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Effects of a healthy diet based on seed-rich vegetables on the gut microbiota and intrinsic brain activity in perimenopausal women: A pilot study on cognitive improvement

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Brain functional changes and gut microbiota dysbiosis have been observed in perimenopausal syndrome (PMS). We evaluated the effects of a plant-based daily diet enriched with *Raphanus sativus L.* (RSL, radish seed) on the gut microbiota composition, gastrointestinal symptoms, resting-state local spontaneous brain activity, and neuropsychology in perimenopausal women. For 12 weeks, the participants were instructed to adhere to a controlled, *Raphanus sativus L.*-rich plant-based diet (a mean RSL intake of 5 g/day). Two test days were organized: before and after the nutritional intervention. The fecal microbiota composition, gastrointestinal symptoms, resting-state brain function, and neuropsychology were assessed twice. A longitudinal single-arm study was conducted on 24 perimenopausal women. The Montreal Cognitive Assessment (MoCA) scores tended to improve in the visuospatial/executive function subitem and in the total score after the diet. The participants presented elevated amplitude of low-frequency fluctuation (ALFF) values in the left middle occipital gyrus, the left precentral gyrus, and the left middle cingulum gyrus. The abundances of the phyla *Synergistetes* and *Verrucomicrobia* were positively correlated with the ALFF values of the left middle occipital gyrus, left precentral gyrus, and left middle cingulum gyrus. These data suggest that specific gut microbes may modulate intrinsic brain activity and cognitive function in perimenopausal women. A plant-based RSL-rich diet has beneficial effects on the gut microbial composition and brain function of perimenopausal women.

Keywords Perimenopausal syndrome, *Raphanus sativus L.*, Diet, Gut microbiota, Resting-state functional magnetic resonance imaging, Interaction

Abbreviations

AD	Alzheimer's Disease
ALFF	Amplitude of Low-Frequency Fluctuation
AAL	Anatomical Automatic Labeling
IQR	Interquartile Range
FALFF	Fractional Amplitude of Low-Frequency Fluctuation
Lefse	Linear Discriminant Analysis Effect Size
LDA	Linear Discriminant Analysis
KI	Kupperman Index
KEGG	Kyoto Encyclopedia of Genes and Genomes

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MoCA	Montreal Cognitive Assessment
MRI	Magnetic Resonance Imaging
MMSE	Minimum Mental State Examination
MNI	Montreal Neurological Institute
OUT	Operational Taxonomic Unit
PMS	Perimenopausal Syndrome
PCR	Polymerase Chain Reaction
PCoA	Principal Coordinate Analysis
PICRUSt	Phylogenetic Study of Communities By Reconstruction Of Unobserved States
RSL	Raphanus Sativus L
rs-fMRI	Resting-State Functional Magnetic Resonance Imaging
ReHo	Regional Homogeneity
ROI	Regions of Interest
SFE	Sulforaphene

Perimenopause is the transitional period before menopause and is accompanied by depletion of ovarian follicles and fluctuating hormone levels in women[1]. Perimenopausal syndrome (PMS) is a series of symptoms caused by fluctuations in estrogen levels around the time of menopause, which lead to both physical and psychological disorders in women (hot flashes, night sweats, sleep disturbances, depressive symptoms, vasomotor dysfunction, mood disorders, etc.)[2]. Hormonal disorders and cytokine dysregulation are the main causes of PMS[1–4]. Perimenopausal symptoms not only impair quality of life but also may be associated with diabetes[5], cardiovascular diseases[6,7], cancer[8], and neurodegenerative diseases[9]. Menopausal women have been reported to suffer from cognitive dysfunction, learning disabilities, and memory decline[10,11]. In addition, the menopausal transition increases the risk of Alzheimer's disease (AD)-related brain changes and is considered a period of neurological transition[9,12]. PMS poses a serious threat to the physical and mental health of middle-aged and older women; therefore, more attention should be given to the prevention and treatment of PMS.

The gut microbiota is a population of microorganisms found in the gastrointestinal tract and is closely associated with health and disease[13]. It has been suggested that the gut microbiota composition is influenced by the estrogen level and that the gut microbiome also significantly affects the estrogen level[14,15]. Gut microbiota dysbiosis can occur in PMS[16]. In estrogen-sufficient women, the intestinal microbiota is diverse, with a predominance of beneficial bacteria[17]. In pregnant women with PMS, the relative abundance of beneficial bacteria such as *Aggregatibacter*, *Lactobacillus,* and *Bifidobacteria* significantly decreased, whereas those of harmful bacteria such as *Enterobacter* increased[16,18,19]. In addition, the gut microbiota may undergo specific compositional changes during perimenopause[19,20]. Therefore, maintaining the gut microbial balance is crucial to the health of perimenopausal women. There is a bidirectional relationship between the gut microbiota and the brain, referred to as the microbiota-gut-brain-axis[21]. Preliminary evidence suggests that the gut microbiota is an important environmental factor that closely correlates with brain function (including cognition, mood, and social behavior) and is potentially linked to neurodegenerative diseases, such as AD[22–26].

Interactions between the gut and the brain form a complex network involving neuronal, endocrine and immune pathways[24,27,28]. Because it is difficult to describe the human brain cellular network precisely, few studies have established a direct link between the gut microbiota and brain function in vivo[19,29]. In patients with amnestic mild cognitive impairment, there is a specific pattern of interaction between the gut microbiota and intrinsic brain activity or mental function. the relative abundances of *Bacteroides*, *Ruminococcaceae*, *Clostridiaceae*, *Blautia*, and *Veillonellaceae* are correlated with the inherent activity of different brain areas. Members of *Clostridium* (*Lachnospiraceae* and *Blautia*), as well as *Veillonellaceae*, are associated with cognition[29]. Four weeks of probiotic intake from fermented dairy products in healthy women led to significant changes in the intrinsic activities of the resting brain[30]. They grouped healthy women into *Bacteroides*-abundant and *Prevotella*-abundant groups according to genus-based clusters. The *Prevotella* group presented decreased hippocampal activity when negative valence images were taken[31]. To the best of our knowledge, no studies to date have detailed the interactions between the gut microbiota and brain function in women with PMS. However, abnormal spontaneous activities in multiple brain regions (frontal, temporal, and hippocampal areas) have been detected in perimenopausal women during resting states[32–37]. These changes may be related to emotional and cognitive impairments and potentially represent the neural mechanisms of cognitive dysfunction in perimenopausal women.

Analysis of intrinsic brain activity is crucial for understanding the pathogenesis of PMS and its early detection[32,38]. Resting-state functional MRI (rs-fMRI) detects brain activity when a subject is awake but not performing a specific task or responding to external stimuli[39]. It reflects the spontaneous activity of the central nervous system in the basal state[40]. Common measures of rs-fMRI include regional homogeneity (ReHo), which assesses the consistency of activity between adjacent voxel regions[39], and the amplitude of low-frequency fluctuation amplitude (ALFF) and fractional ALFF (fALFF), which measures the intensity of regional spontaneous brain activity[41,42]. The rs-fMRI technique has also been used to evaluate brain function in perimenopausal women[33,34,43]. Fluctuating estrogen levels during perimenopause exacerbate PMS-related cognitive decline through multiple mechanisms: (1) Reduced estrogen directly inhibits BDNF-dependent synaptic plasticity in the hippocampus, impairing memory encoding efficiency[44,45]; (2) Estrogen receptor β-mediated disruption of gut barrier integrity promotes translocation of microbiota-derived LPS into circulation, triggering neuroinflammation[15,46]; and (3) Luteal-phase allopregnanolone metabolic dysregulation causes GABAergic signaling dysfunction, amplifying anxiety-induced executive function impairment[47]. These interconnected pathways form a vicious "estrogen-gut-brain axis" cycle, which may be interrupted by a Raphanus sativus L.-rich diet through modulation of gut microbiota metabolism (e.g., increased SCFAs) and activation of the Nrf2 antioxidant pathway[48].

Diet is one of the most important factors regulating behavioral and mental health throughout life[49]. Moreover, diet is one of the key determinants of the composition of the gut microbiota[50,50]. One review reviewed the beneficial effects of diet on brain health[51]. *Raphanus sativus* L. (RSL) belongs to the cruciferous family and is edible and used as a traditional medicine. It has over 1000 years of history for treating food stagnation, constipation, abdominal pain, and bloating[52].RSL and its major chemical constituent, sulforaphene (SFE) promote gastrointestinal motility through multitarget and multichannel activities[52]. Therefore, it is presumed that RSL acts on specific microbes in the intestine. While murine models suggest sulforaphene may modulate tau pathology via PI3K/Akt pathway[53], human translational evidence remains to be established. In addition, some population studies have shown that eating more cruciferous vegetables can prevent age-related cognitive decline in healthy older adults[52,54,55]. Few studies have investigated the relationship between diet and functional connectivity in brain networks in the resting state, which may underlie cognitive function[36,56]. A healthy diet may support cognitive ability despite differences in large-scale network connectivity in the resting state[57]. The diet also has regulatory effects on multiple pathways that connect the gut microbiota to the brain[58,59]. Animal studies have demonstrated the potential benefits of a healthy diet in mediating microbiota–brain interactions[60,61]. However, to our knowledge, no clinical studies have shown the role of dietary interventions in the interactions between the gut microbiota and brain function. In this study, we aimed to evaluate the effects of a plant-based RSL-rich dietary intervention on brain function and the gut microbiota in perimenopausal women and the correlations of the gut microbiota profile with intrinsic cerebral activity (as evaluated by ALFF with rs-fMRI) and cognitive function.

We selected Raphanus sativus L. seeds as the core dietary intervention based on their unique phytochemical composition and clinical advantages. As a traditional medicinal food documented in the Chinese Pharmacopoeia (2020 edition), RSL is particularly rich in sulforaphene and other bioactive isothiocyanates, which have demonstrated both neuroprotective effects[62] and gut microbiota-modulating properties[63]. Compared to other cruciferous vegetables like broccoli, RSL offers superior clinical applicability, containing 2.3-fold higher glucosinolate precursors[48] while maintaining excellent storage stability, making them particularly suitable for long-term intervention studies at the 5 g/day dosage, which has shown no reported adverse effects[48]. The mechanisms linking cognitive changes to the gut microbiota-brain axis in perimenopausal women remain poorly understood, with limited evidence for dietary interventions in this population. Through a longitudinal intervention design, this study systematically evaluates the effects of a Raphanus sativus L. (RSL)-enriched plant-based diet on gut microbiota composition, intrinsic brain activity, and cognitive function in perimenopausal women. The findings may provide novel insights into gut-brain interactions and lay the groundwork for developing safe, accessible dietary strategies to support mental health during menopausal transition.

Results

Baseline characteristics of the study participants

Of the 24 subjects who met the eligibility criteria, 14 dropped out or were excluded, and 10 completed the study. Recruitment took place from December 2021 to May 2022, and the dietary intervention was carried out from August to October 2022, with a follow-up visit one month after the end of the intervention to determine whether the subjects experienced any discomfort. This study was designed as a single-arm longitudinal exploratory investigation. A total of 10 participants were ultimately included (attrition rate: 58.3%). Based on post-hoc power analysis using G*Power 3.1 (paired t-test, two-tailed $\alpha = 0.05$, effect size $d = 0.6$), the current sample size achieved a statistical power of 0.42, indicating that only large effects ($d \geq 1.0$) could be detected with this study design. The baseline characteristics of the participants were collected on the first day of the intervention study (Table 1). The flowchart of the study (adapted from CONSORT 2010) is presented in Fig. 1.

Characteristics	Mean ± SD or *n* (%)
Age (years)	49.3 ± 4.11
Height (cm)	157.6 ± 6.17
Body weight (kg)	53.45 ± 7.45
BMI (kg/m^2)	21.90 ± 2.75
Drinking, n (%)	2 (20%)
Smoking, n (%)	0 (0%)
Married (%)	10 (100%)
Education level college or above, n(%)	9 (90%)
Premenopausal, n (%)	5 (50%)
Postmenopausal, n (%)	5 (50%)
MMSE score	28.9 ± 1.10
MoCA score	25.4 ± 1.78

Table 1. Baseline characteristics of participants ($n = 10$). Note: Data are expressed as means ± SEMs. MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment.

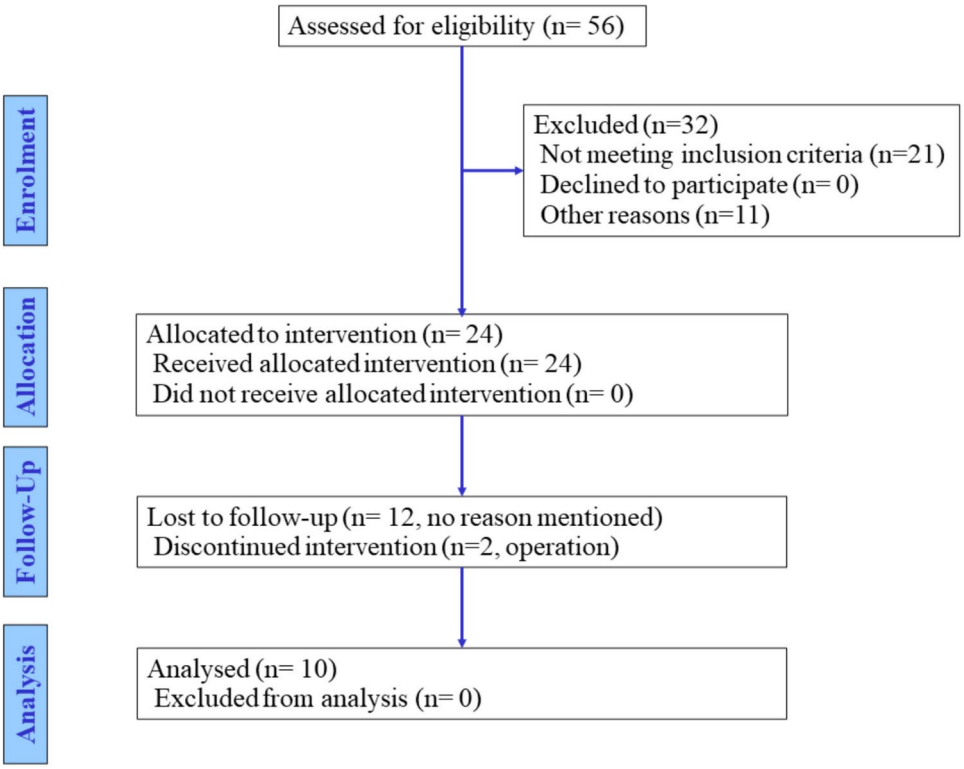

Fig. 1. Flowchart of the study (adapted from CONSORT 2010 Flow Diagram). The study was approved by the Ethics Committee of the Affiliated Hospital of Guizhou Medical University (2021 Ethics No. (675)). The clinical registration number is ChiCTR2400084007. Written informed consent was obtained from all participants before inclusion in this study. All experiments and procedures were carried out in accordance with ethical and biosafety protocols approved by the Institutional guidelines.

MoCA sub-item	T0	T1	*P* value
Visuospatial/executive function	3.50 ± 0.71	4.10 ± 0.88	0.051
Naming	2.90 ± 0.32	3.00 ± 0.00	0.343
Attention	5.50 ± 0.71	5.90 ± 0.32	0.104
Language	2.00 ± 0.67	2.10 ± 0.88	0.758
Abstraction	1.90 ± 0.32	2.00 ± 0.00	0.343
Delayed recall	3.60 ± 1.17	3.50 ± 1.18	0.811
Orientation	6.00 ± 0.00	5.90 ± 0.32	0.343
Total	25.4 ± 1.78	26.6 ± 1.96	0.089

Table 2. Comparison of sub-item scores of MoCA during the dietary. Note: Data are expressed as means ± SEMs and were analyzed by a Paired Sample *t*-test followed by Tukey post hoc tests. $n = 10$. T0, the day before initiation of the nutritional intervention. T1, end of the dietary intervention (12 weeks).

Neuropsychological assessments

The results of the seven sub-items of the MoCA are shown in Table 2. There was a trend toward increased visuospatial/executive function and total score at T1 (at the end of dietary intervention) compared with those at T0 ($P = 0.051$, $P = 0.089$). The scores of the other sub-items of the MoCA did not significantly differ between T0 and T1.

Gastrointestinal symptoms

To assess the effect of probiotics on intestinal health, the participants completed a general health questionnaire comprising items on bowel habit improvement at weeks 0, 4, 8, and 12. There was a significant increase in burping rates at week 12 compared with week 0 ($P < 0.05$) (Fig. 2a), whereas bloating, abdominal pain, early satiety, acid reflux, abdominal burning sensation, poor appetite, stomach heaviness after eating, indigestion, nausea, poor bowel motility, and dissatisfaction with digestive function were unaffected (Table 3). We observed no effects of the dietary intervention on stool time or consistency (assessed by the Bristol stool scale) (Fig. 2).

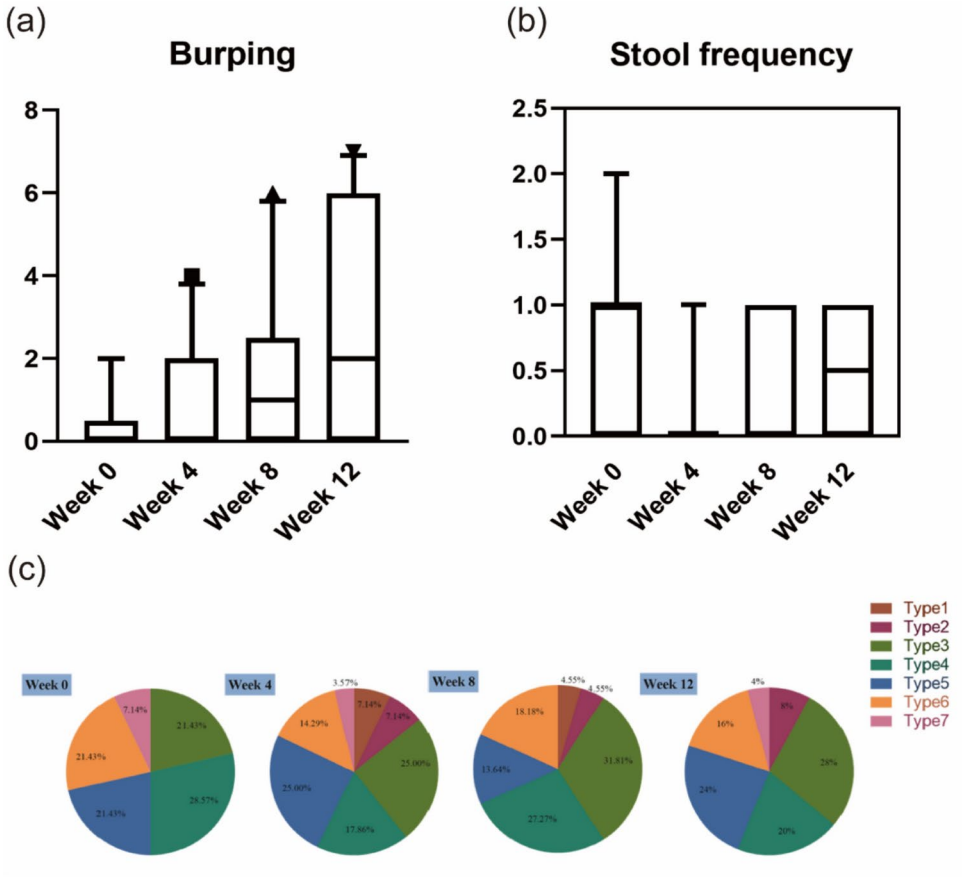

Fig. 2. Gastrointestinal symptoms. (**a**) Scores of burping measured at each visit ($n = 10$). (**b**) Frequency of defecation ($n = 10$). (**c**) Percentage of subjects in each category of stool type assessed by the Bristol stool scale ($n = 10$). Type1: Separate hard lumps, like nuts (hard to pass); Type2: Sausage-shaped but lumpy; Type3: Like a sausage but with cracks on its surface; Type4: Like a sausage or snake, smooth and soft; Type5: Soft blobs with clear-cut edges (passed easily); Type6: Fluffy pieces with ragged edges, a mushy stool; Type7: Watery, no solid pieces, Type 1–2 are considered hard; 3–5 are normal; 4 is the ideal state; 6–7 are soft. Data were analyzed by a Friedman test followed by Dunn's post hoc tests.

	W0	W4	W8	W12	x^2	p-value
Bloating	0(0 ~ 5)	0(0 ~ 2.5)	0(0 ~ 4.25)	0(0 ~ 4.5)	1.125	0.771
Abdominal pain	0(0 ~ 1.5)	0(0 ~ 0.75)	0(0 ~ 2)	0(0 ~ 2)	1.421	0.701
Early satiety	0(0 ~ 0)	0(0 ~ 2)	0.5(0 ~ 2)	0(0 ~ 0)	4.056	0.256
Acid Reflux	0(0 ~ 0)	0(0 ~ 0)	0(0 ~ 2.25)	0(0 ~ 0)	4.875	0.181
Abdominal burning sensation	0(0 ~ 3)	0(0 ~ 0.5)	0(0 ~ 0.5)	0(0 ~ 2)	1.096	0.778
Burping	0(0 ~ 0.5)	0(0 ~ 2)	1(0 ~ 2.5)	2(0 ~ 6)	9.393	0.024
Poor appetite	0(0 ~ 1)	0(0 ~ 2)	0(0 ~ 0.5)	0(0 ~ 2)	1.56	0.668
Stomach heaviness after eating	0(0 ~ 2)	0(0 ~ 0.5)	0(0 ~ 2.5)	2.5(0 ~ 4)	6.382	0.094
Indigestion	3(0 ~ 4.25)	0(0 ~ 2.75)	2(0 ~ 3.25)	2.5(0 ~ 4)	4.176	0.243
Nausea	0(0 ~ 2.25)	0(0 ~ 0)	0(0 ~ 0)	0(0 ~ 0)	4.714	0.194
Feeling of poor bowel motility	3(0 ~ 4.25)	3(0 ~ 3.25)	3(0 ~ 3)	1(0 ~ 4.25)	1.246	0.742
Dissatisfaction with digestive function	0(0 ~ 5.25)	1(0 ~ 2.5)	2(0 ~ 5.25)	2(0 ~ 5.25)	3.722	0.293

Table 3. Gastrointestinal symptoms during the dietary intervention. Note: Data are analyzed by a Friedman test followed by Dunn's post hoc tests. n = 10.W0 is the first test day, taking place before the nutritional intervention. W4 is the second test day, taking place 4 weeks after the start of the nutritional intervention. W8 is the third test day, taking place 8 weeks after the start of the nutritional intervention. W12 is the end test day, the end of the dietary intervention (12 weeks).

Stool frequency significantly improved ($P < 0.05$) (Fig. 2b). Figure 2c shows the results of the Bristol Stool Scale assessment during the dietary intervention.

Microbial diversity and gut microbial composition

The analysis of the alpha diversity of the gut microbiome revealed that the Good's coverage index, which represents coverage, increased significantly, whereas no significant changes were found in the other indices (Fig. 3a). There was no notable difference in terms of beta diversity (Fig. 3b). Next, we analyzed changes in the gut microbial composition at the phylum and genus levels after 12 weeks of dietary intervention and detected differences in the phylum and genus abundances between T0 and T1. We listed the top 10 most abundant phyla and genera at T0 and T1, respectively (Fig. 3c, d). At the phylum level, the phyla *Firmicutes* and *Bacteroidetes* decreased significantly during dietary intervention, while the abundances of *Proteobacteria*, *Actinobacteria*, *Verrucomicrobia* and *TM7* decreased. Linear discriminative analysis (LDA) effect size (Lefse) revealed that the abundances of the genera *Pseudomonas* and the family *Oxalobacteraceae* were relatively high, whereas those of the phyla Actinobacteria and genera *Shigella*, *Subdoligranulum*, *Aeromonas* and *Actinomyces*, the order *Aeromonadales*, and the family *Actinomycetaceae* were relatively low during dietary intervention (Fig. 3e, f). Three KEGG pathways ($P < 0.05$) enriched at T1 were not apparent at T0, including the lysosome, metabolism of xenobiotics by cytochrome P450 and cyanoamino acid metabolism (Fig. 3g).

Brain function

Compared with the baseline values, the participants demonstrated significantly elevated ALFF values in the left middle occipital gyrus, the left precentral gyrus, and the left middle cingulum gyrus after the dietary intervention ($P < 0.05$, FDR corrected) (Fig. 4). The detailed data from the ALFF analysis are summarized in Table 4.

Associations between the cognitive test score, brain activity, and gut microbiome abundance

Finally, we analyzed the correlations between the relative abundance of the gut microbiome, intrinsic brain activities (i.e., ALFF values), and cognitive function via Pearson's correlation analysis. The results revealed that the correlations between the gut microbiota abundance and internal brain activity differed before and after the dietary intervention (Fig. 5). At baseline (T0), there were positive correlations between the relative abundance of the phylum *Tenericutes* and the ALFF value of the left middle occipital gyrus (Occipital_Mid_L), as well as between the relative abundance of the genus *Roseburia* and the ALFF values of Occipital_Mid_L, the left precentral gyrus (Precuneus_L), and the left middle cingulum gyrus (Cingulum_Mid_L). Moreover, after 12 weeks of the plant-based RSL-rich diet (T1), the abundances of the phyla *Synergistetes* and *Verrucomicrobia* were positively correlated with Occipital_Mid_L, Precuneus_L, and Cingulum_Mid_L. The abundance of the phylum *Tenericutes* was positively correlated with Occipital_Mid_L and Cingulum_Mid_L.

Next, we assessed the associations between the relative abundances of the altered gut microbiome and the cognitive test scores (Fig. 6). Before dietary intervention (T0), the relative abundance of the phylum *Synergistetes* was positively correlated with visuospatial/executive function test scores. The phylum *Proteobacteria* and genus *Weissella* were negatively correlated with the naming score. The phylum *Bacteroidetes*, as well as the genera *Bacteroides* and *Coprococcus,* were negatively correlated with the Language score. The genera *Bifidobacterium* and *Roseburia* were negatively correlated with the abstraction score. After 12 weeks of the plant-based RSL-rich diet, we observed a positive correlation between the genus *Faecalibacterium* and the language score. The relative abundance of the genus *Roseburia* was negatively correlated with the delayed recall score. The genus *Ruminococcus* was negatively correlated with attention test scores.

Discussion

Perimenopause is not only a period of physiological transition (menstrual disorders, osteoporosis, cardiovascular disease, diabetes, tumors and other pathological changes)[64] but also a period of neurological transition (depression, mood disorders, insomnia, cognitive decline, increased risk of neurodegenerative diseases, etc.)[65], and a significant proportion of women suffer from problems associated with perimenopause, which seriously affects their quality of life[66]. In addition, the intestinal microbiota can become dysregulated in perimenopausal women. Healthy dietary patterns not only positively regulate the gut microbiota but also have a positive effect on brain health[67]. Dietary patterns such as the Mediterranean diet and calorie-restricted diets are beneficial to cognition and brain function, reducing the risk of neurodegenerative diseases such as AD[66,68]. Regulation of the gut microbiome may positively affect neural pathways and contribute to the regulation of several neurochemical and neurometabolic pathways through complex gut–brain interactions, thereby slowing the process of cognitive decline[69,70]. Therefore, we first explored the effects of a plant-based RSL-rich diet on the gut microbiota and brain function in perimenopausal women. We detected changes in the gut microbial composition, cognitive function and intrinsic brain activity following dietary intervention, followed by further analysis of the associations between these parameters. In this study, we observed a decrease in the relative abundance of several harmful gut bacteria and increased brain activity in the resting state in several brain regions associated with cognition in perimenopausal women after dietary intervention, and the associations between the relative abundance of specific bacterial taxa and intrinsic brain activity paralleled the changes in cognitive domains. Though this was initially designed as a pilot study, the achieved power supports the reliability of our findings regarding the primary outcomes. The limited statistical power (0.42) suggests our findings should be interpreted as preliminary evidence, consistent with the exploratory nature of this pilot study. Future randomized controlled trials with larger samples are needed to confirm these observations.

The Montreal Cognitive Assessment (MoCA) domains exhibit specific neuroanatomical substrates with direct clinical implications: visuospatial/executive performance relies on dorsolateral prefrontal-parietal

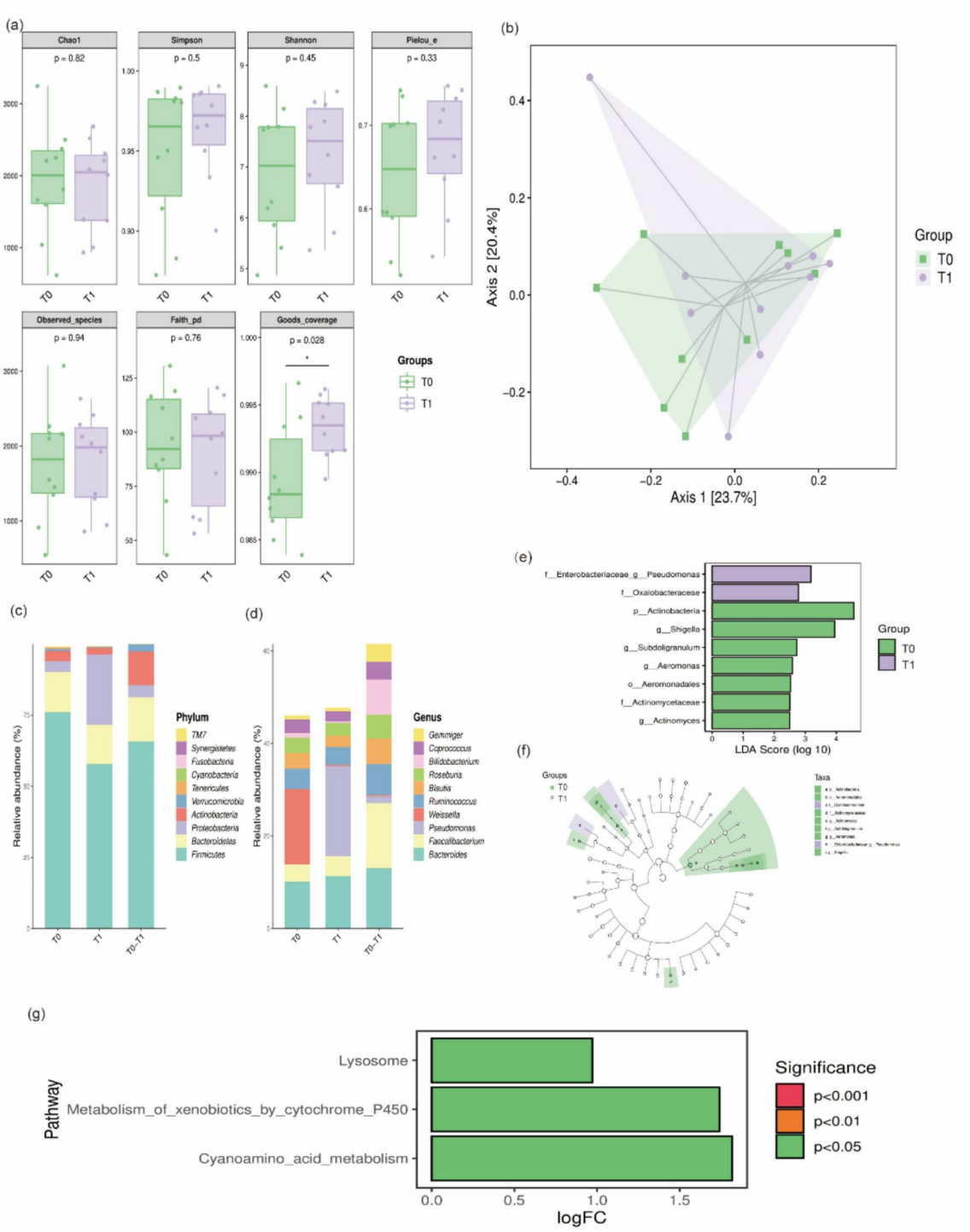

Fig. 3. Changes in microbial composition after 12-week dietary intervention. (**a**): Alpha-diversity indices. The "Chao1" and "Observed species" indices represent the richness, the "Shannon" and "Simpson" indicate diversity, the "Faiths_pd" index represents evolutionary diversity, the "Pielou_e" evenness index represents evenness, and the "Goods_coverage" index represents coverage. (**b**): Beta-diversity indexes. Principal coordinates analysis (PCoA) of the beta–diversity index Weighted UniFrac (**b**). (**c-d**): Gut microbiome composition at the level of major phylum (**c**) and genus (**d**) in T0 and T1. (**e-f**): Linear discriminant analysis (LDA) effect size (Lefse) plot and cladogram representing the unique bacterial signatures identified at T0 and T1. (**f**), family; (**g**), genus. (**g**): KEGG metabolic pathway analysis. A positive value of logFC on the horizontal axis represents an upward adjustment (log2(fold change)) of T1 relative to T0. Data are mean ± SEM and were analyzed by the Kruskal-Wallis test followed by Dunn's post-test: *P < 0.05. T1 vs T0.

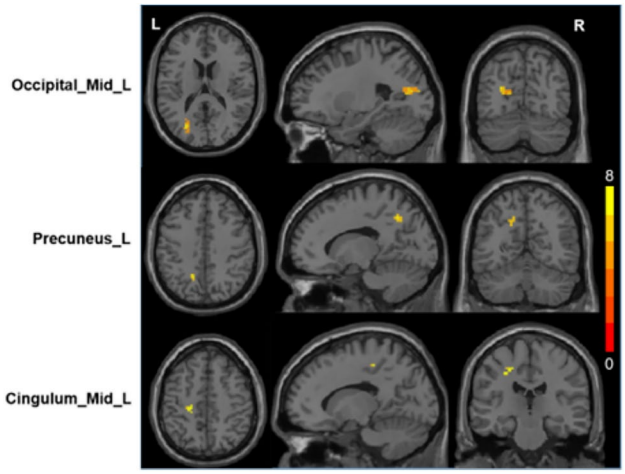

Fig. 4. Brain regions exhibiting enhanced amplitude of low-frequency fluctuations (ALFF) after the dietary intervention.

Brain region (AAL)	Cluster size (mm^3)	MNI coordinates (x, y, z)	Peak intensity	*P* value
Occipital_Mid_L	42	-26, -80, 15	8.2504	< 0.001
Precuneus_L	26	-15, -57, 42	6.5057	0.005
Cingulum_Mid_L	15	-15, -30, 42	5.3787	0.046

Table 4. Brain regions exhibiting significant differences in amplitude of low-frequency fluctuations (ALFF) between pre- and post-nutritional intervention. Note: False discovery rate (FDR) correction for multiple comparisons, $P < 0.05$. Abbreviation: AAL, Anatomical Automatic Labeling; MNI, Montreal Neurologic Institute; Occipital_Mid_L, Left Middle Occipital Gyrus; Precuneus_L, Left Precentral Gyrus; Cingulum_Mid_L, Left Middle Cingulum Gyrus.

circuits (2.1 ± 0.4-point deficit post-DLPFC lesions, $p < 0.01$)[71], naming accuracy reflects anterior temporal lobe integrity ($r = 0.52$ with ATL atrophy, $p = 0.003$)[71], language function depends on arcuate fasciculus white matter microstructure (FA $\beta = 0.34$, $p = 0.02$), while delayed recall strongly predicts hippocampal volume loss[72]. These region-domain mappings enhance the interpretability of cognitive declines in perimenopausal women, particularly when correlating MoCA subscores with our observed ALFF changes in corresponding brain regions. Given the small sample size, as expected, we did not observe large clinical effects. As perimenopause may lead to subcognitive abilities, we performed a MoCA assessment before and after the dietary intervention. We reported that visuospatial/executive function tended to increase from a baseline total score of 25.4 ± 1.78 to 26.6 ± 1.96 at the end of the dietary intervention. Although there was no significant difference, this finding suggests that our dietary intervention may have contributed to the overall cognitive profile. Because of the low internal reliability of the scales and the small sample size, these results must be treated with caution. Many dietary patterns, such as the most studied Mediterranean diet[73] and the Norwegian and Japanese diets[74], have shown protective effects on slowing cognitive decline. These diets have also been associated with a reduced risk of diseases such as depression and AD[75]. Notably, the neuroprotective effects of our RSL-rich dietary intervention align with established benefits of the Mediterranean diet – a pattern repeatedly associated with reduced cognitive decline in longitudinal studies[76]. While Mediterranean diets exert protection through polyphenol-rich olive oil and fish-derived ω-3 fatty acids, our plant-based RSL regimen operates via distinct yet complementary mechanisms: (1) sulforaphene-mediated Nrf2 activation (vs. hydroxytyrosol in olive oil), and (2) gut microbiota modulation favoring SCFA-producing taxa (similar to Mediterranean-induced increases in RSL). This convergence of health effects through divergent pathways underscores the versatility of dietary approaches in perimenopausal neuroprotection.

Based on the characteristics of the dietary regimen (consumption of RSL at 5 g/day), we analyzed whether a 12-week nutritional intervention would affect gastrointestinal symptoms. The results showed that our dietary regimen did not have adverse effects on overall intestinal health, except for the reported increased frequency of burping, which could be attributed to the daily consumption of RSL. RSL is a dual-use food, and its beneficial effects on the gastrointestinal tract such as eliminating food stagnation and bloating, promoting intestinal motility, and ameliorating constipation[77,78]. During the dietary intervention, we observed a significant increase in the frequency of bowel movements, which the consumption of RSL also be explained. However, as this study employed a whole-diet intervention, these effects may also be the result of interactions between various dietary components. In the future, we will conduct a randomized controlled trial to compare the impact of the whole

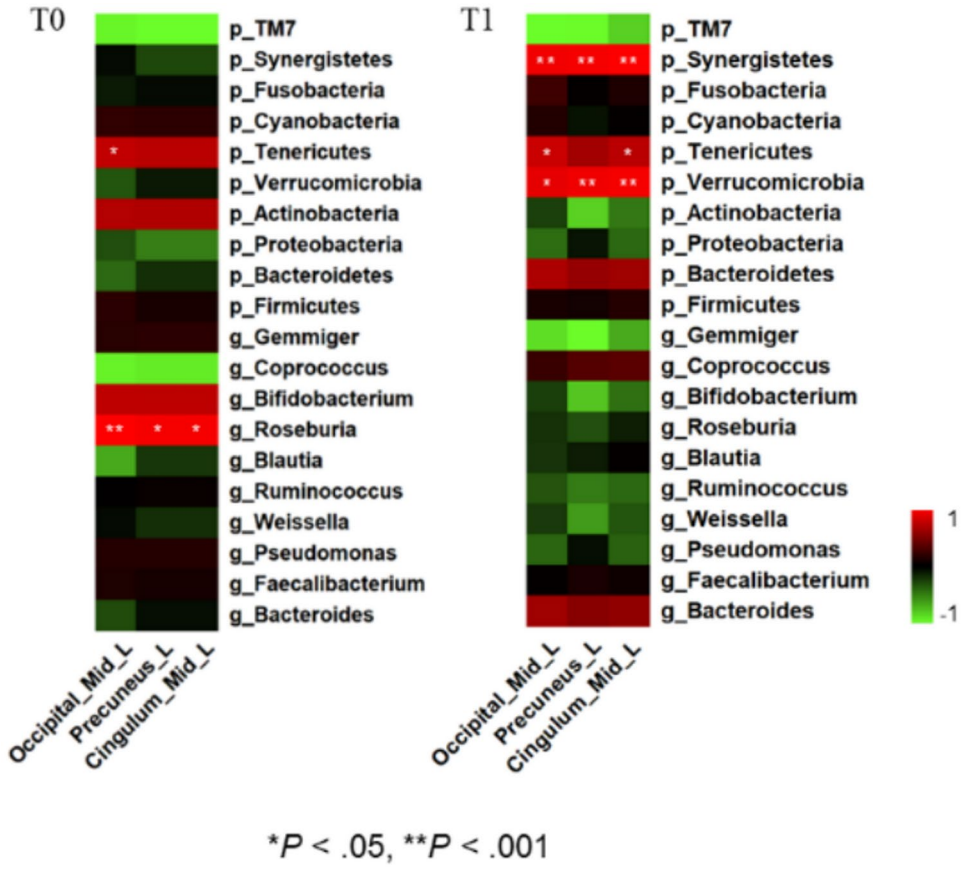

Fig. 5. Different patterns of mapping between gut microbiota and intrinsic brain activity between T0 and T1. Heatmap shows Pearson's correlation analysis between the relative abundance of fecal microbiomes and ALFF value. $^{*}P < 0.05$, $^{**}P < 0.001$ T1 vs T0.

diet with and without the addition of RSL. These results must be treated with caution due to the low internal reliability of the scales and the small sample size.

There is often individual variation in the gut microbiome, even when patients consume the same foods. Our microbiome data are of some benefit because each patient is under their own control[79]. Although there was no significant difference in β diversity after 12 weeks of the plant-based RSL-rich diet, we detected a trend toward increased α diversity and a substantial difference in the Good's coverage index. Additionally, several gut bacterial phyla, families, and genera were altered between T0 and T1. For example, the phyla *Firmicutes* and *Bacteroidetes* decreased at T1, and the *Firmicutes*: *Bacteroidetes* ratio (F/B) decreased. The F/B ratio is greater in postmenopausal women[19], and a lower F/B ratio is considered to offer health benefits[80]. Indeed, the abundances of *Oxalobacteraceae* and *Pseudomonas* increased, whereas those of *Actinobacteria* and *Aeromonas* decreased after dietary intervention. Several studies have reported the beneficial health effects of *Oxalobacter formigenes*, which can degrade oxalate and thus reduce the risk of kidney stone formation[81]. Decreases in *Actinobacteria*, *Aeromonas* and *Shigell* suggest an improvement in dysbiosis[82]. *Aeromonas* and *Shigella* are enteric pathogens that cause diarrhea[83,84]. Here, we found that RSL improved intestinal health, most likely by lowering *Aeromonas* and *Shigella*. More targeted research is needed to validate these findings. *Pseudomonas* is pathogenic, with its cell walls being rich in inflammatory components, such as LPS, which is known to induce low-level systemic inflammation[85]. Whether this increased level of pathogenic bacteria is due to dietary intervention or to the patients themselves cannot be concluded owing to the lack of references to relevant studies. At this stage, in our overall knowledge of dietary intervention and the microbiome of perimenopausal women, there is no specific marker to define an improvement in dysbiosis. Therefore, more studies are needed to explore the possible markers in perimenopausal women and the effects of dietary interventions on specific markers.

This study revealed increases in resting-state activity in the left middle occipital gyrus, the left precuneus, and the left medial cingulum gyrus in perimenopausal women after 12 weeks of dietary intervention. Abnormal spontaneous activities in multiple brain regions during the resting state have been reported to be altered in perimenopausal women. ALFF was significantly elevated in the left rectus gyrus of perimenopausal women compared with premenopausal women. The regions with reduced ALFF during perimenopause include the left superior temporal gyrus, the left inferior frontal gyrus, and the left insula[43]. Although the results of our study did not correspond to those of Liu et al., the left middle occipital gyrus found in this study is involved in visuospatial perception and the visual network. Kawagoe et al. reported reduced occipital blood perfusion in patients with

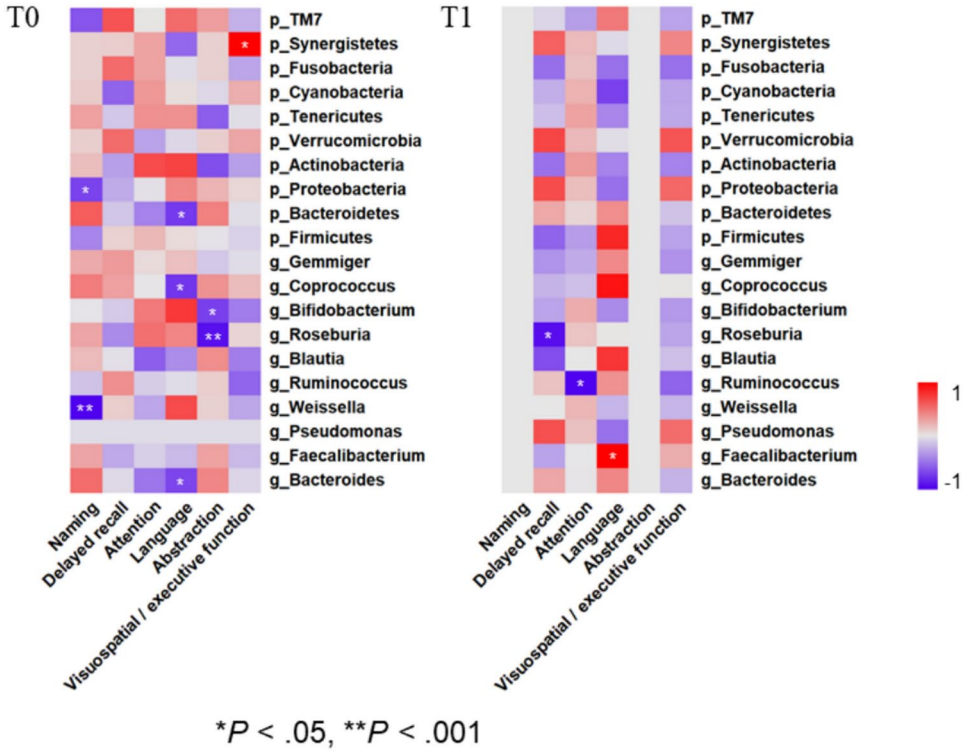

Fig. 6. Different gut microbiota and cognitive function correlations between T0 and T1. Heatmap showing correlations between fecal microbiome and cognitive test scores of all subjects. $^{*}P < 0.05$, $^{**}P < 0.001$.

mild cognitive impairment[86]. Studies in patients with early mild cognitive impairment have revealed that the decline in working memory tends to occur first in the visual field rather than the verbal field. This may indicate an early compensatory response to neurodegenerative changes in response to neural injury[87,88]. This result corresponds to the results of our MoCA test. Therefore, our dietary intervention may prevent cognitive impairment at an early stage by affecting the middle occipital gyrus. The functional roles of the precuneus are unclear. Yeager et al. proposed associations of the precuneus with body awareness, complex cognition, and visual processing and that the precuneus had functional connections to other cortical areas associated with executive function, including the dorsolateral prefrontal cortex and the inferior parietal lobe[89]. Therefore, our dietary intervention may increase cognitive control and executive function in perimenopausal women by increasing the ALFF value of the left precentral gyrus. Abnormal dynamic functional connectivity of the left middle cingulum gyrus and hippocampus in patients with mild cognitive impairment[90]. Patients with temporal lobe epilepsy have language and cognitive deficits as well as lower right–left middle cingulum gyrus activity than healthy participants[91]. Therefore, the elevated ALFF in the left middle cingulum gyrus may be associated with cognitive improvement. Our dietary intervention may prevent cognitive decline in perimenopausal women through early improvements in visuospatial perception and visual network function.

In this study, we also observed changes in the pattern of association between intrinsic brain activity and the gut microbiome in perimenopausal women after dietary intervention. At baseline, there was a significant positive association between the genus *Roseburia* and brain activity in all three brain regions in perimenopausal women. *Roseburia intestinalis* has been shown to be a beneficial intestinal bacterium that helps prevent intestinal inflammation and maintain energy homeostasis[92]. These findings suggest that *Roseburia intestinalis* may have a positive regulatory effect on brain function in perimenopausal women, as previously reported[93]. However, as studies on the microbiota-gut-brain axis in PMS are rare, further investigations into the mechanisms of brain-active bacteria and intrinsic brain function in PMS are needed. Compared with the baseline values, after 12 weeks of dietary intervention, significant positive correlations were found for the phyla *Synergistetes*, *Verrucomicrobia* and *Tenericutes* with all three brain regions. In this study, we also found a positive correlation between the phylum *Synergistetes* and visuospatial/executive function scores on the MoCA at baseline. In humans, the phylum *Synergistetes* is more frequently seen and isolated in the oral cavity and may contribute to periodontal diseases. *Synergistetes* represents a poorly characterized phylotype[94]. There are fewer types isolated from feces, and little is known about the role of the phylum *Synergistetes* in other diseases[95]. Owing to the small sample size in our study, further investigations are needed to demonstrate the causal relationship between the phylum *Synergistetes* and health improvement as well as brain health. The phylum *Verrucomicrobia* is associated with longevity[96], and our results suggest that this phylum may play a beneficial role by positively modulating brain function to improve cognitive function in perimenopausal women. Finally, our correlation analysis of the gut microbiome and the MoCA cognitive test revealed a greater number of gut bacterial species that were negatively correlated with each score in the MoCA test in perimenopausal women before the dietary intervention than in those after the

intervention. These findings suggest that our 12-week healthy diet intervention may have increased the diversity and abundance of the gut microbiota (although not reflected in the diversity analysis) and that these increases in the gut microbiome are beneficial to the understanding of perimenopausal women. In the future, cross-sectional and longitudinal studies with larger sample sizes are needed to verify these findings. Perimenopausal women lack safe, effective non-pharmacological interventions for cognitive decline and abnormal brain activity. This study provides the first longitudinal evidence that a *Raphanus sativus* L. (RSL)-enriched plant-based diet may modulate spontaneous neural activity (ALFF) through specific gut microbiota (e.g., *Synergistetes* and *Verrucomicrobia*), while enhancing visuospatial/executive function. These findings advance our understanding of microbiome-gut-brain axis regulation in perimenopausal syndrome and establish a practical foundation for dietary cognitive protection strategies.

There are several limitations to our study. (1) Due to the small sample size, larger clinical effects and low internal reliability were not observed. (2) Because of the small number of cases recruited, all participants were included in the experimental group to obtain as many significant results as possible. A single-arm study was conducted, and a strictly randomized controlled study was not performed. This may have also had an impact on the experimental results, which need to be further optimized in subsequent studies. (3) Daily consumption of RSL was a major feature of this study. We have high expectations for the therapeutic effects of RSL in this trial. However, since a whole-diet intervention was used, these effects may also be the result of the interaction of various dietary components. In the future, we will conduct a randomized controlled trial to compare the effects of adding and not adding RSL to the whole diet. (4) At this stage, our overall understanding of dietary interventions and the microbiome of perimenopausal women does not yet have specific markers to define improvements in dysbiosis. Therefore, additional studies are needed to explore possible markers in perimenopausal women and the effects of dietary interventions on specific markers. (5) Studies on the microbiota-gut-brain axis in premenstrual syndrome patients are uncommon because our results may not be adequately understood. Therefore, more studies are needed in the future to investigate the mechanisms of brain-active bacteria and intrinsic brain function in perimenopausal syndromes.

We conducted a longitudinal single-arm study. After a 12-week plant-based dietary intervention enriched with Raphanus sativus L., perimenopausal women tended toward improvements in the visuospatial/executive function subscale and total scores and changes in the gut microbial composition. The participants presented elevated ALFF values in the left middle occipital gyrus, left precentral gyrus and left middle cingulum gyrus. These brain regions are associated with cognitive performance. The abundances of the phyla *Synergistetes* and *Verrucomicrobia* were positively correlated with the ALFF values of the left middle occipital gyrus, left precentral gyrus, and left middle cingulum gyrus, and the abundance of the phylum *Tenericutes* was positively correlated with the left middle occipital gyrus and left middle cingulum gyrus. These findings suggest that specific gut microbes may modulate intrinsic brain activity and cognitive function. In conclusion, our study suggests the beneficial effects of a plant-based diet on the gut microbial composition and brain function of perimenopausal women. These findings provide supportive evidence for studies of dietary interventions that modulate the gut microbiota and brain function. This study demonstrates the feasibility and guidance of dietary interventions (plant-rich dietary interventions and increased seed intake) to promote health and improve quality of life in perimenopausal women. Innovative demonstration of the role of dietary interventions in the interaction between the gut microbiota and brain function.

Materials and methods

Study participants

Healthy perimenopausal women were recruited consecutively from the Menopause Clinic, Affiliated Hospital of Guizhou Medical University, from February 2021 to May 2022. All participants were recruited from the outpatient clinic after a comprehensive assessment by an obstetrician-gynecologist, met the criteria for perimenopause, which is defined as the transitional stage from 1 year before the onset of obvious menstrual disorders to the complete disappearance of menstruation, and received the domestic modified Kupperman index (KI) test (Supplementary Table 1) at the hospital. The inclusion criteria were as follows: aged 45–55 years; clinical symptoms, signs, and a diagnosis of perimenopausal syndrome; and no previous hormone supplementation therapy. The exclusion criteria were as follows: consumption of antibiotics, probiotics, prebiotics, or synbiotics within 2 months before fecal sample collection; severe malnutrition; infection; drug or alcohol addictions; irritable bowel syndrome; and inflammatory bowel disease; combined severe liver, kidney, lung, heart, brain, and hematopoietic system diseases; other serious primary diseases; schizophrenia; schizoaffective disorders; or primary affective disorders; and MR incompatibility; and severe auditory, visual, or motor deficits hampering cognitive testing. Here, we used the CONSORT Reporting Guidelines[97]. Fifty-six candidates were enrolled, and 24 were eligible for this study after screening. The flow of participant employment is provided in Fig. 1. The study was approved by the Ethics Committee of the Affiliated Hospital of Guizhou Medical University (2021 Ethics No. (675)). Trial registration: Chinese Clinical Trial Registry (ChiCTR), ChiCTR2400084007. Registered 09 May 2024—Retrospectively registered, https://www.chictr.org.cn/showproj.html?proj=197060. All included subjects signed an informed consent form.

Study design

In this longitudinal single-arm study, all participants underwent a 12-week dietary intervention. They underwent neuroimaging, as well as MMSE and MoCA testing, before and at the end of the dietary intervention. Stool samples were also collected at the two time points. In addition, the participants completed a general health questionnaire comprising items on bowel habit improvement at weeks 0, 4, 8, and 12 (Fig. 7).

The MMSE is used as a global measurement of cognitive status, and a total score of ≥ 27 indicates normal cognition[98]. The Beijing version of the MoCA is the most widely used version in mainland China and exhibits

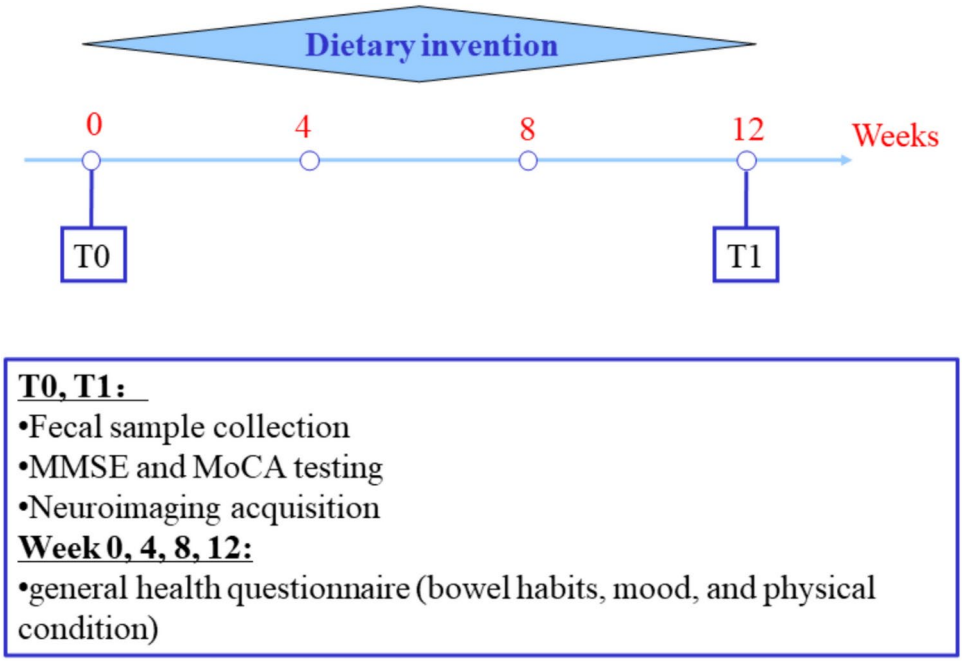

Fig. 7. Experimental protocol. Subjects included in this study received 12 weeks of dietary intervention. During the two weeks of nutritional intervention, they completed a general health questionnaire and Bristol stool scale to report their intestinal tolerance, appetite feelings, stool frequency, and consistency to this particular diet. at baseline before intervention (T0, Week 0), 4 weeks (Week 4) and 8 weeks (Week 8) after the start of the nutritional intervention, and 12 weeks at the end of the nutritional intervention (T2, Week 12).

good internal consistency and general criterion-related validity[99]. It includes seven cognitive domains (i.e., visuospatial/executive function, naming, attention, abstraction, language, delayed memory, and orientation), with a total score of ≥ 26 indicating normal function. The MoCA adds 1 point for individuals who have ≤ 12 years of education for education adjustment. The general health questionnaire comprises items measuring gastrointestinal symptoms (bloating, abdominal pain, early satiety, acid reflux, abdominal burning sensation, burping, poor appetite, stomach heaviness after eating, indigestion, nausea, poor bowel motility, and dissatisfaction with digestive function). Each item had a 4-point scale ranging from 1 ("not at all") to 4 ("very much") for both symptom severity and frequency; thus, the total score was 8. Lower scores indicate improvement. In addition, the subjects completed a questionnaire on 24-h stool frequency and consistency (Bristol stool scale)[100].

Dietary intervention

The diet was developed by a registered nutritionist. The participants were asked to adhere to their daily diet for 12 weeks (see supplementary information for sample menus). Regular online interviews were performed with participants to inform them of meal planning and evaluate their adherence. The participants supplied their own food on the basis of a daily meal plan, food list, and other provided material. To investigate the effect of RSL consumption on perimenopausal women, we formulated a plant-based RSL-rich (5 g/d) diet. RSL belongs to the cruciferous family, and to avoid the intake of other cruciferous vegetables, no other cruciferous vegetables were added to the diet except for RSL. Overall, our dietary intervention provided a controlled, nutrient-dense regimen (≈1300 kcal/day) with optimized macronutrient distribution (protein ≈65 g/day, carbs < 100 g/day, fiber ≈40 g/day), emphasizing RSL (5 g/day) alongside neuroprotective ingredients (e.g., ginkgo, salmon, flaxseed). Throughout the study, the participants received daily multivitamin supplements (B-complex vitamins, vitamin D [400–1000 IU/day], and vitamin C [100–300 mg/d]) and restricted the use of supplements with antioxidant or ketone-inducing effects (i.e., fish oil, coconut oil, and medium-chain triglyceride supplements). In addition, all participants, the experimenter, and the nutritionist had a communication group where they could contact the dietitian during the intervention with any questions they had. Patients were encouraged to post pictures of their daily diets to the communication group, and if they forgot, our lab staff visited them in a private chat to ensure their adherence. During the intervention, we ask about and record any adverse events for participants, and they can also choose to withdraw at any time if they wish. At the end of the intervention, we will maintain the communication group for one month for follow-up.

Analysis of the gut microbiota composition in the stool samples

Each participant was asked to collect a fresh fecal sample. Fecal samples were self-collected by each participant and preserved at − 80 °C until analysis. Genomic DNA was extracted from 500 mg of each stool sample via an Omega M5636-02 kit (Omega, CT, USA), and the DNA was quantified via Nanodrop (Thermo Scientific, Waltham, MA). The quality of the extracted DNA was assessed via 1.2% agarose gel electrophoresis. The DNA samples

were subjected to PCR amplification via the primer pair 338F: 5'-ACTCCTACGGGAGGCAGCA -3' and 806R: 5'-GGACTACHVGGGTWTCTAAT-3' to amplify the V3-V4 region of the bacterial 16S rRNA gene. The PCR products were purified and sequenced via the Illumina® MiSeq platform. Initial screening of raw downstream data from high-throughput sequencing on the basis of sequence quality. Sequence denoising or OTU clustering was performed according to the QIIME2 dada2 analysis process. The alpha diversity level of each sample was assessed on the basis of the distribution of ASVs/OTUs in different samples and the appropriateness of sequencing depth reflected by sparse curves. The beta diversity of the microbiome was analyzed via principal coordinate analysis (PCoA) with unweighted and weighted UniFrac distances. LEfSE (linear discriminant analysis [LDA] effect size) was used to identify significantly different bacterial taxa after the dietary intervention compared with those at baseline[101]. The open-source bioinformatics tool PICRUSt (Phylogenetic Study of Communities by Reconstruction of Unobserved States) was used to explore the metabolic and other functional activities of the intestinal bacterial community[102]. On the basis of the 16S rRNA gene sequencing results, we predicted the metabolic function of the sample's flora, identified differential pathways, and obtained the species compositions of specific pathways. The inferred gene families were annotated according to KEGG (Kyoto Encyclopedia of Genes and Genomes) Orthology (KOs) and then folded into KEGG pathways to generate functional pathways[103].

Neuroimaging acquisition and analysis

MRI data acquisition

All participants underwent brain MRI examination on a 3.0 T MR scanner (Discovery MR 750w, GE Healthcare, Milwaukee, WI) with a 24-channel head/neck coil at Guizhou People's Hospital. They were asked to remain awake and keep their eyes closed during the scan.

High-resolution whole-brain 3D T1-weighted brain anatomical images were collected via volumetric three-dimensional magnetization via a rapid-acquisition gradient-echo sequence with the following parameters: TR/TE = 8.5/3.2 ms, TI = 450 ms, FA = 15°, FOV = 256 mm × 256 mm, matrix = 256 × 256, thickness = 1 mm, resolution = 1 mm × 1 mm × 1 mm, 176 axial slices, scan time = 273 s, accelerated by ARC parallel acquisition technology with acceleration factor R = 2. The rs-fMRI images were obtained via an echo-planar imaging sequence (repetition time = 2000 ms, echo time = 30 ms, flip angle = 70°, field of view = 216 mm × 216 mm, matrix = 72 × 72, thickness = 3.5 mm, slice gap = 0.5 mm, slices = 34, voxel size = 3 mm × 3 mm × 4 mm, volume = 250). The participants with obvious motion artifacts and metal artifacts were excluded from the analysis. In addition, plain brain MR scans (axial T2, sagittal T2, and axial T2 FLAIR) were performed to exclude organic lesions in the brain.

Data preprocessing

Data preprocessing was performed via SPM12 (http://www.fil.ion.ucl.ac.uk/spm) and the Data Processing Assistant for Resting-State fMRI (DPARSF; http://www.restfmri.net) [104,105] in the MATLAB 2018a (Mathworks, Natick, MA) platform. (1) The functional images of the first 10 time points were removed to ensure that the subjects had adapted to the scanning environment. (2) The remaining 240 images were corrected for time differences and small movements, and the signals collected at different times were modified for time point matching. (3) Subjects with head maximum movement > 2.0 mm in the x-, y-, or z-axis or with angular rotation > 2.0° were excluded. (4) The generated images were normalized to the Montreal Neurological Institute (MNI) standard space. The T1 images were first coregistered to the mean functional images after motion correction and then resampled to a 3 × 3 × 3 mm isotropic voxel size. (5) Linear detrending was performed to remove noise from long-term physiological shifts, residual movement-related artifacts after realignment, and instrumental instabilities. (6) The white matter signal, cerebrospinal signal, and head motion parameters (Friston 24 parameters) were regressed out except for the global signal. (7) Low-frequency filtering (0.01–0.1 Hz) was applied to eliminate high-frequency physiological noise.

ALFF analysis

The ALFF was computed via the DPARSF toolbox. The ALFF is defined as the mean amplitude of the rs-fMRI signal within the frequency band of 0.01–0.1 Hz for each voxel and was used to measure the level of basal neural activity in the brain. This approach can reflect the spontaneous neural activity of each voxel in the resting state. The specific calculation procedures are as follows: (1) The time series of each voxel was transformed into the frequency domain via the Fourier transform to obtain the power spectrum. (2) The square root of the power spectrum at each frequency point was computed. (3) The mean square root across all frequency points was deemed the ALFF value, indicating the intensity of spontaneous activity. (4) The ALFF of each voxel was normalized to the global mean ALFF within the brain mask. The normalized ALFF value for each voxel should be approximately 1. (5) The normalized ALFF maps of each subject were spatially smoothed using a 6 × 6 × 6 mm^3 full width at the half-maximum Gaussian kernel. In addition, the brain regions that exhibited between-group differences were identified as regions of interest (ROIs). The ALFF values of all the ROIs were calculated for each subject via the REST plus toolbox[104-106].

Statistical analyses

A priori power analysis using G*Power 3.1 software to determine the appropriate sample size. Statistical analysis was performed via SPSS version 26.0 (SPSS Inc., Chicago, IL) and GraphPad Prism 8 (GraphPad Software, Inc.). The results are presented as percentages, means ± standard deviations, or medians with interquartile ranges (IQRs). The data were analyzed with paired samples *t* tests with Tukey post hoc tests if they were parametric and with Friedman tests with Dunn post hoc tests if they were nonparametric. Normality was assessed by the Shapiro–Wilk test. Statistically significant differences between the gut microbiome groups were calculated via the Kruskal–Wallis test followed by Dunn post hoc analysis. Correlations between the relative abundance of

the fecal gut microbiota, cognitive levels, and ALFF values in active brain regions were performed via Pearson's correlation analysis. The Pearson correlation coefficient R and the P values were calculated via Python in Excel. All the statistical tests were two-sided, and $P < 0.05$ was considered statistically significant.

Data availability

The data that support the findings of this study are available from the corresponding author, Yi Zhu, upon reasonable request. Raw data have been deposited to National Center for Biotechnology Information (NCBI) under the BioProject number PRJNA1248708.

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Caihui Guo (First Author): Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing—original draft, Writing—review & editing; Zhenliang Xiong: Formal analysis, Writing—original draft; Lin Yang: Investigation, Resources, Project administration, Funding acquisition; Mingxian Bai: Investigation; Xianchun Zeng (Corresponding Author): Resources, Writing—Review & Editing, Supervision, Project Administration; Yi Zhu (Corresponding Author): Conceptualization, Resources, Writing—Review & Editing, Supervision, Project Administration.

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Declarations

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Affiliated Hospital of Guizhou Medical University (2021 Ethics No. (675)). The clinical registration ID: ChiCTR2400084007, URL: https://www.chictr.org.cn/showproj.html?proj=197060 (Retrospectively registered).

Consent for publication
Informed consent was obtained from all individual participants included in the study.

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