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Original Article

Correlation between daily physical activity and intestinal microbiota in perimenopausal women

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ABSTRACT

This study aimed to examine the correlation between daily physical activity levels and gut microbiota during the perimenopausal period. To explore the physiopathological traits of perimenopause women, and provide a theoretical basis for the development of menopause exercise intervention program. In this study, daily physical activity was assessed using the ActiGraph wGT3X-BT wearable sensor, and 16S rRNA sequencing was employed to analyze gut microbiota diversity and composition. Comparative analysis was conducted to evaluate the association between physical activity and specific phyla and genera in the gut microbiota, comparing perimenopausal women to youth women. The results showed perimenopausal women exhibited significantly lower levels of daily physical activity, including Kcals, metabolic equivalents (METs), and moderate-to-vigorous physical activity (MVPA), compared to youth women. The composition of the gut microbiota was markedly different between the two groups. Specifically, the abundance of *Acidobacteria*, *Chloroflexi, Nitrospirae*, and *Gemmatimonadetes* were lower at the phylum level ($p < 0.01$). *Collinsella, Ruminococcus gnavus, Rothia, Haemophilus, Sphingomonas, Lactobacillales,* and *Lactococcus* were lower at the genus level, while *Phascolarctobacterium, Paraprevotella, Acinetobacter, Flavonifractor,* and *Intestinimonas* exhibited a significant increase ($p < 0.05$, $p < 0.01$). Furthermore, a positive correlation was observed between *Chloroflexi* or *Sphingomonas* and physical activity (kcals, METs, and MVPA), while a negative correlation was found between *Intestinimonas* and physical activity (METs: $p < 0.01$, and MVPA: $p < 0.05$). Doubtlessly, the perimenopausal period is associated with lower levels of physical activity and distinct changes in gut microbiota. *Sphingomonas* may serve as a sensitive bacterium closely linked to physical activity.

1. Introduction

The human intestinal tract serves as the primary site for nutrient absorption and metabolism. Over a long evolutionary period, a symbiotic relationship has developed between the host and the parasitic microbial community in the intestines, influencing various physiological processes throughout the host's lifespan.[1] Scientific investigations have revealed that the intestinal microbiota functions as an additional "organ" within the human body, exerting effects on diverse processes such as dietary fiber and protein metabolism, as well as immune system regulation.[2] The perimenopausal stage represents a unique period in a woman's life and is characterized by altered hormone levels that can result in symptoms such as hot flashes, fatigue, and insomnia. The majority of current research on female menopause focuses on the relationship between intestinal flora and estrogen levels. Menopause related symptoms, such as sadness, osteoporosis, and metabolic disorders, are associated with intestinal flora.[3] Physical activity could improve menopausal symptoms by regulating body function as a positive intervention.[4]

Physical activity refers to activities that result in energy expenditure at the basal metabolic level, which closely intertwined with the body's substance metabolism and diseases such as hyperlipidemia, hypertension, and hyperglycemia. The physical activity qualitatively (such as low, moderate, and high) are assessed by collecting the data of MVPA, METs, and the related metabolic index, such as Body Mass Index (BMI) , body fat percentage and energy utilization.[5–7] However, there is currently a

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Abbreviation

METs	metabolic equivalents
MVPA	moderate-to-vigorous physical activity
Y group	youth group
M group	middle-aged group
BMI	Body Mass Index
MVPA/d	average daily time of moderate-to-vigorous physical activity
MET-min/w	metabolic equivalent minutes per week
F/B	*Firmicutes* to *Bacteroidetes*
NOB	nitrite-oxidizing bacteria
SLE	systemic lupus erythematosus

scarcity of research exploring the relationship between daily physical activity levels in menopausal women and the composition of intestinal flora, as well as the corresponding metabolites in *vivo*. In this study, collecting fecal samplers from youth group (with high levels of physical activity as the control group) and middle-aged group (with lower levels of physical activity), we analyzed the relationship between daily physical activity and intestinal flora differences among women of different ages. The study would provide useful data for exploring the pathological mechanism in perimenopause, and suggest the climacteric exercise intervention program in clinic.

2. Methods

2.1. Research subjects

The 59 research participants for this study randomly consisted of female students and faculty members from Guangzhou University and Guangzhou University of Traditional Chinese Medicine. They were divided into two groups based on age: the youth group (Y group, comprised of students aged 19–23 years) and the middle-aged group (M group, consisting of faculty members aged 45–55 years). The youth group participants maintained moderate-to-vigorous physical activity 1 hour (h) a day. All subjects taking in the school canteen daily, were in good health, free from any major diseases. Stool sampling was avoided during menstruation.

2.2. Ethical approval

Prior to participation, the subjects provided informed consent and were aware of the study and agree to publish it. The study protocol was approved by the Ethics Committee of Gaozhou Hospital of Traditional Chinese Medicine, Guangzhou University of Traditional Chinese Medicine (NO. Y [2021]16).

2.3. Research methods

2.3.1. Body composition and physical activity monitoring

Body composition was assessed using the domestic DHM-200 height-weight tester and Tsinghua Tongfang BCA-1A tester. To monitor physical activity intensity, energy utilization, and metabolic equivalents (METs), participants wore the ActiGraph wGT3X-BT human exercise energy monitor around their waist, which continuously recorded data for 48 h. The sampling interval was set at 30 seconds (s) per epoch, with a frequency of 30 Hz.

2.3.2. Intestinal flora detection

Approximately 2 g of fresh fecal samples from the middle internal part were collected using sterile tubes and sampling spoons. The samples were stored at −80 °C and transported on dry ice. The total DNA was extracted using MN NucleoSpin 96 Soi and the quality inspection was qualified. Primers were designed according to the conserved region, sequencing adapters were added to the end of primers, and PCR amplification. OMEGA DNA purification kit was used for passivation, then PCR products were quantified and homogenized. Constructing PE library, and amplicon sequencing libraries were constructed based on the Illumina platform by Beijing Bemaker Biotechnology Co, Ltd. After data filtering and quality assessment, the data were subjected to bioinformatics analysis. (Primer sequence: F: ACTCCTACGGGAGGCAGCA, R: GGACTACHVGGGTWTCTAAT; Amplification condition: 94 °C 5 min, [94 °C 30 s, 50 °C 30 s, 72 °C 40 s] × 25, 72 °C 7 min).

2.3.3. Data processing and analysis

Data were analyzed and processed using SPSS 26.0 software. Normally distributed data were presented as mean ± standard deviation, and *t*-tests were used for between-group comparisons. Non-normally distributed data were analyzed using non-parametric rank sum tests. Colony diversity analysis was performed using Shannon and Chao1 indices, PCoA, and binary Jaccard methods. Relative abundance differences were analyzed using ANOVA and LEfSe analysis. The correlation between physical activity and characteristic microbiota was assessed using Spearman analysis. Statistical significance was considered at $p < 0.05$.

3. Results

3.1. Analysis of differences in daily physical activity among women of different ages

When comparing the middle-aged group to the youth group, no significant differences were observed in Body Mass Index (BMI) and body fat percentage. However, the middle-aged group showed significantly lower average daily energy utilization, average daily time of moderate-to-vigorous physical activity (MVPA/d), and METs compared to the youth group ($p < 0.05$, $p < 0.01$). Please refer to Table 1 and Fig. 1 for further details.

3.2. Characterization of intestinal flora in perimenopausal women

The α-diversity analysis results revealed no significant difference ($p > 0.05$) in species abundance (Fig. 2A) and species diversity (Fig. 2B) between the Y group and M group. However, the Principal Coordinate Analysis (PCoA) demonstrated a significant clustering of flora distribution between the M and Y groups, as depicted by the oval circles representing 95% confidence ellipses (Fig. 2C). The Permanova analysis further confirmed a significant difference in β-diversity between the samples of the different subgroups ($R = 0.308$, $p = 0.001$), indicating a notable distinction in the structure of intestinal flora between the youth and middle-aged groups (Fig. 2D). Please refer to Fig. 2 for visual representation.

Table 1

Differences in body composition and daily physical activity among women of different ages.

Group	*n*	BMI	Body fat percentage (%)	kcals/d	MVPA/d (minutes)	METs
Y	29	22.16 ± 3.17	28.38 ± 4.51	369.06 ± 156.63	68.71 ± 15.61	1.44 ± 0.26
M	30	23.32 ± 2.96	29.64 ± 5.82	290.47 ± 93.34$^{\#}$	54.30 ± 18.82$^{\#\#}$	1.16 ± 0.08$^{\#\#}$

Abbreviations: Y, Youth group; M, Middle-aged group; BMI, Body Mass Index; kcals/d, kilocalaries/day; MVPA/d, average daily time of moderate-to-vigorous physical activity.

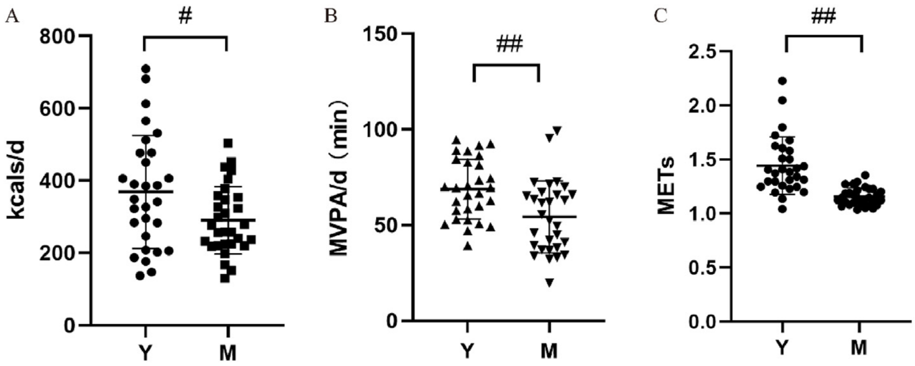

Fig. 1. Changes in indicators of daily physical activity among women of different ages: (A) The average daily energy consumption. (B) The average daily time of moderate-to-vigorous physical activity. (C) Metabolic equivalents. (# $p < 0.05$; ## $p < 0.01$) Abbreviations: kcals/d, kilocalories/day; MVPA/d, average daily time of moderate-to-vigorous physical activity.

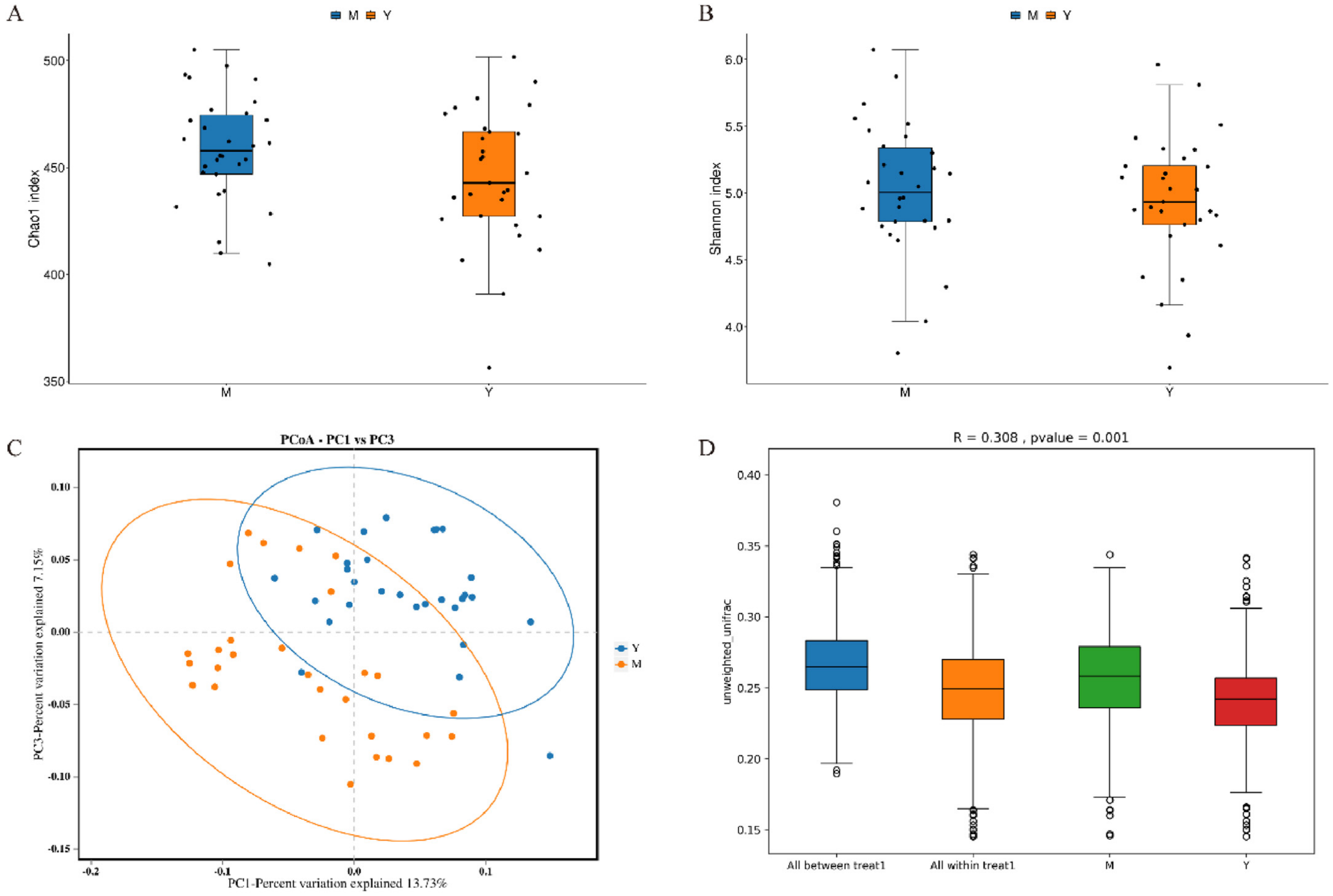

Fig. 2. Analysis of intestinal flora diversity in women between youth and middle-aged group: (A) The Chao1 index. (B) The Shannon index. (C) The Principal Co-ordinate Analysis. (D) The beta diversity analysis of different group by Unweighted Unifrac method. ($n = 29$–30).

At the phylum level, significantly lower were observed in *Acidobacteria, Chloroflexi, Nitrospirae,* and *Gemmatimonadetes* in the middle-aged group ($p < 0.01$). The ratio of *Firmicutes* to *Bacteroidetes* (F/B) did not exhibit a significant change (refer to Fig. 3A and B). Regarding the genus level, significant reductions were observed in *Collinsella, Ruminococcus gnavus, Rothia, Haemophilus, Sphingomonas, Lactobacillales, Lactococcus,* and others in the middle-aged group. Conversely, *Phascolarctobacterium, Paraprevotella, Flavonifractor, Acinetobacter, Intestinimonas,* and others were significantly higher in the middle-aged group (see Fig. 3C).

Functional gene prediction analysis revealed lower levels of oxidative stress tolerance (*Klebsiella, Enterobacteriaceae*), biofilm formation (*Collinsella, Bifidobacterium*), and kinetic elements (*Eubacterium, Streptococcus*) in the middle-aged group compared to the youth group. Additionally, higher levels of Gram-negative bacteria (*Phascolarctobacterium, Bacteroides, Akkermansia*) were observed in the middle-aged group. These findings suggest a lower level in the abundance of bacteria associated with immune resistance and stress tolerance in perimenopausal women. Moreover, the increased presence of Gram-negative organisms with an outer membrane structure, primarily producing LPS

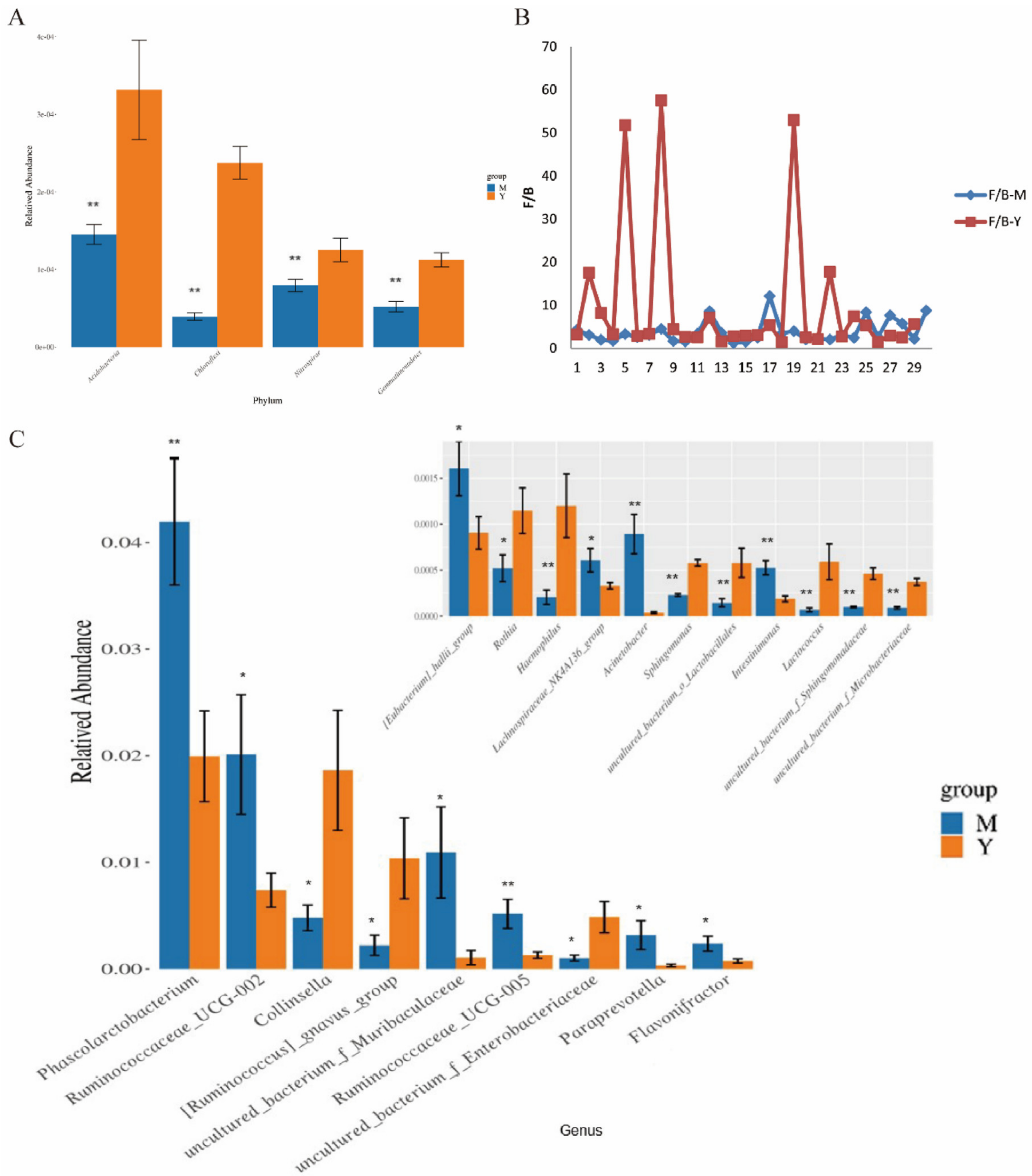

Fig. 3. Analysis of differential intestinal flora in women of different ages: (A) The number of Acidobacteria, Chloroflexi, Nitrospirae, and Gemmatimonadetes in the youth and middle-aged group. (B) The ratio of Firmicutes to Bacteroidetes (F/B). (C) Comparison of the content of each bacterial group at genus level. ($n = 29–30$, $*p < 0.05$; $**p < 0.01$).

endotoxin, may impair the body's defensive capacity (refer to Fig. 4).

3.3. Correlation analysis between daily physical activity and characteristic flora

The correlation analysis of the above screened characteristic flora with daily energy utilization value, moderate-to-vigorous physical activity (MVPA) and METs revealed that METs had the strongest correlation with the characteristic flora, followed by MVPA and then daily energy utilization value. METs showed significant positive correlation with *Acidobacteria*, *Chloroflexi*, *Nitrospirae*, *Sphingomonas*, *Enterobacteriaceae*, and with *Intestinimonas*, *Phascolarctobacterium* showed a negative correlation. Notably, *Sphingomonas* has the strongest correlation with daily physical activity indicators, suggesting that it may be a sensitive genus for exercise interventions. See Table 2.

4. Discussions

4.1. Changes in the daily physical activity of perimenopausal women

As individuals age, the functioning of all body systems gradually declines, leading to a relative reduction in physical activity. Perimenopause represents a unique phase in the female physiological process, often accompanied by osteoporosis and metabolic disorders due to hormonal changes. BMI serves as a comprehensive indicator of the body's nutritional status, integrating factors such as exercise and diet. Studies

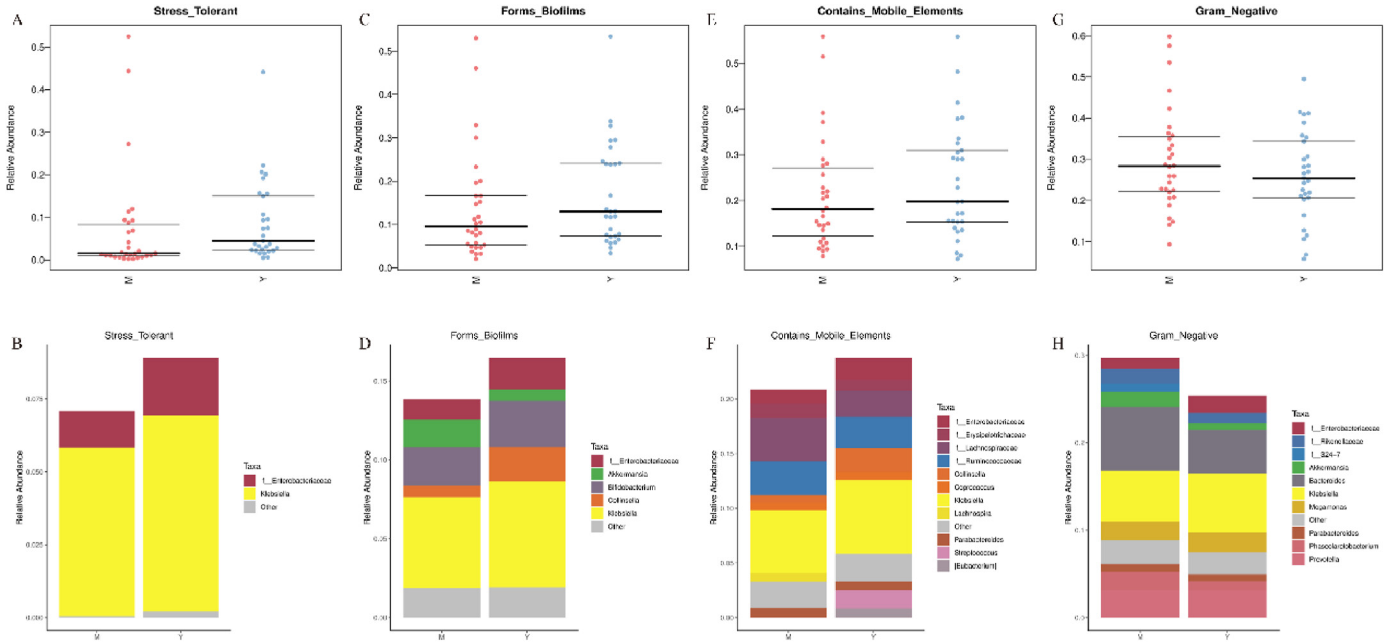

Fig. 4. Functional prediction analysis of different intestinal flora in women of different ages: (A, B) The prediction analysis of levels of oxidative stress tolerance. (C, D) Prediction of biofilm formation. (E, F) Analysis of kinetic elements. (G, H) The levels of Gram-negative bacteria. (n = 29–30).

Table 2
Correlation coefficient R values of daily physical activity indicators and characteristic flora (n = 59).

	kcals/d	MVPA/d	METs
Acidobacteria	0.338 6**	0.204 1	0.441 2**
Chloroflexi	0.286 4*	0.317 3*	0.543 5**
Nitrospirae	0.184 9	0.308 3*	0.557 3**
Gemmatimonadetes	0.291 2*	0.121 2	0.238 1
Sphingomonas	0.315 1*	0.424 2**	0.661 4**
Enterobacteriaceae	0.231 7	0.261 9*	0.426 2**
Intestinimonas	−0.186 9	−0.294 9*	−0.44 9**
Phascolarctobacterium	−0.060 8	−0.105 7	−0.290 7*

*$p < 0.05$; **$p < 0.01$.
Abbreviations: kcals/d, kilocalories/day; MVPA/d, average daily time of moderate-to-vigorous physical activity.

have indicated that BMI, exercise, and physical activity are independent risk factors for osteoporosis in perimenopausal women. In a study involving 151 adults, higher levels of physical activity were associated with a decreased prevalence of cardiovascular disease. Specifically, for each increase of 1 000 MET-min/w in physical activity level, the risk of cardiovascular disease in women decreased by 7%. Therefore, physical activity can serve as an evaluative parameter for assessing the risk of cardiovascular disease.[8,9] Notably, physically active women (engaging in moderate and high-intensity physical activity) did not exhibit significant differences in blood pressure and markers of vascular function before and after menopause.[10] In the present study, energy utilization, MVPA, and METs were significantly reduced in menopausal women compared to young women, despite no significant differences in BMI. These findings suggest a substantial reduction in daily physical activity among women at this stage, potentially contributing to adverse effects such as osteoporosis and abnormalities in cardiovascular and cerebrovascular function.

4.2. Relationship between characteristics of intestinal flora and daily physical activity in perimenopausal women

Intestinal microbiota plays an important role in metabolic function, immunity, food digestion, endocrine function and nerve signaling maintaining physiological homeostasis, several factors are greatly affected, such as physical activity, diet, age, gender, etc.[11,12] Short-term dietary intervention can rapidly change the diversity of intestinal flora.[13] Long-term dietary differences in different regions also affect intestinal flora. Yatsunenko et al. found that America has a significantly smaller diversity of gut microbiota than the African. Respectively, America is dominated by a low-fiber diet while African has a plant-based polysaccharide diet.[14] Physical activity prevented dietary changes. It alters the gut microbiota in a mouse model of high fat diet-induced obesity.[15] In this study, all subjects from the same area with small regional differences and similar food cultures, which can reduce the impact on intestinal microbes.

Firmicutes and *Bacteroidetes* are the two predominant phyla in the gut microbiota, and the ratio of *Firmicutes* to *Bacteroidetes* (F/B) is commonly used to assess the composition of gut flora in different individuals. Liping Zhao et al. found that obese individuals tend to have lower levels of *Bacteroidetes* in their intestines, and the proportion of *Bacteroidetes* increases after weight loss.[16] Additionally, the F/B ratio has been found to be associated with age, with significantly higher values observed in elderly individuals aged 60–69 years compared to children aged 0–9 years.[17] In our study, the middle-aged group consisted of women aged 45–55 years, representing an age difference of approximately 20–25 years compared to the youth group. No significant changes in BMI and F/B ratio were observed between the two groups. *Acidobacteria* is a widely distributed bacterial phylum, particularly in soil ecosystems. *Chloroflexi* is found in various environments and plays a role in biogeochemical cycling, particularly in soil and marine habitats. *Nitrospirae* and *Chloroflexi* are both nitrite-oxidizing bacteria (NOB) that are widely distributed in marine ecosystems and contribute to nitrogen cycling.[18] While dominant bacteria such as *Acidobacteria* have been extensively studied, their physiological and pathological functions remain largely unknown. For instance, *Acidobacteria* has been reported to be lacking in patients with functional dyspepsia, and it participates in carbohydrate and nitrogen metabolism in premature infants.[19,20] Furthermore, *Acidobacteria, Nitrospira,* and *Gemmatimonadetes* have been found to be significantly decreased in patients with systemic lupus erythematosus (SLE), suggesting their involvement in the body's immune system.[21] In our study, we observed no significant changes in dominant gut bacteria in perimenopausal women, but there was a significantly lower level in the overall proportion of NOB bacteria. *Acidobacteria, Chloroflexi,* and

Nitrospirae showed a significant positive correlation with METs values, as well as a certain degree of positive correlation with moderate-to-high intensity physical activity and average daily energy utilization. This suggests that these non-dominant phyla may participate not only in carbohydrate and nitrogen metabolism but also as effector phyla regulated by daily physical activity.

Collinsella is capable of producing ursodeoxycholic acid in the intestine, which possesses anti-inflammatory and antioxidant effects. One study found a negative association between *Collinsella* and osteoporosis.[22] Additionally, the ratio of intestinal *Collinsella* has been inversely correlated with the mortality rate of individuals infected with COVID-19.[23] *Rothia* is involved in the production of short-chain fatty acids, particularly butyric acid, which plays a role in energy production, intestinal protection, and anti-inflammatory effects. *Rothia* has been negatively associated with diseases such as ulcerative colitis, irritable bowel syndrome, colorectal cancer, obesity, type II diabetes, as well as age-related conditions like Parkinson's disease, chronic fatigue syndrome, and cardiovascular diseases including atherosclerosis, coronary artery disease, and hypertension.[24,25] *Lactobacillales* and *Lactococcus* are considered beneficial gut flora and are commonly utilized in the production of probiotics and fermented dairy products. Interestingly, in contrast to our study, *Collinsella, Rothia, Lactobacillales,* and *Lactococcus* were significantly decreased in stool samples from the perimenopausal women group, suggesting that alterations in the characteristic flora of women at this age are associated with an increased risk of abnormal glucolipid metabolism, cardiovascular disease, and osteoporosis.

Sphingomonas is a genus characterized by a unique lipopolysaccharide structure and represents a newly re-isolated class of genera from Pseudomonas. It is widely distributed in various natural habitats and has implications for human health.[26] Various bacterial extracts containing *Sphingomonas* have been found to possess anti-aging effects, largely attributed to the production of unique carotenoids with antioxidant and environmental stress tolerance effects, thanks to intracellular catalase and oxidase.[27] Moreover, *Sphingomonas* extracts have been shown to inhibit the expression of SA-β-galactosidase and cell cycle inhibitors (p21, p16), consequently delaying cellular senescence.[28] In our study, we observed a significant downregulation of intestinal *Sphingomonas* in perimenopausal women, which exhibited a significant positive correlation with daily physical activity-related indicators including energy utilization, moderate-to-vigorous physical activity, and metabolic equivalents. This suggests that *Sphingomonas* may serve as a sensitive effector genus in response to daily physical activity interventions. Increasing daily physical activity may enhance the abundance of this genus, exerting antioxidant and anti-aging effects while reducing the occurrence of adverse events in menopausal women.

Phascolarctobacterium, an obligate anaerobic and gram-negative bacterium, produces short-chain fatty acids and associated with host metabolism and mood. The abundance of this bacterium gradually increases with age, a high-fat diet and weight gain, but decreases in individuals over 60 years old. *Phascolarctobacterium* can utilize succinic acid produced by other bacteria to participate in the body's metabolism.[29] *Acinetobacter,* on the other hand, is an obligate anaerobic, non-fermenting, non-motile *coccobacillus* that is often conditionally pathogenic. It is susceptible to infections when the body's resistance is reduced and is one of the main opportunistic pathogens causing nosocomial infections because of the ability of *A. baumannii* to withstand desiccation and to form biofilms. *Acinetobacter* can lead to various infections, including respiratory tract infections, bloodstream infections, and wound infections et.[30] In our study, the abundance of both *Phascolarctobacterium* and *Acinetobacter* were significantly increased in perimenopausal women compared to youth women, and *Acinetobacter* showed a negative correlation with METs, suggesting that these genera may be associated with age, weight gain, and host metabolism, leading to an increased risk of potential pathogenicity. Furthermore, *Paraprevotella, Flavonifractor, Intestinimonas,* and other genera were enriched in the intestinal tract of perimenopausal women in our study. Due to the lack of relevant studies, the precise function of *Intestinimonas* remains unclear, although it was found to be negatively correlated with MVPA and METs. This suggests that daily physical activity may have some effect on *Intestinimonas*.

5. Conclusions

In this study, we compared the daily physical activity and characteristic intestinal flora in youth women to assess the impact on menopausal women. We observed a significant decrease in daily physical activity (MVPA, METs) among menopausal women, even before changes in body fat percentage. Among these indicators, METs demonstrated the strongest correlation with characteristic flora. Notably, we found that *Sphingomonas* exhibited the most significant correlation with daily physical activity indicators, implying that it may serve as a sensitive genus for exercise interventions.

Ethical approval statement

All subjects gave informed consent before participating , and the study procedure was approved by the Ethics Committee of Gaozhou Hospital of Traditional Chinese Medicine, Guangzhou University of Traditional Chinese Medicine (NO. Y [2021]16).

Authors' contributions

Yanqiu Zheng: Writing – review & editing, Writing – original draft, Data curation. **Junyu Ke:** Writing – review & editing, Investigation. **JinBin Song:** Investigation. **Xuyu Li:** Resources, Methodology, Investigation. **Rongman Kuang:** Resources, Methodology, Investigation. **Haiyan Wang:** Resources, Methodology, Investigation. **Shuang Li:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Yanwu Li:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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