)	34 (72%)
Post-secondary school education, *n* (%)	21 (91%)	20 (83%)	41 (87%)
Employed, *n* (%)	18 (78%)	23 (96%)	41 (87%)
Household income above $74,999, *n* (%)[a]	13 (57%)	13 (54%)	26 (55%)
Health factors			
Current smoker, *n* (%)	1 (4%)	2 (8%)	3 (6%)
Alcohol intake, grams/day—*M* (SD)[b]	4.8 (6.5)	3.0 (4.8)	3.9 (5.7)
Any medication use, *n* (%)	18 (78%)	13 (59%)	31 (69%)
Stool consistency—*M* (SD)	3.8 (1.1)	4.3 (1.2)	4.0 (1.2)
Body mass index—*M* (SD)	36.8 (3.6)	34.8 (3.2)	35.8 (3.5)
Weight, kilograms—*M* (SD)	101 (10.1)	94.1 (10.3)	97.3 (10.6)
Waist circumference, centimeters—*M* (SD)	107 (8.7)	103 (9.1)	105 (9.1)
Hip circumference, centimeters—*M* (SD)	124 (9.1)	119 (7.1)	122 (8.5)
Low physical activity, *n* (%)	13 (57%)	18 (75%)	31 (66%)
Peri/menopausal, *n* (%)[c]	7 (30%)	11 (46%)	18 (38%)

Note: stool consistency measured using the Bristol Stool Form Scale; low physical activity measured using the International Physical Activity Questionnaire. Abbreviations: M, Mean; *n* (%), number and proportion of participants; VLED, very low-energy diet; SD, standard deviation.
[a]Data available for 22 in the food-based group and 23 in the supplement-based group (*n* = 45).
[b]Data available for 18 in the food-based group and 19 in the supplement-based group (*n* = 37).
[c]Data available for 19 in the food-based group and 22 in the supplement-based group (*n* = 41).

in the supplement-based group (mean change: 1.34, 95% CI: 0.58–2.09). For *Ruminococcus_A*, the food-based group experienced a statistically significant decrease (mean change: −0.86, 95% CI: −1.35 to 0.37) compared to a non-significant increase in the supplement-based group (mean change: 0.19, 95% CI: −0.23 to 0.62). Additionally, the family that remained statistically significant after correction for multiple comparisons was an uncharacterized family of the Christensenellales order, *GCA_900066905* (mITT β: −1.19, 95% CI: −1.81 to −0.57) (Table S8; Figure S2). The food-based group experienced a statistically significant decrease in Christensenellales_*GCA_900066905* (mean change: −0.33, 95% CI: −0.60 to −0.06) compared to a statistically significant increase in the supplement-based group (mean change: 0.87, 95% CI: 0.29–1.44).

Functional potential (MetaCyc pathways)

No significant between-group differential changes in functional alpha diversity were observed (Table S3). We observed between-group differential changes in six MetaCyc groups ($p < 0.05$); however, none of these remained statistically significant after adjustment for multiple comparisons (Table S9).

Secondary clinical outcomes

For secondary clinical outcomes, there was a between-group differential change over time ($p < 0.05$) in hip circumference (mITT β: 3.29 cm, 95% CI: 0.17–6.42), albumin concentration (mITT β: −1.60 g/L, 95% CI: −2.86 to −0.33), and constipation symptoms (mITT β: 19.5, 95% CI: 3.59–35.5) (Table S3); however, these did not remain statistically significant after correction for multiple comparisons. For hip circumference, the food-based group experienced a smaller, non-significant decrease (mean change: −1.18 cm, 95% CI: −3.40 to 1.03) compared to a larger, statistically significant decrease in the supplement-based group (mean change: −4.53 cm, 95% CI: −6.87 to −2.20). For albumin concentration, the food-based group experienced a smaller, statistically significant increase (mean change: 1.32 g/L, 95% CI: 0.48–2.15) compared to a larger, statistically significant increase in the supplement-based group (mean change: 2.91 g/L, 95% CI: 1.90–3.92). For constipation symptoms (where higher scores reflect more favorable ratings), the food-based group experienced a greater, non-significant improvement (mean change: 1.95, 95% CI: −6.20 to 10.1) compared to a statistically significant worsening in the supplement-based group (mean change: −18.5, 95% CI: −33.1 to −3.98).

Post hoc exploratory analyses

Correlation analyses

Changes in species-level Shannon index showed a weak inverse association with changes in serum leptin ($r = -0.37$, $p = 0.024$), suggesting that greater increases in the Shannon index tended to be associated with either smaller increases or greater decreases in serum leptin concentrations. Weak positive associations were observed between changes in the Shannon index and changes in bloating/flatulence ($r = 0.31$, $p = 0.059$) and abdominal pain ($r = 0.31$, $p = 0.061$), suggesting that greater increases in the Shannon index tended to be associated with greater increases (more favorable ratings) or smaller decreases in these gastrointestinal symptoms (Figure 3).

Species-level contributions to the Shannon index

Decomposition of the Shannon index into species-level contributions revealed modest differences between groups. The food-based group showed a slightly greater number of

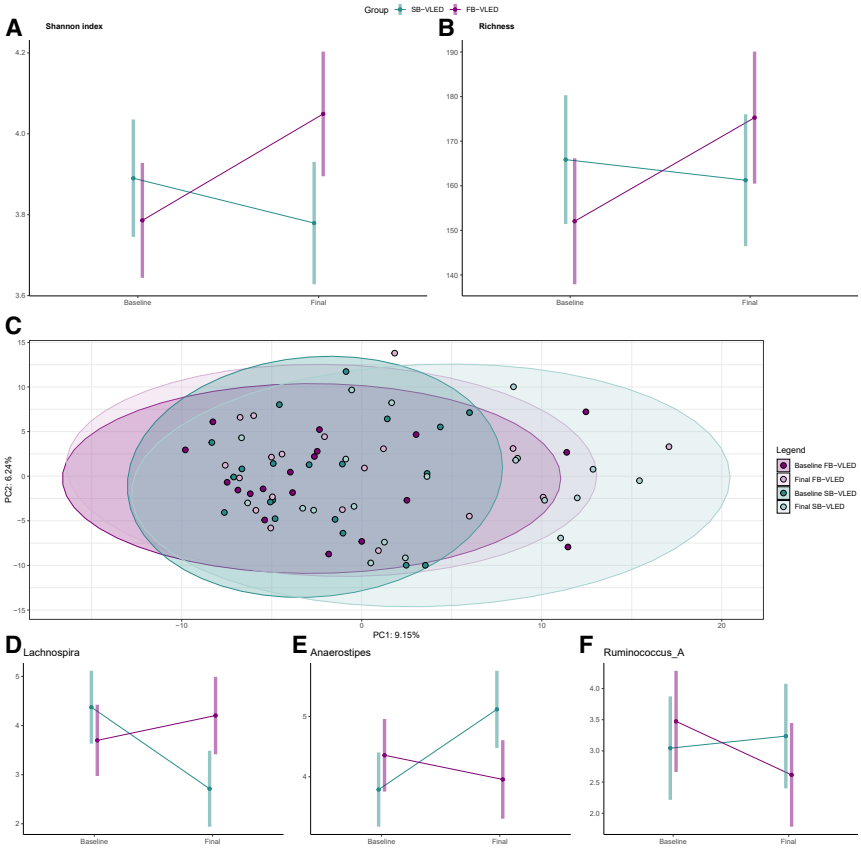

Figure 2. Between-group differential changes in gut microbiome alpha diversity, beta diversity, and genus-level composition during food- vs. supplement-based VLEDs over three weeks

(A and B) Interaction plots for the estimated marginal means of (A) species-level Shannon index (primary outcome) and (B) species richness, in participants who received a food-based very-low-energy diet (FB-VLED; purple) versus supplement-based very-low-energy diet (SB-VLED; blue) from baseline to week 3 (final). Vertical bars represent 95% confidence interval.

(C) Principal-component analysis of Aitchison distances in those that received a food-based very-low-energy diet (FB-VLED; purple) versus supplement-based very-low-energy diet (SB-VLED; blue) at baseline and week 3 (final). The scatterplot shows the first two principal components (PC1 and PC2), explaining 9.15% and 6.24% of the variance, respectively. Each point represents one biological replicate (an individual sample). Ellipses around each group represent the 95% confidence interval, illustrating the spread and central tendency of the samples within each group. The ellipses provide a visual indication of the multivariate normal distribution of the data points, helping to identify the degree of overlap or separation between different groups.

(D–F) Interaction plots for the estimated marginal means of the three most statistically significant and well-characterized bacterial genera that remained significantly different between groups over time after correction for multiple comparisons ($q < 0.1$): *Anaerostipes*, *Lachnospira*, and *Ruminococcus_A*. (A, B, D–F) Points represent estimated marginal means derived from all biological replicates (individual participant samples) within each group and time point, with vertical bars representing 95% confidence intervals. No technical replicates were performed.

species with increased contributions to diversity (16 increased contribution vs. 14 decreased contribution) compared to the supplement-based group (13 increased contribution vs. 17 decreased contribution) (Figure 4). Across both groups, 45 distinct species were identified as major contributors. Of these, 15 species were shared between groups, and each of their contributions changed in the same direction in both groups. The remaining 30 species were group specific: 15 unique to the food-based group and 15 unique to the supplement-based group, with both increases and decreases in contribution observed.

To capture broader community shifts beyond these top contributors, we examined the net contribution of all other species (represented by the "Other_net" bar) in each group. The food-based group showed a large positive contribution from other species, suggesting a more diffuse restructuring of the community beyond just the most prominent taxa that contributed to the increase in Shannon index observed in this group. In contrast, in the supplement-based group, this contribution was small and negative, showing that the top 30 species contributed the most to the modest reduction in the Shannon index observed in this group.

To further contextualize these shifts, we classified the top 30 species in each group as either dominant (≥1% mean relative abundance) or subdominant (<1%) at baseline. In both groups, most species were subdominant at baseline (20 in the food-based group and 21 in the supplement-based group) rather than dominant (10 in the food-based group and 9 in the supplement-based group), indicating that changes in the Shannon index were largely contributed to by less abundant members of the community in both groups.

Phylum-level taxonomic composition

Aggregating species-level relative abundances at the phylum level showed the gut microbiome was broadly dominated by bacteria belonging to Firmicutes (now Bacillota) and Bacteroidota (previously Bacteroidetes) in both groups and at both time points (Figure 5). Overall, phylum-level profiles remained relatively stable across groups and over time, with no substantial compositional shifts observed at this taxonomic level.

DISCUSSION

We present evidence showing differential effects of food- versus supplement-based VLEDs on gut microbiome alpha diversity, beta diversity, and the relative abundances of eight genera and one family in women with high BMI. Consistent with our hypotheses, and despite comparable weight loss and nutrient intakes (carbohydrate, protein, sugar, sodium, and fiber), the food-based group exhibited greater increases in the species-level Shannon index compared to the supplement-based group. The food-based group also showed more pronounced increases in species richness, smaller shifts in beta diversity, and, similar to

Table 2. Modified intention-to-treat (n = 45) analyses of the within-group changes from baseline, and between-group differential changes over time, in bacterial genera and families after consuming a food-based versus supplement-based very-low-energy diet for 3 weeks

Taxon	Level	Food-based VLED within-group change from baseline to week 3, (mean, 95% CI)	Supplement-based VLED within-group change from baseline to week 3, (mean, 95% CI)	Between-group differential change from baseline to week 3 (unadjusted β, 95%CI)	p value	q value
GCA_900066905	genus	−0.30 (−0.54 to −0.07)	0.90 (0.32–1.49)	−1.21 (−1.82 to −0.60)	0.000	0.056
Lachnospira	genus	0.55 (−0.08 to 1.19)	−1.67 (−2.68 to −0.66)	2.17 (0.99–3.34)	0.001	0.056
Anaerostipes	genus	−0.41 (−1.01 to 0.20)	1.34 (0.58–2.09)	−1.73 (−2.67 to −0.79)	0.001	0.056
Lachnospiraceae_MIC9331	genus	−0.08 (−0.31 to 0.16)	1.27 (0.50–2.04)	−1.35 (−2.13 to −0.57)	0.001	0.079
Ruminococcus_A	genus	−0.86 (−1.35 to −0.37)	0.19 (−0.23 to 0.62)	−1.05 (−1.68 to −0.43)	0.002	0.079
UBA11774	genus	1.58 (0.54–2.61)	−0.59 (−1.45 to 0.26)	2.12 (0.81–3.43)	0.002	0.079
CAG_103	genus	0.15 (−0.51 to 0.82)	−1.70 (−2.71 to −0.69)	1.91 (0.73–3.10)	0.002	0.079
Clostridium_A	genus	0.26 (−0.47 to 1.00)	2.06 (1.19–2.92)	−1.78 (−2.89 to −0.68)	0.002	0.079
Christensenellales_*GCA_900066905*	family	−0.33 (−0.60 to −0.06)	0.87 (0.29–1.44)	−1.19 (−1.81 to −0.57)	0.000	0.028

The taxa presented in the table (n = 9) are those with evidence (q < 0.1) of between-group differential changes over time, based on the modified intention-to-treat analyses.
Note: unadjusted model: ∼ group*time point.
To avoid ambiguity in taxa sharing the identifier GCA_900066905, we refer to the genus-level taxon as GCA_900066905 and the corresponding family-level taxon as Christensenellales_GCA_900066905.
Adjusted q value: the p value adjusted for multiple comparisons using the Benjamini-Hochberg procedure.
Abbreviations: β, beta-coefficient; CI, confidence interval.

alpha diversity patterns, largely divergent directional changes across genus- and family-level taxa. Although we hypothesized more favorable changes in the food-based group, we observed no statistically significant differences between the two diet groups in terms of changes to other secondary outcomes, including species- and phylum-level composition, functional potential, or clinical outcomes such as anthropometrics, serum biomarkers, mental health, sleep, or gastrointestinal parameters. Hence, the observed differences in microbial diversity and community structure offer preliminary support for potential microbiome-mediated effects of VLED format, with further research needed to assess the persistence and clinical relevance of these changes over longer time frames.

Diversity differences and clinical implications

To our knowledge, no randomized trials have compared the effects of food- versus supplement-based VLEDs on the gut microbiome. While previous studies have examined the impact on the gut microbiome of supplement-based VLEDs alone,[17] as well as supplement- and food-based weight loss interventions in general (not VLEDs),[20,21] we directly investigated the role of diet format within a VLED context. We observed differential impacts of these two types of VLEDs on alpha diversity, with consistent increases in the food-based group across both the Shannon index and richness, while the supplement-based group showed non-significant decreases, reflecting divergent ecological responses. A recent meta-analysis reported a non-significant pooled increase in alpha diversity following food-based weight loss interventions,[22] aligning in direction with the statistically significant increases in the Shannon index and richness observed in our food-based VLED group. In contrast, supplement-based diets have been associated with statistically significant increases in alpha diversity in prior single-arm studies,[22] which diverges from the modest, non-significant reductions observed in our supplement-based VLED group, despite similar weight loss. Notably, the between-group difference in the Shannon index persisted across sensitivity analyses and was even more pronounced after adjustment for alcohol intake, suggesting the robustness of our findings and the potential influence of other non-study foods or drinks. As both groups in our study achieved comparable weight loss, the divergent alpha diversity trajectories are more likely attributable to diet format than to energy restriction alone. Our exploratory decomposition of the Shannon index provides some insight into potential underlying contributors to this divergence. In both groups, most top-contributing species were subdominant at baseline, consistent with previous findings that low-abundance taxa contribute substantially to diversity shifts.[23] However, based on visual inspection, the distribution of these contributions appeared to differ between groups. In the supplement-based group, changes to the Shannon index (i.e., alpha diversity) were largely accounted for by a small number of species, with limited influence from the broader microbial community. Conversely, the food-based group exhibited a broader pattern of change, suggesting that shifts extended beyond the top 30 species and were more evenly distributed across the community. This may reflect greater evenness and/or the emergence of previously low-abundance species not captured among the top contributors, in response to the food-based intervention. These findings underscore the value of decomposing diversity metrics to disentangle community-level shifts from the contributions of

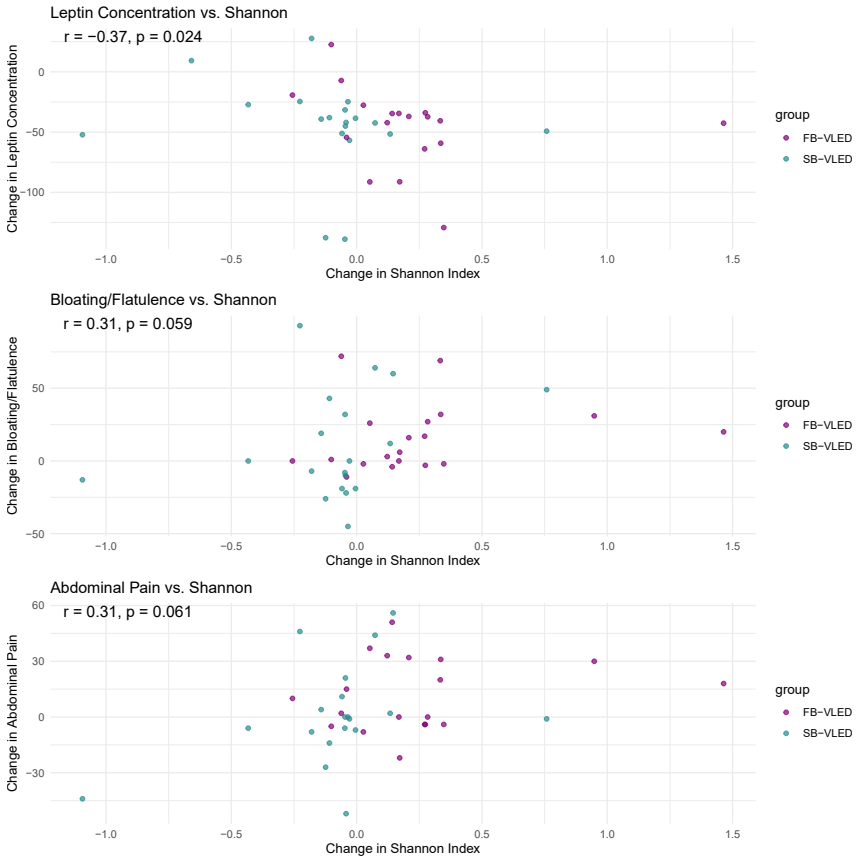

Figure 3. Associations between changes in species-level Shannon index and changes in clinical outcomes over the 3-week intervention period

Scatterplots show Spearman correlation coefficients (r) and corresponding *p* values between changes in gut microbial alpha diversity (Shannon index) and changes in leptin concentration, bloating/flatulence symptoms, and abdominal pain symptoms. Each point represents one biological replicate (the paired change in an individual participant). No technical replicates were performed. Data were pooled across intervention groups (food-based very-low-energy diet [FB-VLED, purple]; supplement-based very-low-energy diet [SB-VLED, blue]).

specific taxa. They also suggest that the food-based VLED may have promoted broader microbial restructuring compared to the supplement-based VLED.

In our study, we also observed shifts in beta diversity in both groups, with a greater shift observed in the supplement-based group. While there is inconsistent evidence of the effects of supplement-based VLEDs or food-based weight loss diets on gut microbiome beta diversity,[17,20,21] the greater shift in the supplement-based group reflects a more pronounced compositional response to this type of diet. In contrast, the smaller beta diversity shift in the food-based group suggests internal restructuring toward more diverse and evenly distributed communities, without marked divergence between individuals. Although beta diversity does not directly measure microbial stability, the smaller shifts in the food-based group indicate a broader microbial response and potentially less ecological disruption. Together, the alpha and beta diversity findings reflect fundamental differences in food processing between the two VLEDs, such as food matrix complexity, fiber diversity, or additive exposure, warranting further investigation. They also highlight the potential of food-based VLEDs to promote a more favorable microbial profile even under substantial energy restriction.

Taxonomic differences and clinical implications

During the 3-week study period, we observed between-group differential changes in eight genera and one family. For 12 of these genera and one family, within-group estimates showed opposite patterns of change between the food- and supplement-based groups, mirroring the divergence observed in alpha diversity. As noted in the results section, we focused on the three most significant and well-characterized genera (*Lachnospira*, *Anaerostipes*, and *Ruminococcus_A*) to enhance interpretability and avoid overinterpreting changes in less well-defined taxa.

The between-group difference for *Lachnospira* appeared to be driven by the more substantial, statistically significant decrease in the supplement-based group, versus the smaller, non-significant increase in the food-based group. *Lachnospira* is a pectinolytic genus that ferments complex plant-derived polysaccharides such as pectin and polygalacturonic acid[24]—dietary fibers abundant in fruits, vegetables, and legumes but largely absent from highly processed foods. Its abundance is directly associated with greater fruit and vegetable intake[25–27] and is typically higher in healthier populations.[28–30] The marked reduction in *Lachnospira* in the supplement-based group may reflect reduced availability of fermentable substrates in that diet, while the preservation in the food-based group may indicate protection of fiber-degrading niches through greater fiber diversity and intact plant matrices. These shifts reinforce the possibility that certain fiber-degrading species were better maintained in the context of the food-based VLED, suggesting that lower levels of food processing may help preserve fiber-degrading taxa relevant to microbial resilience.

Anaerostipes is a butyrate-producing genus within the *Lachnospiraceae* family that ferments carbohydrates into short-chain fatty acids[31] such as acetate, lactate, and butyrate[32–34]—metabolites that contribute to gut barrier integrity and have anti-inflammatory effects.[35,36] In our study, the between-group difference for *Anaerostipes* appeared to be primarily influenced by the more pronounced, statistically significant increase in the supplement-based group, compared to the smaller, non-significant decrease in the food-based group. The increase in *Anaerostipes*

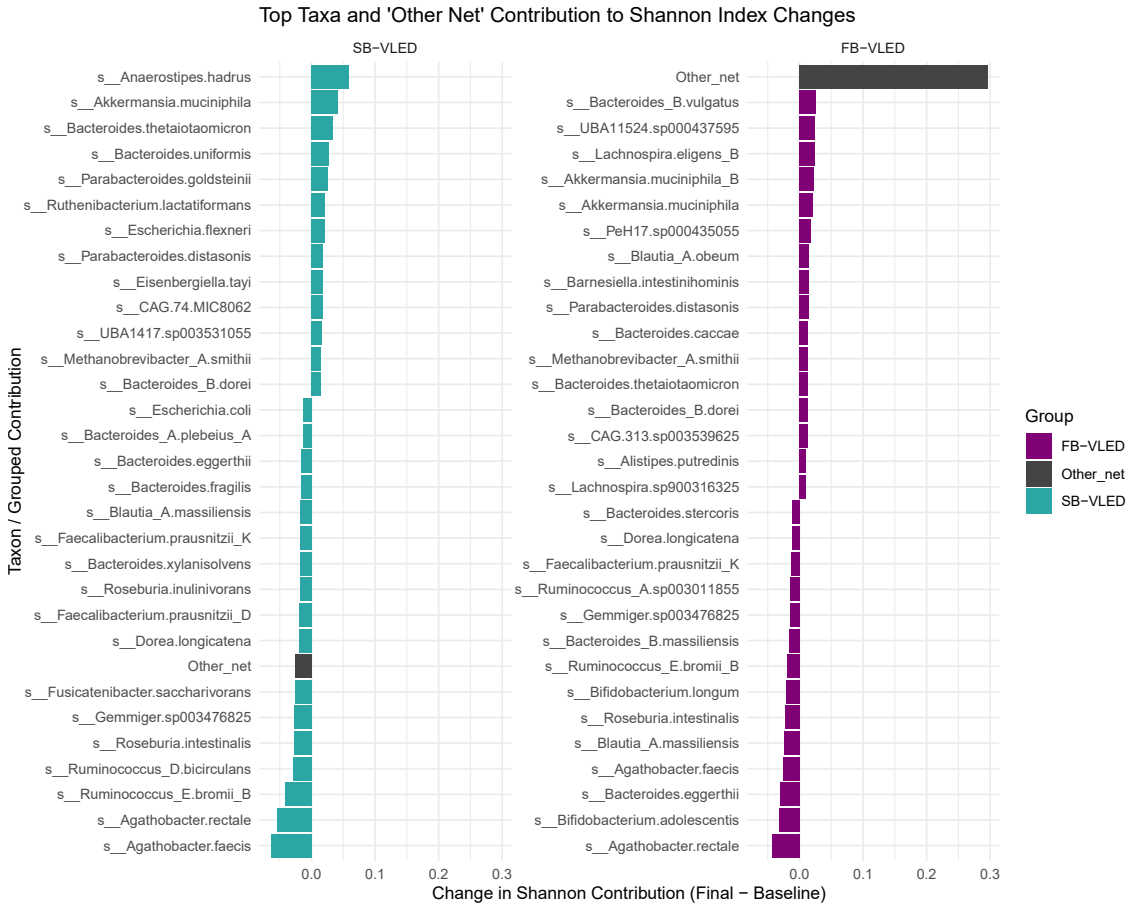

Figure 4. Species-level decomposition of the Shannon index over the 3-week intervention period

Bar plots show the 30 species with the largest absolute changes in contribution to Shannon diversity, alongside the grouped contribution of all other taxa ("Other_net"), separately for each group (supplement-based very-low-energy diet [SB-VLED, blue]; food-based very-low-energy diet [FB-VLED, purple]). Positive values indicate increased contribution to Shannon diversity at week 3 relative to baseline, and negative values indicate decreased contribution. Species are ordered by net change within each group. Bars represent group-level averages calculated across all biological replicates (individual participants). No technical replicates were performed.

within the supplement-based group may be explained by the inclusion of inulin—a fermentable prebiotic fiber known to selectively promote this genus in feeding studies.[37] While butyrate production is generally considered beneficial,[35,36] this increase occurred alongside broader reductions in diversity in the supplement-based group, suggesting that targeted enrichment of specific genera may not compensate for wider ecological disruptions. The non-significant decrease in *Anaerostipes* in the food-based group may reflect lower levels of isolated prebiotic fibers, but this was offset by broader preservation of microbial diversity, highlighting the complexity of dietary influences on microbial ecology.

Ruminococcus_A is a taxonomically diverse genus known for fermenting complex carbohydrates into various fermentation products including acetate, formate, and succinate.[38] Some species within this genus also degrade host-derived mucins,[39] potentially influencing gut barrier function. In our study, the food-based group experienced a statistically significant decrease in *Ruminococcus_A*, while the supplement-based group showed a small, non-significant increase. Although the functional significance of this genus remains uncertain due to its heterogeneity, some *Ruminococcus* taxa have been linked to inflammatory diseases,[40] whereas others are considered fiber degraders.[41,42] The reduction observed in the food-based group may therefore reflect suppression of potentially mucin-degrading or pro-inflammatory strains, although further strain-level resolution is needed. These findings add to the growing recognition that shifts in gut microbiome composition must be interpreted in the context of both functional potential and broader community dynamics.

While several species and predicted functional pathways showed between-group differences, none passed correction for multiple comparisons and are not discussed further due to high false-positive risk in small-sample, high-dimensional settings. Similarly, no between-group differences were observed for MetaCyc alpha diversity, and no functional pathways passed multiple testing correction, which may reflect the well-recognized functional redundancy of the gut microbiome, where taxonomic shifts do not always lead to measurable differences in

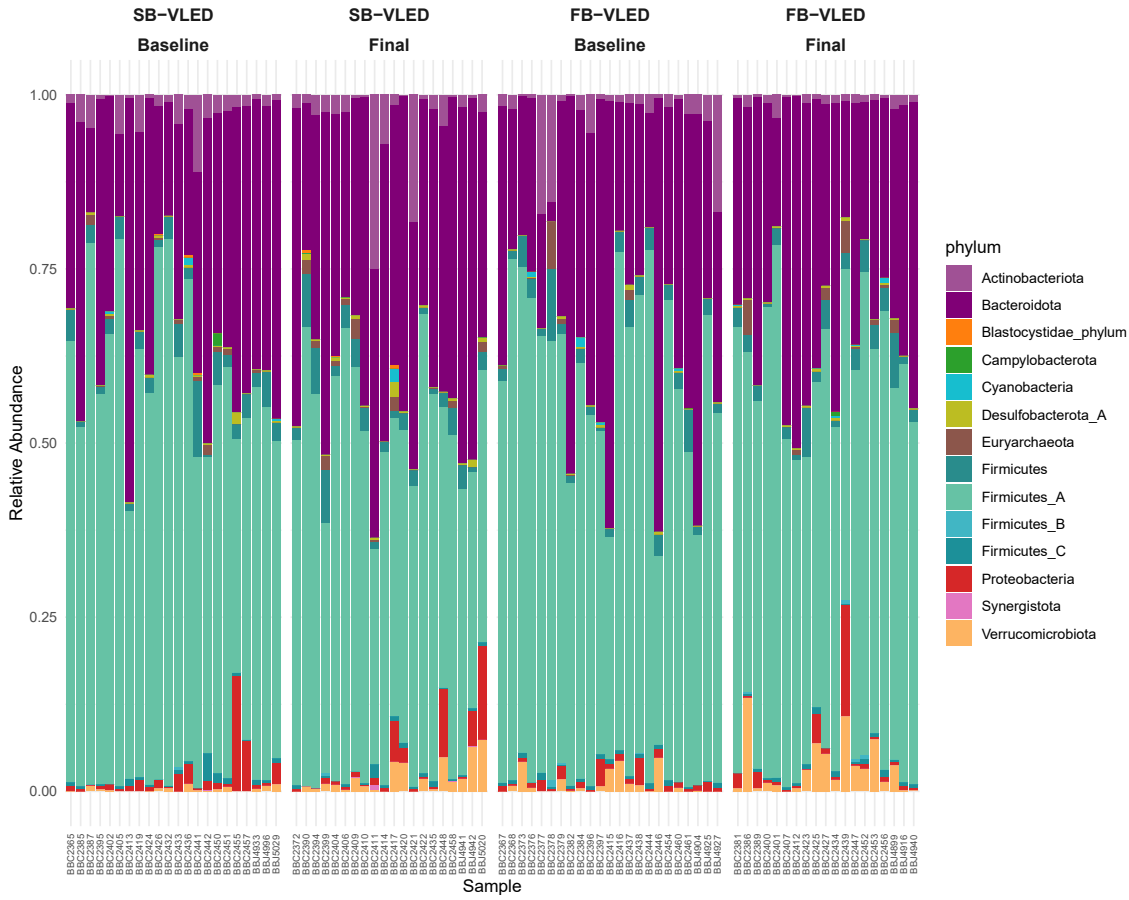

Figure 5. Relative abundance of major gut microbial phyla, stratified by group (SB-VLED; FB-VLED; purple) and time point (baseline; week 3 [final])

Each stacked bar represents one biological replicate (an individual participant sample) after compositional transformation. Colors denote phylum identity as indicated in the legend. Taxonomy follows Microba's database (mixture of older and Genome Taxonomy Database [GTDB] nomenclature); Firmicutes_A–C and Desulfobacterota_A are GTDB-defined clades within their respective former phyla. No technical replicates were performed.

predicted functional capacity.[43] At a broader taxonomic level, post hoc analyses showed that phylum-level profiles remained relatively stable across both groups and time points, with no marked shifts in dominant phyla observed. These limitations highlight the need for larger, longer-term studies to validate and extend the current findings.

Clinical outcomes and implications

Preliminary analyses revealed between-group differences in hip circumference, constipation symptoms, and albumin concentration before correction for multiple comparisons. Specifically, the food-based group showed a smaller and non-significant reduction in hip circumference, a marginal and non-significant improvement in constipation symptoms, and a less pronounced increase in albumin concentration. In contrast, the supplement-based group exhibited statistically significant and more pronounced changes in all three parameters, namely a greater reduction in hip circumference, a worsening in constipation symptoms, and a larger increase in albumin concentration. However, none of these differences remained significant after multiple comparisons correction, likely reflecting the nature of the interventions (both active rather than active versus placebo), the short study duration, small sample size, and low baseline symptom burden in this non-clinical sample. Although our trial was not powered for secondary clinical outcomes, we highlight these three outcomes with nominal statistical significance as potential signals, explored below as hypothesis-generating leads. Notably, adverse events were twice as common in the supplement-based group, suggesting broader health impacts that may not have been captured by our selected measures and warrant further investigation.

Hip circumference, which reflects both adiposity and lower-body muscle mass, is inversely associated with cardiometabolic risk and premature mortality according to observational studies.[44–46] The relatively minor reduction in hip circumference in the food-based group may suggest better preservation of lower-body muscle mass and/or lower reduction in gluteal fat mass compared to the supplement-based group, which showed a larger reduction. While further body composition data are required, preservation of muscle mass aligns with prior evidence

linking whole-food-derived protein to muscle maintenance[47] and highly processed food intake to muscle loss.[48] Similarly, the worsening of constipation symptoms in the supplement-based group, despite similar reported fiber intake, may reflect a limited diversity of soluble fiber sources, consistent with previous reports of higher constipation prevalence on supplement-based VLEDs[49] and evidence that fiber type and diversity influence gut function.[50,51] Since the supplement-based VLED included vitamin- and mineral-fortified products, the smaller increase in albumin concentrations, a marker of liver function and nutritional status,[52] in the food-based group may reflect differences in micronutrient content and fortification.[52,53] Higher serum albumin concentrations can also reflect dehydration,[54] which may partially account for the greater increases observed in the supplement-based group, given that dehydration is a commonly reported side effect of supplement-based VLEDs. However, albumin changes over short periods are typically minimal in healthy populations, making the clinical relevance unclear.

To further explore potential links between gut microbiome changes and clinical outcomes, we conducted post hoc correlation analyses between changes in the species-level Shannon index and changes in selected outcomes. Small-to-moderate inverse correlations with leptin and positive correlations with bloating/flatulence and abdominal pain were observed. The statistically significant inverse correlation of alpha diversity with leptin is particularly noteworthy, given leptin's links to metabolic flexibility and inflammation,[55] though causal pathways remain speculative. While the other correlations did not reach statistical significance ($p > 0.05$), their consistency in direction and magnitude with observed microbial and preliminary clinical shifts suggests potential relevance that warrants further investigation.

Future directions

Our study adds to the growing evidence suggesting that changes in gut microbiota composition may represent one pathway through which diets high in ultra-processed foods (UPFs) affect health. While our study was not specifically designed to investigate the impact of UPFs, as defined by the widely used Nova food classification system,[11] the supplement- and food-based VLEDs can broadly be characterized as high and low UPF, respectively. Existing epidemiological evidence links high-UPF diets to poorer health outcomes, including greater risks of cardiovascular disease, type 2 diabetes, common mental disorders, and mortality, as well as gut-related conditions such as inflammatory bowel diseases, functional gastrointestinal disorders, and intestinal cancers.[12,56] However, an oft-cited limitation of this literature is the lack of causal and mechanistic evidence explaining these associations.[57] In 2023, the UK's Scientific Advisory Committee on Nutrition highlighted ongoing uncertainty as to whether the adverse health effects attributed to UPFs are independent of their typically poor nutritional profile and noted the scarcity of evidence in specific population subgroups.[57] Our study helps address this gap by focusing on individuals with high BMI using two nutritionally adequate VLEDs that differed in processing-related characteristics. The supplement-based VLED was comprised almost entirely of highly processed industrial ingredients, including macronutrient isolates (e.g., calcium caseinate, sodium caseinate, and medium chain triglycerides) and food additives (e.g., emulsifiers and nonsugar sweeteners such as citric and fatty acid esters of glycerol [472c] and aspartame [951], respectively). Conversely, the food-based VLED consisted primarily of ingredients containing intact, minimally processed foods that retained their physical structure (e.g., chopped and cooked vegetables, legumes, and whole grains), with minimal inclusion of heavily processed industrial ingredients and none in approximately half of the meals. These differences may partly explain the observed divergence in gut microbial composition between groups, despite both diets' similar nutrient and low-energy profiles, highlighting the gut microbiome as a potential intermediary between dietary processing level and health outcomes.

Emerging mechanistic evidence from *in vitro* and animal studies, and a small number of human studies, suggests that the characteristic ingredients of UPFs, such as emulsifiers, nonsugar sweeteners, colors, and micro- and nano-particles, may reduce gut microbial diversity, increase intestinal permeability, and promote intestinal inflammation.[12,58,59] In contrast, intact food structures and matrices, along with polyphenols, phytonutrients, and diverse fibers found in whole foods, may positively modulate the gut microbiome.[60,61] While much of this research has focused on individual components, UPFs typically comprise complex mixtures of food additives and other industrial ingredients that may interact in cumulative or synergistic ways, while containing little to no whole foods. For example, a recent *in vitro* study showed that food additive mixtures commonly consumed in UPFs can elicit combined effects not detected in single-substance testing.[62] These findings underscore the need to consider the broader food matrix and processing-related features of diets when evaluating their health effects. Although research on UPF-rich diets and the human gut microbiome is expanding, few intervention studies have tested their effects under controlled conditions. Our study contributes to this effort by demonstrating that two VLEDs, designed to be isoenergetic and nutritionally matched but with varying processing characteristics, were associated with distinct gut microbial responses. Further research is required to isolate the specific components responsible for these shifts, elucidate their mechanisms of action, and inform the design of microbiome-supportive dietary interventions using VLEDs.

Conclusion

Our study provides evidence of the differential impacts of food- versus supplement-based VLEDs on the diversity of the gut microbiome of women with high BMI, even over a short 3-week intervention. Despite similar weight loss and intakes of carbohydrate, protein, sugar, sodium, and fiber, participants in the food-based group experienced significantly greater increases in species-level alpha diversity (Shannon index and richness), smaller shifts in beta diversity, and compositional changes consistent with enhanced preservation of fiber-degrading and health-associated taxa, compared to the supplement-based group. These findings suggest that food-based VLEDs, richer in whole-food components and lower in highly processed industrial ingredients, may more favorably modulate gut microbiome composition compared to supplement-based alternatives. Further investigation in adequately powered, longer-term trials is needed to confirm these findings and identify the specific dietary elements responsible for the observed effects.

Limitations of the study

Several limitations must be considered when interpreting our findings. While the single-blind nature of the trial likely had little impact on the objective gut microbiome measures, knowledge of intervention allocation may have influenced self-reported gastrointestinal and mental health symptoms. Our study was conducted in women with a BMI at or above 30 kg/m^2 and over a relatively short time frame, reducing generalizability to other populations and typical VLED durations of 12 weeks or more. Broader generalizability will require studies with more diverse populations and extended follow-up, particularly to assess the durability of observed effects.

We randomized 47 participants instead of the planned 40 to address missing data, withdrawals, and loss to follow-up; however, mITT principles were maintained, and LMER models, which partially mitigate bias from missing data, showed consistent results aligned with complete case analyses. The large number of outcomes assessed increases the potential for type I errors; however, primary and secondary outcomes were grouped into related categories, and adjustments for multiple comparisons were made for each analysis. There were baseline differences between the groups in country of birth, marital status, employment, medication use, stool consistency, BMI, physical activity, menopause status, and alcohol intake, likely due to the small sample size that was not large enough to balance all prognostic covariates. While sensitivity analyses adjusting for these prognostic covariates yielded similar results to the primary analysis, larger trials are required to confirm findings.

Dietary adherence was measured by self-reported intake using the Easy Diet Diary app. Although we employed strategies to maximize adherence, self-reported dietary assessment methods have limitations that may have influenced results.[63] Future inpatient or metabolic ward settings may help to address these limitations. While the two VLED formats were intended to be isocaloric and nutritionally equivalent, the food-based group reported ~150 kcal/d higher average energy intake and ~18 g/d higher fat intake compared to the supplement-based group. Notably, lower dietary fat intake is often associated with lower microbial diversity,[64,65] yet our food-based group showed increased alpha diversity. Conversely, the supplement-based group had lower dietary fat intake yet showed non-significant reductions in alpha diversity. This finding is somewhat consistent with a prior supplement-based VLED study that reported similar patterns despite lower fat and energy intake compared to a higher-energy, higher-fat conventional weight-loss diet phase.[66] These findings suggest that energy and fat intake alone may not explain microbiome shifts. Lastly, other program differences included a recommended restriction on nonsugar sweeteners in the food-based group and the inclusion of the probiotic species *Lactobacillus plantarum* in some of that group's discretionary snack items. Although present at less than 1% in those options and not detected in any participant fecal samples, suggesting limited probiotic exposure, the possible implications of these dietary differences on our findings remain unclear.

In terms of clinical relevance, our study was not powered for clinical endpoints, and the primary outcomes were gut microbiome measures rather than clinical outcomes. Preliminary evidence suggested nominal between-group differences in hip circumference, constipation symptoms, and albumin concentration; however, none remained statistically significant after correction for multiple comparisons, underscoring the limited clinical utility of these findings at this stage. The higher rate of adverse events in the supplement-based group likewise represents a hypothesis-generating signal only. More broadly, our trial reflects the current state of the field, in which promising microbiome science has yet to be translated into routine clinical care. As highlighted in a recent perspective ("The microbiome for clinicians"), multiple biological, methodological, and translational barriers have prevented the integration of microbiome endpoints into clinical medicine.[67] Although we followed the standardized microbiome reporting initiative STORMS (Strengthening the Organizing and Reporting of Microbiome Studies), alongside CONSORT (Consolidated Standards of Reporting Trials)[68,69] (see STAR Methods), to enhance the translatability and reproducibility of our work, larger, longer-term, and clinically focused trials will be required to determine whether the gut microbiome differences observed here translate into meaningful patient outcomes.

RESOURCE AVAILABILITY

Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Dr. Melissa M. Lane (m.lane@deakin.edu.au).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Raw shotgun metagenomic sequencing data are stored on Deakin University's Research Data Store, a secure institutional repository managed by Deakin University. These data cannot be deposited in an external public repository because participant consent for open sharing was not obtained at recruitment. Participant-level data can be requested from the lead contact with a detailed research proposal outlining the intended use of the data. Data access will only be provided once an appropriate Human Research Ethics Committee (HREC) or equivalent institutional approval is in place and will be managed in line with Barwon Health (primary sponsor) and Deakin University (secondary sponsor) governance requirements.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this work is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization, M.M.L., M.M., A.L., A.O'.N., W.M., and F.N.J. Data curation, M.M.L. and A.J.M. Formal analysis, M.M.L., A.J.M., M.M., M.L., A.L., and M.O'.H. Funding acquisition, F.N.J. and W.M. Investigation, A.J.M., J.B., and L.S. Methodology, M.M.L., M.M., A.L., A.O'.N., W.M., and F.N.J. Project administration, M.M.L. and A.J.M. Resources, R.P. and S.B. Supervision, M.M., A.L., A.O'.N., M.K., M.B., W.M., and F.N.J. Visualization, A.M., M. M., M.L., and M.O'.H. Writing—original draft, M.M.L. and A.J.M. Writing—review & editing: all authors.

DECLARATION OF INTERESTS

M.M.L. is a committee member (2019–present) and former secretary (2022–2024) of the Melbourne Branch Committee of the Nutrition Society of Australia (both unpaid) and has received travel funding support from the International Society for Nutritional Psychiatry Research, the Nutrition Society of Australia, the Australasian Society of Lifestyle Medicine, and the Gut Brain Congress. A. J.M. is immediate past secretary for the International Society for Nutritional Psychiatry Research (unpaid) and is funded through the National Health and Medical Research Council (NHMRC)-supported CREDIT CRE, the Center for Research Excellence for the Development of Innovative Therapies. M.B. is supported by an NHMRC Senior Principal Research Fellowship (1156072). M.B. has received grant/research support from the NIH, Cooperative Research Center, Simons Autism Foundation, Cancer Council of Victoria, Stanley Medical Research Foundation, Medical Benefits Fund, National Health and Medical Research Council, Medical Research Futures Fund, Beyond Blue, Rotary Health, the a2 Milk Company, Meat and Livestock Board, Woolworths Limited, and Avant and the Harry Windsor Foundation and has been a speaker for Abbot, Astra Zeneca, Janssen and Janssen, and Lundbeck and Merck and served as a consultant to Allergan, Astra Zeneca, Bioadvantex, Bionomics, Collaborative Medicinal Development, Janssen and Janssen, Lundbeck and Merck, Pfizer, and Servier—all unrelated to this work. A.L. has received grant, research, or travel support from Deakin University, the University of Melbourne, RMIT University, the National Health and Medical Research Council, Australian Academy of Science, the Jack Brockhoff Foundation, Epilepsy Foundation of Australia, and American Epilepsy Society and has received speakers' honoraria from the European Space Agency and Swisse Australia—all unrelated to this work. A.L. is a named inventor on a patent relating to Prevotella. M.M. has received grant/research support from the NHMRC, Deakin University School of Medicine, Deakin Biostatistics Unit, Institute for Mental and Physical Health and Clinical Translation, Stroke Foundation, and Medibank Health Research Fund. W.M. is currently funded by an NHMRC Investigator grant (#2008971). W.M. has previously received funding from the Cancer Council Queensland and university grants/fellowships from La Trobe University, Deakin University, the University of Queensland, and Bond University. W.M. has received funding and/or has attended events funded by Cobram Estate Pty. Ltd. and Bega Dairy and Drinks Pty Ltd. W.M. has received travel funding from the Nutrition Society of Australia. W.M. has received consultancy funding from Nutrition Research Australia and ParachuteBH. W.M. has received speakers' honoraria from VitaFoods, the Cancer Council Queensland, and the Princess Alexandra Research Foundation. F.N. J. has received competitive grant/research support from the Brain and Behavior Research Institute, the NHMRC, Australian Rotary Health, the Geelong Medical Research Foundation, the Ian Potter Foundation, and the University of Melbourne; industry support for research from Meat and Livestock Australia, Woolworths Limited, the a2 Milk Company, and Be Fit Food; philanthropic support from the Fernwood Foundation, Wilson Foundation, the JTM Foundation, the Serp Hills Foundation, the Roberts Family Foundation, and the Waterloo Foundation; and travel support and speakers' honoraria from Sanofi-Synthélabo, Janssen Cilag, Servier, Pfizer, Network Nutrition, Angelini Farmacéutica, Eli Lilly, and Metagenics. F.N.J. has written two books for commercial publication. She is on the scientific advisory board of the Dauten Family Center for Bipolar Treatment Innovation and Zoe Limited. She is currently supported by an NHMRC Investigator grant L1 (#1194982). The Food & Mood Center has received grant/research support from the a2 Milk Company, Be Fit Food, Meat and Livestock Australia, and Woolworths Limited and philanthropic support from the Fernwood Foundation, Wilson Foundation, the JTM Foundation, the Serp Hills Foundation, the Roberts Family Foundation, and the Waterloo Foundation. A.O'N. receives funding support from the NHMRC (#2009295).

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors used ChatGPT to assist with refining text and statistical code. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the final content of the publication.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Human fecal and blood samples	This paper	N/A
Critical commercial assays		
FLOQSwab-ADT (active drying tube with internal desiccant)	Copan Italia SPA	https://www.copangroup.com/
Alcohol wipes (70% isopropyl alcohol)	Briemarpak	https://www.briemarpak.com.au/
BD Vacutainer® tubes (yellow top)	Becton Dickinson	https://www.bd.com/en-anz/
Butterfly needles and barrels	Becton Dickinson	https://www.bd.com/en-anz/
BD™ Cytometric Bead Array assay platform	BD Biosciences	https://www.bdbiosciences.com/
Radioimmunoassay (Iodine 125) kit for leptin	Merck Millipore	https://www.sigmaaldrich.com/AU/en/
ADVIA® Chemistry kits	Siemens Healthineers	https://www.siemens-healthineers.com/en-au/
DNeasy 96 PowerSoil Pro QIAcube HT Kit	Qiagen	https://www.qiagen.com/au/
QIAcube HT DNA extraction system	Qiagen	https://www.qiagen.com/au/
PowerBead Pro Tubes	Qiagen	https://www.qiagen.com/au/
Quant-IT™ PicoGreen™ dsDNA Assay Kit	ThermoFisher Scientific	https://www.thermofisher.com/
Illumina DNA Prep (M) Tagmentation Kit	Illumina	https://www.illumina.com/
IDT for Illumina DNA/RNA UD Index Sets A-D	Illumina	https://www.idtdna.com/
QIAxcel DNA High Resolution Kit	Qiagen	https://www.qiagen.com/au/
NovaSeq 6000 Sequencing System	Illumina	https://www.illumina.com/
Software and algorithms		
REDCap	REDCap	https://redcap.deakin.edu.au/
FoodWorks Professional	Xyris Software Pty Ltd	https://foodworks.online/editions/foodworks-professional/
R	R Project for Statistical Computing	https://www.r-project.org/
RStudio	Posit	https://posit.co/
Microba Gene and Pathway Profiler (MGPP) v1.0	Microba Life Sciences	https://www.microba.com/
Microba Genome Database (MGDB) v1.0.3	Microba Life Sciences	https://www.microba.com/
MMSeqs2 Release 10-6d92c	Steinegger & Söding, 2017[70]	https://github.com/soedinglab/MMseqs2
enrichM	Woodcroft et al., 2020[71]	https://github.com/geronimp/enrichM
UniProt ID mapping service	UniProt Consortium	https://www.uniprot.org/
Other		
Clinical Trial Registration	Australian New Zealand Clinical Trials Registry	ACTRN12620000301965 at http://anzctr.org.au/
−80°C secure freezer facility	Barwon Health, Geelong, Australia	N/A
Clinical pathology services (blood sample processing and biochemical assays)	Australian Clinical Labs, Clayton, Australia	https://www.clinicallabs.com.au/
Clinical pathology services (serum inflammatory marker assays)	SA Pathology, Adelaide Women's & Children's Hospital, Australia	https://www.sapathology.sa.gov.au/
Dietary Questionnaire for Epidemiological Studies v3.2 scoring service	Cancer Council Victoria, Nutritional Assessment Office	https://www.cancervic.org.au/research/epidemiology/

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

The MicroFit Study was a three-week, investigator-initiated, single-blind, two-arm, parallel-group randomized controlled-feeding trial with computer-generated 1:1 randomization. Women aged 30–65 years with BMI 30–45 kg/m^2 were recruited from a community-based sample in the south-west region of Victoria, Australia. Participants were screened against eligibility criteria online using REDCap,[72,73] and research assistants confirmed eligibility and obtained informed consent via telephone. At the baseline assessment, research assistants collected anthropometric measurements to verify participants' eligibility based on BMI. Eligible participants were randomized to a meal replacement program comprising primarily food-based or supplement-based VLED options. A uniform calorie limit for the VLEDs was chosen to ensure consistency, simplify adherence and monitoring, and enable a more controlled comparison than varying caloric restriction based on individual energy needs. Participants attended in-person study visits at baseline and week three at Australian Clinical Labs (Geelong, Australia), where a phlebotomist collected serum samples after an overnight fast. Participants were provided kits to collect fecal samples at home at baseline and week three, which they sent via prepaid mail to Microba Pty Ltd (Brisbane, Australia) for analysis. Questionnaire data, including sociodemographic, dietary, and health-related factors, were self-reported at both study timepoints by participants at home using REDCap online. Adverse events were monitored from consent until study completion (week 3), recorded by the study team when reported by participants, and assessed for severity and relatedness to the intervention according to Barwon Health research monitoring guidelines. An *a priori* sample size calculation was conducted based on 40 participants (20 in each arm). However, by study completion, we randomized 47 participants to account for missing baseline data and higher than anticipated dropout, and the power calculation was adjusted accordingly. A schematic overview of the study design, including participant characteristics, intervention arms, and assessment timepoints, is presented in Figure S3.

Participants were informed that the purpose of the study was to examine whether VLEDs influence the type and number of gut microbiota in the gastrointestinal tract, and to assess associations between gut microbiota and BMI, weight, waist circumference, blood markers, symptoms of depression, anxiety and stress, perceived wellbeing, sleep patterns, stool consistency, and gastrointestinal symptoms.

This trial was conducted following the principles of the Declaration of Helsinki, received ethical approval from the Barwon Health (19/112) and Deakin University (2018/211) Human Research Ethics Committees and was registered on the Australian New Zealand Clinical Trials Registry (ACTRN12620000301965). This manuscript is presented as per the Consolidated Standards of Reporting Trials (CONSORT) statement and checklist[69] and gut microbiome data are reported as per the Strengthening the Organizing and Reporting of Microbiome Studies (STORMS) checklist.[68] The completed checklists and additional details are provided in the supplemental information (see Tables S11 and S12). The trial and manuscript development did not involve patients or the public owing to the absence of funding to support consumer engagement for this research.

METHOD DETAILS

Recruitment and participants

Community-based recruitment was conducted from May 2021 to February 2022 using online platforms hosted by Deakin University and Barwon Health (e.g., Facebook, Instagram, Twitter, Barwon Health online newsletter), distributing flyers to local general practitioner offices, and through a variety of both paid and free online advertising services.

Inclusion criteria were: female sex, chosen to reduce interindividual heterogeneity; with a BMI between 30 and 45 kg/m^2 to align with guidelines recommending VLEDs for individuals in this BMI range[74]; aged 30–65 years; able to commit to all study procedures, including attending in-person appointments and consuming only the investigational products and recommended extras for the study duration; able to understand study materials and directions presented in English; with access to the internet and a computer, smartphone, or tablet; and able to consent to not to enrolling in another clinical trial while taking part in the study.

Exclusion criteria were: currently consuming VLED products; having a diagnosed food allergy or food intolerance; receiving treatment with medications related to obesity; confirmed/suspected/planned pregnancy, or lactating; diagnosed with or having commenced a new treatment for, anxiety and/or depression within one month before baseline; having gastrointestinal disease or history of major gastrointestinal surgery; having a pre-existing cardiometabolic conditions; having had a heart attack within the past six months; having a diagnosed eating disorder; having other major medical conditions likely to have systemic effects or deemed unfit for study participation by the research team (e.g., type 2 diabetes, prediabetic, insulin resistance); regularly (subjective, as defined in our study protocol and not quantified due to individual variations in interpretation) using opioid-based medications; regularly using recreational or illicit drugs; regularly using sodium-glucose co-transporter-2 inhibitors (i.e., gliflozins); having used antibiotics, prebiotics, and/or probiotics in the month before baseline; and having been enrolled in another clinical trial within the past three months.

Randomization, allocation, and blinding

Eligible participants were randomly assigned in a 1:1 ratio to either the food-based or supplement-based VLED using a computer-generated randomization sequence with randomly ordered blocks of sizes 2 and 4. This sequence was created by a study statistician and input into REDCap online by an independent researcher to ensure allocation concealment from the study investigators. An

unblinded trial coordinator enrolled and informed participants of the VLED program to which they had been assigned. The outcome assessors (research assistants) and all other study investigators, including the analyzing statisticians, remained blinded to the group allocations until data analysis completion. As the study was single-blind, participants were aware of their group allocations and instructed not to discuss their diet with the outcome assessors to preserve blinding.

Interventions

The VLEDs were intended to be matched in overall energy (800–900 kcal per day), macronutrient profiles, sugar, sodium, and fiber. The supplement-based VLED comprised three daily total meal replacement options (16 items). Participants chose from a selection of powdered shakes in banana, caramel, chocolate, coffee, mocha, strawberry, and vanilla flavors; soups in chicken and vegetable flavors; bars in almond butter, berry, cappuccino, chocolate, and cereal flavors; and, desserts in chocolate and lemon crème flavors. On average, approximately 70% of the composition of these options was made of extracted, refined, fractionated, modified, and/or isolated proteins (e.g., calcium caseinate), carbohydrates (e.g., maltodextrin), fats (e.g., medium chain triglycerides), and fibers (e.g., fructo-oligosaccharide), as well as added vitamins (e.g., vitamin B1) and minerals (e.g., potassium citrate), and additives like emulsifiers (e.g., citric and fatty acid esters of glycerol [472c]), non-sugar sweeteners (e.g., aspartame [951]), flavors (e.g., unspecified "flavor"), colors (e.g., curcumin [100]), thickeners and stabilizers (e.g., vegetable gum [414]). The remaining 30% consisted primarily of whole powdered milk. The food-based VLED comprised three daily total meal replacement options (55 items) and a discretionary snack (11 items). Participants chose from a selection of pre-prepared meals, including various porridge, egg, and vegetable dishes for breakfast, with lunches and dinners composed of baked and stuffed items, casseroles, curries, meatballs, pasta, rice dishes, stir-fries, and soups. On average, approximately 93% of the composition of these options was made of vegetables (e.g., green cabbage), fruits (e.g., banana), whole grains (e.g., oats), beans (e.g., cannellini beans), legumes (e.g., chickpeas), lean meats (e.g., chicken), dairy cheeses (e.g., ricotta), nuts (e.g., almond meal), seeds (e.g., flaxseed), herbs (e.g., parsley), and spices (e.g., cinnamon). The remaining 7% of the composition consisted primarily of protein isolates (e.g., whey protein isolate), as well as additives such as emulsifiers (e.g., soy lecithin [322]), non-sugar sweeteners (e.g., stevia [960]), flavors (e.g., vanilla extract), thickeners and stabilizers (e.g., guar gum [412]), with approximately less than 1% of the composition of discretionary snacks including an added and isolated fiber (e.g., oligofructose) and the probiotic *Lactobacillus plantarum* (now *Lactiplantibacillus plantarum*). The full list of the options in each group and their ingredients are detailed in the supplemental information (see Table S13).

Participants in both groups selected their meal replacements online weekly, with all consumables and home delivery provided free of charge. They could choose any three meal replacements to consume at any time throughout the day. Participants were also permitted to include additional 'recommended extras' foods from a predetermined list (see Table S14). The supplement-based group was recommended to consume at least two cups of low starch vegetables. The food-based group was recommended to include one additional fruit or protein snack and three serves of side salads or vegetables. Non-sugar sweeteners, diet jelly desserts, and sugar-free lollies and gum were not recommended for daily use in the food-based group, with diet cordial and diet soft drinks recommended as occasional options. The supplement-based group were recommended to consume these items *ad libitum*, with no restrictions. Participants logged their daily food consumption using the Easy Diet Diary application (app) throughout the entire study period. These data were used to monitor adherence to the approximately 900 kcal/d target and to assess macronutrient intake, which was analyzed using Australian food composition databases via the FoodWorks Professional nutrient analysis software (Xyris Software Pty Ltd, Brisbane, Australia.[75,76] Several strategies were employed to optimize reporting accuracy and minimize burden within the practical constraints of a free-living trial. Participants received standardized and group-specific dietary guidance, used the app daily (pre-populated with trial consumables and featuring 'search as you type' and barcode functionality, with demonstrated feasibility, acceptability, and relative validity[77]), and were supported by weekly staff contact. Additionally, the provision of food, particularly meal replacements, can enhance dietary adherence by increasing convenience and allowing for tighter control compared to participant-prepared meals.[78,79]

Sample size

An *a priori* sample size calculation was conducted, informed by a similar study available at the time of protocol development that used alpha diversity metrics, including the Shannon index.[80] The Shannon index was selected as the primary outcome for this manuscript due to its inclusion in the sample size calculation, as well as its widespread use and sensitivity in detecting between-group differences in microbiome research.[81] A sample size of 40 participants (20 in each arm) had 80% power with an alpha of 5% to detect large between-group difference effect sizes (d = 0.91) in the Shannon index. An *a posteriori* power calculation based on 45 participants had 80% power to detect effect sizes of d = 0.85.

Sample collection

Fecal samples (~15g) were collected by participants at baseline and week eight using a Copan Italia SPA FLOQSwab in an active drying tube, including an internal desiccant to preserve samples at room temperature for up to four weeks. Participants were instructed to collect their fecal sample within 48 h after their in-person appointments. Fecal samples were sent directly to Microba via post at room temperature where they were stored at −80°C until further processing. Fasted blood samples (40mL) were collected and stored at ACL as per standard procedures.

DNA extraction

Fecal samples were extracted using the DNeasy 96 PowerSoil Pro QIAcube HT Kit (Qiagen 47021) in a 2mL deep well plate format with a modified initial processing step on the QIAcube HT DNA extraction system (Qiagen 9001793). Mechanical lysis was performed with PowerBead Pro beads (Qiagen 19311). DNA was quantified using a high-sensitivity dsDNA fluorometric assay (QuantIT, ThermoFisher, Q33120), with samples needing to reach a minimum of 0.2 ng/μL for quality control.

Library preparation

Libraries were constructed using the Illumina DNA Prep (M) Tagmentation Kit (Illumina, 20018705) with IDT for Illumina DNA/RNA UD Index Sets A-D (Illumina 20027213-16), modified to accommodate processing in a 384-plate format. Individual libraries were pooled in equimolar amounts and assessed using a high-sensitivity dsDNA fluorometric assay (QuantIT, ThermoFisher, Q33120) and visualized with capillary gel electrophoresis using the QIAxcel DNA High Resolution Kit (Qiagen, 929002).

Shotgun metagenomic sequencing

Samples were sequenced on the NovaSeq6000 (Illumina) using v1.5 300bp PE sequencing reagents to a target depth of 3Gb, with a minimum of 2Gb (approximately 7–16 million paired-end reads per sample). Sequence data were reviewed for yield and quality, with known control samples included in each run to monitor for background contamination. Paired-end DNA sequencing data were demultiplexed and adaptor trimmed using Illumina BaseSpace Bcl2fastq2 (v2.20) with one mismatch allowed in index sequences. Reads were quality trimmed and residual adaptors removed using Trimmomatic v0.39 with parameters: -phred33 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 CROP:100000 HEADCROP:0 MINLEN:100. Human DNA was removed by aligning reads to the human genome reference assembly 38 (GRCh38.p12) using bwa-mem v0.7.17 with a minimum seed length of 31 (-k 31). Alignments were filtered using SAMtools v1.7, and reads mapping to the human genome with >95% identity over >90% of the read length were flagged as human DNA and removed.

Species profiles and functional potential

Species profiles were obtained using the Microba Community Profiler (MCP) v1.0 and the Microba Genome Database (MGDB) v1.0.3, with reads assigned to genomes within MGDB to estimate and report the relative cellular abundance of species clusters. Quantification of gene and pathway abundance was performed using the Microba Gene and Pathway Profiler (MGPP) v1.0 against the Microba Genes (MGENES) database v1.0.3. Open reading frames from genomes in MGDB were clustered against Uniref. 90 (release 2019/04) using MMSeqs2 with 90% identity over 80% of read length. Gene clusters were annotated with Uniref. 90 identifiers and linked to Enzyme Commission and Transporter Classification Database annotations via the UniProt ID mapping service. Enzyme Commission annotations were then used to determine MetaCyc pathway encoding using enrichM, with pathways classified as encoded if completeness exceeded 80%. DNA sequencing read pairs aligning with gene sequences from any protein within an MGENES protein cluster were summed. The abundances of encoded pathways for species detected by MCP were calculated by averaging the read counts of all genes for each enzyme in the pathway.

Data preparation

Data normalization was performed by down-sampling to a standardized number of reads (7,000,000) before profiling within the MCP. One sample (food-based, week 3) had low read count (2,779,002) and was removed in sensitivity analyses. As per recommendations to account for variation in sequencing depth,[82,83] alpha diversity was calculated using raw count data, with rarefaction applied to match the smallest total number of prokaryotic reads across all samples (4,139,908). All other secondary gut microbiome outcome analyses, including beta diversity, taxonomic composition, and functional potential, were conducted using non-rarefied data. CLR transformations were utilized before conducting beta-diversity and differential abundance statistical tests (species, genus, family, and phylum) given the compositional and non-normal nature of microbiome relative abundance data.

Alpha diversity, beta diversity, taxonomic composition, and functional potential

Alpha diversity, which summarizes community structure within a sample, was evaluated at the species-level using two metrics: the Shannon index (primary outcome) and richness (secondary outcome). The Shannon index provides a comprehensive measure of community structure by considering both the number of detected species (richness) and how evenly distributed the species are (evenness); communities with higher numbers of detected species and more even distributions of these species will result in a higher Shannon index. Richness, which quantifies only the number of different species present in each sample, was chosen as a secondary outcome measure of alpha diversity due to its more limited capacity to capture community structure. In addition, alpha diversity was assessed at the functional level using MetaCyc pathway annotations as a secondary outcome. Beta diversity, also a secondary outcome, which summarizes between-sample differences in community structure, was calculated using Aitchison distances, defined as the Euclidean distance between CLR-transformed samples, via the *stats*[84] package. Principal Component Analysis (PCA) was used to reduce data dimensionality for visualization in two dimensions to identify patterns or clusters of samples within the dataset using the *Tjazi*[85] package. Additionally, taxonomic composition was assessed by identifying and quantifying bacterial species, genera, families, and phyla using shotgun metagenomic sequencing. The analysis of functional potential, focused on MetaCyc pathways, was also conducted to assess the microbiome's functional capabilities. Throughout, we report traditional phylum- and

genus-level taxonomic names for clarity and continuity, while acknowledging recent taxonomic reclassifications.[86] For certain taxa (e.g., *GCA-900066905*), the same identifier is used at both the family and genus levels due to incomplete taxonomic classification in reference databases.

Secondary clinical outcomes

Anthropometric measurements included height (stadiometer), weight (electric scales), and hip and waist circumferences (measuring tape). BMI was calculated as: BMI = (weight, kg)/(height, m).[2] Serum inflammation markers (homocysteine, IL-β, IL-6, and TNF-α) were assayed using the BDtm Cytometric Bead Array platform (SA Pathology, Adelaide Women's and Children's Hospital). Serum leptin was measured using Merck Millipore radioimmunoassay kits (Royal Prince Alfred's Central Sydney Pathology Services). Other serum biomarkers (glucose, insulin, liver function markers (ALT, GGT, ALP, AST, total bilirubin, albumin, protein, and globulin), and lipid markers (total cholesterol, HDL, LDL, non-HDL, LDL/HDL ratio, cholesterol/HDL ratio, triglycerides) were analyzed using Siemens' ADVIA Chemistry kits (Australian Clinical Labs, Victoria). Self-reported measures included: mental health symptoms using the Depression Anxiety Stress Scale-21 (DASS-21),[87] with higher scores indicating more severe symptoms; perceived well-being using the World Health Organization Wellbeing Scale (WHO-5),[88] with higher scores indicating better well-being; sleep-related difficulties using the Athens Insomnia Scale (AIS),[89] with higher scores indicating more severe issues; gastrointestinal symptoms using the Visual Analogue Scale for Irritable Bowel Syndrome (VAS-IBS),[90,91] with higher scores indicating more favorable symptom ratings ("very good"); stool consistency using the Bristol Stool Form Scale (BSFS),[92] a 7-point scale spanning from firmest (1) to softest (7) stool, with a mean score calculated for each participant across one week; and physical activity using the International Physical Activity Questionnaire-Short Form (IPAQ-SF),[93] with categorical scores estimated (i.e., low, moderate, high).

QUANTIFICATION AND STATISTICAL ANALYSIS

All statistical analyses were conducted using R in the RStudio environment.[94] Participant baseline characteristics were summarized with mean and standard deviation for continuous variables (or median and interquartile range where appropriate) and frequency and percentage for categorical variables. To assess both primary and secondary outcomes, we conducted our main analyses using a modified intention-to-treat (mITT) approach, which included all participants who provided baseline data, regardless of follow-up completion. We also conducted secondary complete case analyses, which included only participants who provided both baseline and week three data, to confirm the robustness of the results. We used linear mixed-effects regression (LMER) models to estimate between-group (food-based vs. supplement-based) differential changes (week three vs. baseline) in species-level alpha diversity, as measured by the Shannon index (primary outcome), and secondary outcomes using the *lme4*[95] package. The models included participant as a random effect, and group allocation, nominal time point, and the interaction between diet group and time point (i.e., diet group x time point) as fixed effects. The interaction estimated the between-group differential changes from baseline to week three using beta-coefficient point estimates (β) with 95% confidence intervals (95%CI) and *p*-values (two-tailed; $p < 0.05$ for significance).[96] The supplement-based VLED group was set as the reference group. A positive β indicates a greater increase or smaller decrease in the food-based group compared to the supplement-based group. Conversely, a negative β reflects a greater increase or smaller decrease in the supplement-based group. When both groups change in the same direction (e.g., both increase or both decrease), the β reflects a difference in the magnitude of change. When groups change in opposite directions, the β reflects the divergence in trend, with the sign indicating which group changed more in the positive (or less in the negative) direction. Importantly, the β alone does not convey the absolute direction of change in either group; this must be determined by examining within-group estimates or visualized using change-from-baseline plots. Mean within-group changes (with 95%CIs) are provided to infer directionality, however should be interpreted with caution due to the limited sample size and low statistical power. Missing covariate and secondary outcome data were imputed using the *mice*[97] package (five imputations), with predictive mean matching employed for continuous outcomes (alcohol intake) and proportional odds logistic regression employed for ordered factors (household income, menopause status). Interaction plots of the estimated marginal means were created using the *emmeans*[98] package.

For beta diversity, we used complete cases to create individual CLR component-wise change scores (baseline minus week three) and then used permutational analysis of variance using adonis2 via the *vegan*[99] package with 999 permutations to calculate the between-group differential change (i.e., beta diversity change ~ group). We report the R-squared (r^2) statistic, providing a measure of the proportion of variance explained by the grouping factor (i.e., diet group) in the model.

We applied the Benjamini-Hochberg procedure[100] to adjust for multiple comparisons. Outcomes were grouped into related categories (e.g., gastrointestinal outcomes) and multiple comparisons testing was conducted within each category ($q < 0.1$ for significance given the small sample size). In post hoc sensitivity analyses for the primary outcome, we adjusted for prognostic covariates individually due to the limited sample size. These included country of birth, marital status, employment, medication use, stool consistency, BMI, physical activity, menopause status, and alcohol intake. Adjustments were based on baseline group imbalances, assessed using Standardized Mean Differences (>0.25, a threshold considered reasonable for acceptable standardized biases[101]). BMI was the sole anthropometric covariate considered due to its high correlation with weight and hip and waist circumferences. Additional models adjusted for age and postmenopausal status (SMDs <0.25), included to confirm the robustness of findings. One sample with a low read count was also removed.

Post hoc exploratory analyses

All post hoc analyses described below were exploratory and descriptive in nature. Consequently, no formal statistical inference was conducted, and no correction for multiple testing was applied. *p*-values are reported for transparency where relevant but should be interpreted with caution. We first examined post hoc Spearman's correlations (r) between changes in our primary outcome (species-level Shannon index) and changes in secondary clinical outcomes over the three-week intervention period. Data were pooled across intervention groups. Only associations with r values $\geq$ 0.30 in magnitude were reported, consistent with thresholds indicating small-to-moderate or greater effect sizes.[102]

To explore the microbial contributors of within-sample alpha diversity change, we decomposed the Shannon diversity index into species-level contributions at both baseline and week three. Raw species-level metagenomic counts were transformed to relative abundances within each sample. For each taxon, its contribution to the Shannon index was calculated as $-p \cdot \log(p)$, where p is the relative abundance. Mean contributions were then averaged within each group and timepoint, and the change in contribution (week three minus baseline) was computed for each taxon. To facilitate interpretation and reduce potential distortion from low-impact species, we identified the top 30 taxa with the largest absolute changes in Shannon contribution within each group. All remaining species were aggregated into a single "Other_net" category, capturing the summed net contribution of taxa outside the top 30, thus preserving the total change in diversity. Bar plots were generated to visualize the top 30 contributing species and the Other_net category within each intervention group. This descriptive visualization was used to qualitatively examine patterns in taxon-level contributions to Shannon diversity change across groups.

To further provide some ecological context, the top 30 contributing species were also classified as dominant or subdominant based on their mean relative abundances at baseline across all samples in both arms: taxa with $\geq$1% mean relative abundance were considered dominant, while those <1% were classified as subdominant.[103,104] This threshold was applied to distinguish between more prevalent community members and rarer taxa, helping to contextualize whether the top contributors to the change in Shannon index in each group were relatively abundant (i.e., dominant) or less abundant (i.e., subdominant) species at baseline.

Finally, to assess overall microbial community structure, taxonomic data were aggregated from species to phylum level using the tax_glom() function in the *phyloseq*[105] package. To facilitate compositional comparisons across samples, counts were transformed to relative abundances by dividing each taxon's count by the total count for that sample, using the *microbiome*[106] package. The resulting phylum-level relative abundances were visualized using stacked bar plots generated with *ggplot2*, stratified by intervention group (food-based vs. supplement-based) and time point (baseline vs. week three).

ADDITIONAL RESOURCES

Australian New Zealand Clinical Trials Registry Identifier: ACTRN12620000301965.