

Gut Microbiota Associated with Effectiveness and Responsiveness to Mindfulness-Based Cognitive Therapy in Improving Trait Anxiety

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Research

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Abstract

Objective

The mindfulness based interventions have been widely demonstrated effective on reducing stress, alleviating mood disorders and improving quality of life; however, the underlying mechanisms remained to be fully understood. Along with the advanced research in microbiota-gut-brain axis, this study aimed to explore the impact of gut microbiota on the effectiveness and responsiveness to mindfulness-based cognitive therapy (MBCT) among high trait anxiety populations.

Design

A standard mindfulness-based cognitive therapy (MBCT) was performed among 21 young adults with high trait anxiety. A total of 29 healthy controls were matched for age and sex. The differences on gut microbiota between the two groups were compared. The changes of fecal microbiota and psychological indicators were also investigated before and after the intervention.

Results

Compared with healthy controls, we found markedly decreased bacterial diversity and distinctive clusters among high trait-anxiety populations, with significant overgrowth of bacteria such as *Streptococcus*, *Blautia*, *Romboutsia*, and decrease of genera such as *Faecalibacterium*, *Coprococcus_3*, *Lachnoclostridium*. Moreover, MBCT attenuated trait-anxiety and depression, improved mindfulness and resilience, and turned gut microbiota more close to that of healthy controls. Notably, high burden of intestinal *Subdoligranulum* pre-MBCT was associated with an increased responsiveness to MBCT. Decreases in *Subdoligranulum* post-MBCT were indicative of ameliorated trait anxiety. The tryptophan metabolism pathways were significantly over-represented among high-responders compared to low-responders.

Conclusion

The significantly increased diversity post-MBCT added evidence to gut-brain communication, and highlighted the utility of mycobiota-focused strategies for promoting effectiveness and responsiveness of the MBCT to improve trait anxiety.

Trial registration: Chicttr.org.cn, ChiCTR1900028389. Registered 20 December 2019, <http://www.chicttr.org.cn/edit.aspx?pid=47167&htm=4>

Introduction

As proposed by Spielberger [1, 2], trait anxiety described as a relatively stable disposition referring to a tendency within individuals to judge a situation as more threatening than it actually was. It was marked that people with trait anxiety might react excessively anxious and nervous while facing dangerous or

uncertain situations. Trait anxiety were reported significantly associated with panic attacks, impaired cognitive functions, harm avoidance, obsessive-compulsive disorder, and other psychopathological disorders [3–5]. The prevalence of trait anxiety revealed an estimated 30% among Chinese medical professionals [6]. This prevalence was responsible for mission failures, poor medical outcomes, significant decrements in tolerance and resilience, and eventually resulted in reduced quality of life. Therefore, considering the increasing tasks and missions among medical professionals such as disaster rescues and epidemics of infectious diseases, it was imperative to alleviate trait anxiety and to promote mental health among medical professionals through structured systematic interventions.

According to the diathesis - stress model [7], individuals with high trait anxiety inherited a genetic tendency toward a mental disorder. Due to the limited advances in psychopharmacology in recent years and the increasing reports on relapse rates and adverse events, it was essential to explore alternative psychotherapies to help patients with mental disorders. In recent years, mindfulness based interventions have gained worldwide popularity since it has been widely demonstrated effective on reducing stress [8, 9], alleviating mood disorders such as depression, anxiety [10, 11] and bipolar disorder [12], relieving pain and improving quality of life in clinical or non-clinical populations [13, 14]. Research has also revealed that mindfulness was negatively associated with trait anxiety, and mindfulness training could improve trait anxiety [15–17]. Moreover, a study by Dr Cotton revealed that mindfulness-based cognitive therapy (MBCT) could prevent the progression and deterioration of mental health disorders in young adults [18]. Long-term mindfulness training was showed to effectively change the cortex thickness and gray matter density in brain regions, which were associated with learning, memory, attention processes, and mood regulations [5, 19].

Despite of the increasing evidence about the effectiveness of mindfulness, the underlying mechanisms remained to fully understand. It was increasingly suggested that there could be some overlapping among the biological, behavioral and psychosocial mechanisms. Interestingly, accumulating data indicated that the gastrointestinal (GI) microbiota could influence brain functions, mood symptoms and behaviors. Gut microbiota dysbiosis was observed in individuals with mood disorders such as autism, bipolar disorder [20], anxiety and depression. Therefore, it may be a novel way to explore the gut-brain axis to assist and/or strengthen the effectiveness of psychopharmacology and psychotherapy, such as the mindfulness based interventions. For example, previous studies have revealed significant differences on gut microbiota among patients with major depression disorder (MDD) compared to healthy controls. The gut microbiota among MDD patients was characterized by a relative abundance of Firmicutes, Actinobacteria, and Bacteroidetes [21, 22]. Additionally, following a fecal microbiota transplant (FMT) from participants with MDD patients, the depressive-like behaviors was also transferred to germ-free mice. In another animal study, it was found enhanced physiological response to stress among germ-free mice compared to pathogen-free mice, and the altered stress response could be partially corrected if GI microbiota were reconstituted at an early age [20]. Furthermore, the consumption of probiotics was demonstrated to place a positive impact on alleviating stress responses, symptoms of anxiety and depression, and cognitive functions in both clinical studies [23–25] and in animal studies [26]. For instance, in a trial by Reininghaus et al [27], there were significant improvements on attention and

psychomotor processing speed after 1 and 3 months of probiotic supplementation among patients with bipolar disorder. In another study, Liu et al. found that administration of probiotics (*Lactobacillus* sp.) could reduce immobility time in the forced swimming model of depression in mice with early life stress [28]. Taken together, these findings indicated an intimate connection between psychiatry and GI microbiota. However, there was a lack of evidence to examine the effect of gut microbiome on the effectiveness and responsiveness to mindfulness interventions to mental illness.

Therefore, this study aimed: (1) to explore the differences on gut microbiota among high trait anxiety group compared to healthy controls, and to further find out the potential bacterial genera that may contribute to the differences; (2) to examine the effectiveness of MBCT intervention to relieve mood disorders among high trait anxiety group, and to investigate the changes on gut microbiota before and after the intervention; (3) to investigate the potential bacterial genera that may contribute to the responsiveness to MBCT.

Methods

Participants and study design

This intervention trial was the second phase of a Strong Mind Project (SMP). This study was performed at a medical university in China from July 2019 to July 2020. The first phase was a cross-sectional survey to investigate mental health status among young adults from a medical school. In the first phase, questionnaires were distributed to assess participants' trait anxiety, mindfulness, depression, resilience and happiness. The participants who scored ≥ 50 points in the trait anxiety subscale of state-trait anxiety inventory (STAI) were potentially eligible for the second phase of MBCT intervention. Eventually, seventy-six participants in the first phase were invited and forty-one of them agreed to complete a further screening to confirm eligibility for the second phase. The screening rules were inclusion criteria and exclusion criteria as stated below. Young adults who met the following criteria were included: (1) aged ≥ 18 years; (2) the score of trait anxiety subscale ≥ 50 points; (3) a willingness and motivation to follow the study protocol; (4) not currently taking anti-anxiety or anti-depressant medication; and (5) not report previous meditation or mindfulness experience. The exclusion criteria included: (1) history of gastrectomy; (2) diagnosis of mental health disorders such as depression and anxiety; (3) the use of anti-acids and/or gastric mucosal protective drugs and/or antibiotics and/or probiotics 4 weeks prior to the study; and (4) not able to follow MBCT intervention due to physical restraints or other reasons. Reasons for the participant to be discontinued from the study: (1) withdrawal of informed consent; (2) incomplete attendance at the intervention visits due to time conflicts or other reasons; (3) exclusion criteria found after enrollment; and (4) any serious adverse event during the intervention period. According to the screening rules, a total of 25 young adults (intervention group, also known as high trait-anxiety group) were eventually recruited into the intervention. Four subjects withdrew from the study during the intervention.

Healthy controls were recruited from young adults whose trait anxiety scored lower than 50 points. The inclusion criteria were: (1) aged ≥ 18 years; (2) no history of gastrectomy; (3) not current report of depression or anxiety; (4) not currently taking anti-anxiety or anti-depressant medication; (5) not report previous meditation experience; (6) not the current use of anti-acids and/or gastric mucosal protective drugs and/or antibiotics and/or probiotics 4 weeks prior to the study; and (7) willing to take part in. Eventually, a total of 29 young adults were included.

Ethical Consideration

Ethical approval was obtained from the medical ethics committee of army medical university (2019-005-02). This trial was registered at Chinese Clinical Trial Registry (Chictr.org.cn, ChiCTR1900028389). The first participant of the intervention study was enrolled in 30th December. Signed informed consent was obtained from each participant after the procedures were clearly explained.

Mindfulness intervention

The intervention was mindfulness-based cognitive therapy (MBCT), which combined elements of both mindfulness training and cognitive-behavioral therapy (CBT). It was originally developed to prevent relapse of major depression disorders (MDD), but has since been adapted to a range of different populations and contexts. Instead of focused on thought content tradition CBT, the MBCT approach focused on through process, and promoted new way of being with painful affects and challenging circumstances. Our intervention protocol followed the standard structure of MBCT, including the 8-week group-based format, and the length and type of homework assignments.

Measures

In addition to information on demographics including age, gender, weight, height, BMI (weight in kilograms/[height in meters]²), 3-day dietary intake, and current use of meditation, self-report questionnaires were also used to assess participants' trait anxiety, resilience, mindfulness and depression.

Trait Anxiety. The personality of trait anxiety was described as a relatively stable personality trait and anxious tendencies. In this study the trait anxiety was measured by the Chinese version of the trait anxiety subscale of state-trait anxiety inventory (STAI-T), which originally developed by Spielberger and Gorsuch [29, 30]. The trait anxiety subscale included 20 items following a 4-point Likert scale ranging from 1 (almost never) to 4 (almost always). Items 1, 3, 4, 6, 7, 10, 13, 14, 16, and 19 were scored in reverse. The total score for each scale ranges from 20 to 80. The higher the overall score was, a higher trait anxiety level was indicated.

Resilience. The Connor-Davidson Resilience Scale (CD-RISC) [31, 32] was used to assess participants' ability to endure stress or pain and to cope with adversity. The CD-RISC comprised of 25 items, with each item responding on a 5-point Likert scale from 0 (not true at all) to 4 (true nearly all the time). The total

score ranged from 0-100, with higher scores reflecting greater resilience. The scale showed good psychometric properties with a Cronbach's alpha value of 0.89 and test-retest correlation of 0.87.

Mindfulness. The 15-item Mindful Attention Awareness Scale (MAAS) [33] was adopted to measure the participants' capacity of a sustained attention to and awareness of the experience of the present moment in the daily life. Items on the MAAS were reverse-scored and assessed the absence of mindful attention rather than actual mindful moments. The scale scored according to a 6-point Likert scale from 1 (almost always) to 6 (almost never). The elevated scores indicated a greater state of mindfulness. The internal consistency of alpha coefficient was 0.82.

Depression. The 20-item Self-rating Depression Scale (SDS) used to explore the participants' self-reported level of depression [34, 35] SDS was a 4-point Likert scale assessing both affective and somatic symptoms, with 1 = a little of the time to 4 = most of the time. The raw scores ranging from 20 to 80 were converted to standard scores by dividing the sum of the raw scores by 80, and multiplying by 100. The higher the overall score, the more frequent depressive symptoms were experienced. Individuals with SDS standard scored greater than 50 were regarded as having clinically significant anxiety.

Data collection

Psychometric questionnaires, a three-day 24-hour dietary history and fresh stool samples were collected from all participants at baseline (before intervention), at week 8 (at the end of the intervention) and week 12 (4 weeks follow-up of the intervention). Questionnaires were filled out through online website at each time point. The three-day 24-hour dietary history of each participant were recalled and recorded by a trained research assistant. Fecal samples were collected after questionnaires. Participants were asked to return the fecal specimen to the research assistant on the day of sample collection. All stool samples were immediately frozen and stored at -80 °C prior to analyses.

16S rRNA gene sequencing

Total genomic DNA from each fecal sample was extracted utilizing the commercial TIANamp Stool DNA Kit (TIANGEN Biotech Co. Ltd., Beijing, China) according to the manufacturer's instructions. For sequencing, a DNA fragment comprising the bacterial hypervariable regions V3-V4 of the 16S rRNA gene were amplified with primers 341F (5'- CCTAYGGGRBGCASCAG-3', forward primer) and 806R (5'- GGACTACNNGGTATCTAAT-3', reverse primer). The PCR amplifications were performed and purified and then sequenced on an Illumina Miseq high-throughput PE 300 Sequencing platform (Illumina, San Diego, USA) by Novogene Biomedical Corporation (Beijing, China).

Fecal microbiota analysis

Sequence outputs were analyzed using the software package of QIIME2 (Quantitative Insights into Microbial Ecology 2, version 2019. 7) platform on our Linux server (i7-8700K, 64Gb RAM). The bioinformatic analysis process was performed following (1) quality filtering; (2) feature-table rarefaction; and (3) phylogenetic diversity analyses. Then the diversity and composition analysis was performed of

within-sample diversity (α -diversity), between-sample diversity (β -diversity) and PCoA (Principal Coordinates Analysis). Moreover, the linear discriminant analysis of effect size (LEfSe) was performed to identify: (a) taxonomic composition of high trait-anxiety groups compared to controls; (b) changes in the taxonomic composition of the gut microbiota in high trait-anxiety groups before and after mindfulness intervention; and (c) taxonomic composition between the “high responders” and the “low responders” to the mindfulness intervention among high trait-anxiety group. It was considered as significantly discriminant taxa with LEfSe score greater than 2.0 (default) and $p < 0.05$. Regarding the “high responders” and the “low responders” to the mindfulness intervention, “high responders” were regarded as those whose score changes on trait-anxiety subscale before and after the intervention were more than 50% ($n=9$); while the “low responders” were those whose score changes were less than 50% ($n=11$).

Functional pathway prediction

The amplicon sequence variants (ASVs) was performed by PICRUST2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States, version 2) to predict functional pathway from 16S rRNA gene sequences. Then ASVs were categorized into Clusters of Orthologous Groups (COG) and predicted based on the Kyoto Encyclopaedia of Genes and Genomes (KEGG) database.

Statistical analyses

All statistical analyses were conducted using software as follows: SPSS (the Statistical Package for the Social Sciences version 21.0, IBM, US), Graphpad prism (version 8.0, GraphPad Software Corporate, San Diego, US), and R (version 3.4.3, Team 2017) with the ‘ape’ and ‘vegan’ package. Data was presented as mean \pm standard deviation (SD). With regard to the psychological variables including trait anxiety, mindfulness, resilience and depression, continuous scores were used in the analyses of associations with microbial composition and correlation analyses with bacterial abundance. The χ^2 test or Fisher’s exact test were used for analysis of categorical data and Student’s t test or the ANOVA test for analysis of continuous data. Significant differences in α -diversity and β -diversity distances across groups were assessed using the Mann-Whitney U tests. The p value was set at 0.05 (two-sided) throughout all analyses.

Results

Part 1. Sample characteristics

See Table 1 for demographic characteristics, and descriptive information on mental health and daily nutrient consumption at different time points for the high trait-anxiety group (intervention group). The intervention group included 5 females and 16 males, with mean age of 20.38 years (range: 18–23 years). The healthy controls included 7 females and 22 males, with mean age of 20.97 years (range: 19–25 years). The average score of BMI (Body Mass Index) was 21.74 and 22.27 for the intervention group and the healthy controls, respectively. No significant differences were observed on age, BMI score, and the

nutrient elements of protein, fat, carbohydrate and fiber between the intervention group and the healthy controls at baseline ($p>0.05$). Compared to the healthy controls, the participants in the intervention group showed higher level of trait-anxiety and depression, and lower level of mindfulness and resilience ($p<0.001$) (Fig. 1A-D, and Table 1).

Table 1
Demographics and information on mental health and daily nutrients at each time points

| Variables | MBCT intervention (n = 21) | Healthy controls (n = 29) | <i>P</i> |
|------------------------------|----------------------------|------------------------------|----------|
| | Mean (SD) | Mean (SD) | |
| Age | 20.38 ± 1.12 | 20.97 ± 1.72 | 0.180 |
| Weight | 64.04 ± 9.02 | 66.14 ± 10.02 | 0.450 |
| BMI(kg/m²) | 21.74 ± 1.71 | 22.27 ± 2.53 | 0.410 |
| Trait anxiety scale | | | |
| 0w | 54.14 ± 3.95 | 35.90 ± 5.97 | 0.000 |
| 8w | 45.90 ± 8.51 | | |
| 12w | 44.86 ± 9.06 | | |
| Mindfulness scale | | | |
| 0w | 44.67 ± 8.80 | 64.03 ± 9.44 | 0.000 |
| 8w | 53.48 ± 9.71 | | |
| 12w | 51.95 ± 13.08 | | |
| Resilience scale | | | |
| 0w | 50.14 ± 3.95 | 72.45 ± 11.83 | 0.000 |
| 8w | 45.90 ± 8.51 | | |
| 12w | 44.86 ± 9.06 | | |
| Depression scale | | | |
| 0w | 47.10 ± 8.43 | 32.10 ± 6.87 | 0.000 |
| 8w | 37.10 ± 9.27 | | |
| 12w | 41.19 ± 11.09 | | |
| Protein (g) | | | |
| 0w | 81.40 ± 67.87 | 78.80 ± 39.31 | 0.865 |
| 8w | 51.44 ± 32.73 | | |
| 12w | 76.89 ± 36.01 | | |

Note: MBCT, mindfulness-based cognitive therapy; BMI, body mass index; SD, standard deviation.

| Variables | MBCT intervention (n = 21) | Healthy controls (n = 29) | <i>P</i> |
|-------------------------|----------------------------|------------------------------|----------|
| | Mean (SD) | Mean (SD) | |
| Fat (g) | | | |
| 0w | 52.65 ± 35.12 | 54.71 ± 34.27 | 0.836 |
| 8w | 34.26 ± 36.49 | | |
| 12w | 56.33 ± 30.90 | | |
| Carbohydrate (g) | | | |
| 0w | 204.64 ± 123.36 | 198.26 ± 128.98 | 0.861 |
| 8w | 143.96 ± 71.69 | | |
| 12w | 258.10 ± 183.49 | | |
| Fiber (g) | | | |
| 0w | 6.46 ± 4.23 | 7.53 ± 6.42 | 0.509 |
| 8w | 4.51 ± 4.65 | | |
| 12w | 5.94 ± 4.72 | | |
| Calories (kcal) | | | |
| 0w | 1625.55 ± 963.43 | 1599.37 ± 869.89 | 0.920 |
| 8w | 1088.46 ± 691.40 | | |
| 12w | 1848.15 ± 991.19 | | |

Note: MBCT, mindfulness-based cognitive therapy; BMI, body mass index; SD, standard deviation.

Part 2. Different gut microbiota compositions prior to the MBCT

Fecal bacterium composition

There was no significant difference in α -diversity between high trait-anxiety group and healthy controls (Fig. 1E-H). However, significant differences were observed on β -diversity index indicating the extent of the similarity in the microbial communities. As shown in Fig. 1I-N, the gut microbiota of high trait-anxiety group and healthy controls could be significantly divided into two different clusters and separated clearly by PCoA analysis. To further evaluate whether high level of trait-anxiety had an effect on β -diversity, we compared the distance between each pair of samples between high trait-anxiety group and healthy

controls; and significantly higher unweighted UniFrac distance in healthy controls were observed ($p < 0.001$) (Fig. 1O-P). Cluster tree of each fecal sample were shown in Fig. 1Q. See Supplementary Fig. 1 for the difference between high trait-anxiety group and healthy controls on relative abundance at the phylum level, the family level and the genus level in fecal microbiota. A cladogram showing differences in fecal microbiota between high trait-anxiety group and healthy controls was reported in Supplementary Fig. 5(A and C). In the figure, it was showed that *Actionbacteria* phylum was greatly enriched in healthy controls, while *Firmicutes* phylum was consistently higher in high trait-anxiety group.

Gut Microbiota Difference

To investigate the significant bacteria that may contribute to the differences on gut microbiota between high trait-anxiety group and healthy controls, we performed PLS_DA analysis. Almost 20 kinds of bacteria met the standard of $vip_{score} > 2$ in PLS_DA analysis, with the top five bacteria genus were:

Streptococcus, *Blautia*, *Romboutsia*, *Lachnoanaerobaculum* and *Lachnoclostridium* (Supplementary Fig. 2C). Further comparisons were performed on the relative abundance of all the significant bacteria between high trait-anxiety group and healthy controls (Supplementary Fig. 3). Compared to healthy controls, high trait-anxiety group showed significantly lower relative abundance in: *Streptococcus* ($p < 0.0001$), *Blautia* ($p < 0.0001$), *Romboutsia* ($p < 0.0001$), *Escherichia_Shigella* ($p < 0.0001$), *Eubacterium_hallii_group* ($p < 0.001$), *Eggerthella* ($p < 0.001$), and *Allorhizobium_Neorhizobium_Pararhizobium_Rhizobium* ($p < 0.001$), and showed significantly higher relative abundance in: *Lachnoanaerobaculum* ($p < 0.001$), *Lachnoclostridium* ($p < 0.001$), *Rothia* ($p < 0.01$), *Leptotrichia* ($p < 0.01$), *Lachnospiraceae_UCG_010* ($p < 0.01$), *Faecalibacterium* ($p < 0.01$), *Coprococcus_3* ($p < 0.01$), *Eubacterium_eligens_group* ($p < 0.01$), *Atopobium* ($p < 0.01$), *GCA_900066575* ($p < 0.01$) and *Pseudopropionibacterium* ($p < 0.05$).

Part 3. Mbct Intervention Altered Gut Microbiota

Compared to the baseline (time point: 0 week), participants in the high trait-anxiety group experienced significant improvements in mindfulness (Fig. 2B) and resilience (Fig. 2C), and significant decreases on trait-anxiety (Fig. 2A) and depression (Fig. 2D) at the end of intervention (time point: 8 week) and 4-week follow-up after end of intervention (time point: 12 week) (all $p < 0.05$). The community richness and diversity of fecal microbiome shown by α -diversity indices were not significantly different before and after MBCT intervention (Fig. 2E-H). Nevertheless, the intervention group displayed a decreasing β -diversity distance over the time points, indicating that the MBCT intervention had a significant effect on gut microbiota. In accordance with these observations, the PCoA demonstrated changes in pattern of clustering over the period of intervention and follow-up. The gut microbiota before and after intervention was obviously separated into different clusters among high trait-anxiety group (Fig. 2I-N). Interestingly, at the end of intervention (8 week) and 4 week follow-up (12 week), the β -diversity among high trait-anxiety group changed more close to that among healthy controls (Fig. 2O-P). Cluster tree of each fecal sample were shown in Fig. 2Q. A cladogram showing differences in fecal microbiota among high trait-anxiety group over different time points were presented in Supplementary Fig. 5. Notably, at the end of MBCT

(week 8) in high trait-anxiety group, it was found increasing abundance of the phylum of *Actionbacteria*, *Proteobacteria* and *Fusobacteria*.

Changes of significant genera over intervention period

The relative abundance of the 18 bacteria that were found significant between high trait-anxiety group and healthy controls were investigated over the intervention period (Supplementary Fig. 4). Specifically, from baseline to the end and the follow-up, the MBCT intervention resulted in a significant increase in relative abundances of *Streptococcus* ($p < 0.0001$), *Blautia* ($p < 0.0001$), *Romboutsia* ($p < 0.0001$), and *Eggerthella* ($p < 0.001$), while the intervention led to a reduction in *Lachnospiraceae_UCG_010* ($p < 0.01$), *Faecalibacterium* ($p < 0.01$), *Coproccoccus_3* ($p < 0.01$), and *Eubacterium_eligens_group* ($p < 0.01$). Additionally, at the completion of intervention (week 8) and the follow-up (week 12), the relative abundances of the above bacteria went more close to those in healthy controls, which suggesting that MBCT intervention could successfully shifted high trait-anxiety microbiota community toward that of healthy controls.

Part 4. Subdoligranulum relating to the responsiveness to MBCT interventions

To evaluate whether responsiveness to MBCT interventions related to gut microbiota, we compared bacterial profiles between high-responder group and low-responder group. The PCoA plots revealed a significant separation by genotype (Fig. 3C). The Braycurtis and Unweighted_unifrac distance suggested significantly lower β -diversity distance in high-responder group compared to low-responder group (Fig. 3A-B) ($p < 0.01$). The significant bacteria with vipscores > 2 in PLS_DA analysis were: *Subdoligranulum*, *Eubacterium_yurri_group*, and *Ruminococcus* (Fig. 3D). A further comparison was performed on the relative abundance of the above three bacteria between high-responders and low-responders (Fig. 4A, D-E). A significantly lower relative abundance in *Subdoligranulum* were reported in high-responders compared to low-responders (Fig. 4A-C) ($p < 0.05$), whereas no significant difference were found on the other two bacteria (Fig. 4D-E).

To determine whether *Subdoligranulum* levels were related to trait-anxiety, we then performed linear correlation analyses. It was revealed that the abundance of operational taxonomic units (OTUs) in *Subdoligranulum* were positively correlated with the trait-anxiety score in high-responders (Fig. 4B). Conversely, a negative correlation showed between OTUs and trait-anxiety score in low-responders (Fig. 4C). The linear correlation between high-responders and low-responders in genera *Subdoligranulum* levels with other psychometric indicators (mindfulness, resiliency, depression) were presented in Supplementary Fig. 7.

To determine the role of *Subdoligranulum* in affecting responsiveness, a further linear correlation analyse was conducted between the OTUs and the score difference on trait-anxiety before and after MBCT

intervention (week 8 minus week 0). It was illustrated in high-responders that the higher *Subdoligranulum* abundance was, the lower difference scores was achieved (Fig. 5B); although no significance were found. Taken together, the genera *Subdoligranulum* was considered as a potential target that may help strengthen the MBCT intervention response.

Part 5. Tryptophan Metabolism Over-represented Among High-responders

As shown in Fig. 6, heat map of differentially abundant Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were performed to identify potential function pathways that affecting response strength. The values of color in the heat map represented the normalized relative abundance of KEGG pathways. The analysis indicated that genes for tryptophan metabolism were significantly over-represented among high-responders in comparison with low-responders (Fig. 6A). In addition, a further comparison of KEGG pathways between two time points of baseline (week 0) and after MBCT (week 8) were conducted in high-responders group and low-responders group, respectively. In high-responders, a significant enrichment in the proportion of genes related to staphylococcus aureus infection, taurine and hypotaurine metabolism, ascorbate and aldarate metabolism, tryptophan metabolism, and nitrotoluene degradation were obtained after MBCT intervention (week 8) compared to baseline (Fig. 6B). In low-responders, it was observed that a gene families linked to staphylococcus aureus infection, taurine and hypotaurine metabolism and nitrotoluene degradation were significantly increased after MBCT intervention (week 8) compared to baseline (Fig. 6C). However, no obvious difference were found in genes for tryptophan metabolism. Overall, these data suggested that gut microbiota from high-responders was significantly enriched with genes for tryptophan metabolism, which was a potential pathway affecting the response strength towards MBCT intervention.

Discussion

Although an increasing number of studies have reported an altered gut microbiome composition among patients with major depressive disorder (MDD), the profile of gut microbiota among individuals with high trait anxiety remained unclear. This study has revealed significant alterations in the abundance of different genera within Lachnospiraceae, Peptostreptococcaceae, and Ruminococcaceae family. In particular, compared to healthy controls, we found significantly reduced abundance in *Streptococcus*, *Blautia*, *Romboutsia* and *Eggerthella*. Conversely, a significantly increased abundance was revealed regarding *Lachnoanaerobaculum*, *Lachnoclostridium*, *Faecalibacterium*, and *Coprococcus*. In this study, increased expression of the Lachnospiraceae family, within the phylum Firmicutes, was found in the populations with high trait anxiety. Consistent with previous reports in animal study, the abundance of Lachnospiraceae family were correlated with behavioral changes induced by stress [36]. Since the Lachnospiraceae family participated in the breakdown of carbohydrates into short-chain fatty acids (SCFAs) [37]; therefore the increase of Lachnospiraceae family promoted a decline in SCFA concentration, which in turn caused intestinal barrier dysfunction [38].

The *Subdoligranulum* taxa, a gram-negative microbe, non-motile and non-spore-forming, belonged to the families of Clostridia. As a short-chain fatty acid-producing taxa, the major products of *Subdoligranulum* were butyric and lactic acids, together with minor amounts of acetic and succinic acids [39]. However, the functions and roles of *Subdoligranulum* remained inconsistency. On one hand, the richness and abundance in *Subdoligranulum* genera was found positively associated with asthma [40] and diabetes mellitus. Most interestingly, a significantly enriched relative abundance of *Subdoligranulum* was revealed among participants receiving long-term meditation training compared to those who never received any meditation training [41]. Meditation training has been widely used for the treatment of a variety of psychological, cardiovascular, and digestive diseases as well as chronic pain. On the contrary, it was reported under-represent among *Clostridium difficile* infection (CDI) [42], high cancer-related fatigue [43] and food allergy [44]. In this study, we found that the abundance in *Subdoligranulum* was positively correlated with the trait-anxiety score in high-responder group. The reason we assumed was the quality of *Subdoligranulum* genera that auxotrophic for most of the vitamins and the amino acid tryptophan [45], which was generally recognized as candidate for protecting against stress disorders.

Tryptophan and tryptophan metabolites had a wide range of physiological functions and were involved in the modulation of mood, anxiety, the stress response and social behaviours [40]. Tryptophan was the precursor of 5-HT and the human brain had limited storage capacity, therefore requiring a continual replenishment from the intestine. Therefore, the major antidepressants were selective serotonin reuptake inhibitors or combined serotonin/noradrenaline reuptake inhibitors, it was undoubtedly to assume that tryptophan metabolism and serotonin pathways were involved in the mechanism of gut microbiota regulating mood and cognition. Aside from its role in protein biosynthesis, tryptophan metabolite was an essential precursor of peripherally and centrally produced serotonin. Both of clinical studies and rodent studies have revealed that acute tryptophan depletion dramatically inhibited serotonin synthesis and reduced tryptophan concentrations in the brain, resulting in a decrease in central serotonin and related metabolite such as 5-hydroxyindoleacetic acid (5-HIAA) and 5-HT_{1A} receptor binding [46]. Furthermore, there was strong evidence implicating that low serotonin synthesis was associated with depressed mood and impaired cognitive functions [47]. Through various preclinical strategies, it has been established that manipulating microbial colonization in GI tract influences tryptophan availability. Serotonin synthesis occurred peripherally within the gut neurons and enterochromaffin cells and centrally within the neurons of the raphe in the brain stem [48]. Serotonin receptors were detected in brain regions including the cortex, amygdala, and hippocampus, which were involved in cognitive process such as learning and memory. Similarly and specially, in this study, we found the enriched expression of tryptophan metabolism pathways among high-response participants compared to those with low response to MBCT interventions through KEGG pathway analysis. This finding suggested that tryptophan metabolism in gut microbiota may potentially be a pathway to promote response strength in brain to psychotherapy such as mindfulness-based intervention. Further animal studies were needed to confirm the signaling factors that played role in tryptophan pathways.

As always, there were limitations to the current study worth noting. Firstly, as this was a pilot study intended to assess feasibility, acceptability and preliminary efficacy of mindfulness through the lens of

gut microbiota, no control group in comparison with the MBCT intervention group was included. As such, the study design precluded our ability to draw conclusions about the specific effects of MBCT. Secondly, this study utilized a very small homogenous sample of young adults, limiting our generalizability to other groups and likely preventing us from obtaining other statistically significant results. However, we were able to find significant results given the very small sample size.

Conclusion

The current study highlighted differences on gut microbiota between high trait-anxiety and healthy controls. This was a valuable attempt to fill the research gap to find out significant intestinal microbiome that may affect trait-anxiety among young adults. These preliminary results also provided information for further intervention studies to reduce mental disorders through the perspective of gut microbiota. Moreover, we further explored the gut microbiota changes on during the effectiveness to MBCT, which added evidence into the current mechanism of gut-brain-axis of how the psychotherapy took effects in improving mental health. Thirdly, we examined the potential bacterial genera and the possible pathways that influence the responsiveness to MBCT interventions among high trait-anxiety participants. Notably, preexisting high abundance in high trait-anxiety populations prior to MBCT was associated with increased bacterial diversity and a better therapy outcome. These findings supported the feasibility of combining gut microbiota therapy with MBCT to strengthen the effectiveness to alleviate trait-anxiety in this population.

Declarations

Ethics approval and consent to participate

Ethical approval was obtained from the medical ethics committee of army medical university (2019-005-02). This trial was registered at Chinese Clinical Trial Registry (Chictr.org.cn, ChiCTR1900028389). The first participant of the intervention study was enrolled in 30th December. Signed informed consent was obtained from each participant after the procedures were clearly explained.

Consent for publication

Not applicable.

Availability of data and material

Data are available upon reasonable request.

Competing interests

None declared.

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Authors' contributions

ZW, XX, BT, CS, and TW conceived and designed this study. ZW, XX, CJ, FH, CS and TW performed the intervention and collected data. ZW, SL, YX, MY and BT collected and analyzed fecal sample. SL and BT carried out statistical analysis. All authors contributed to interpret the data. ZW prepared the manuscript. XZ, SY, CS and TW revised the manuscript.

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Not applicable.

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Figures

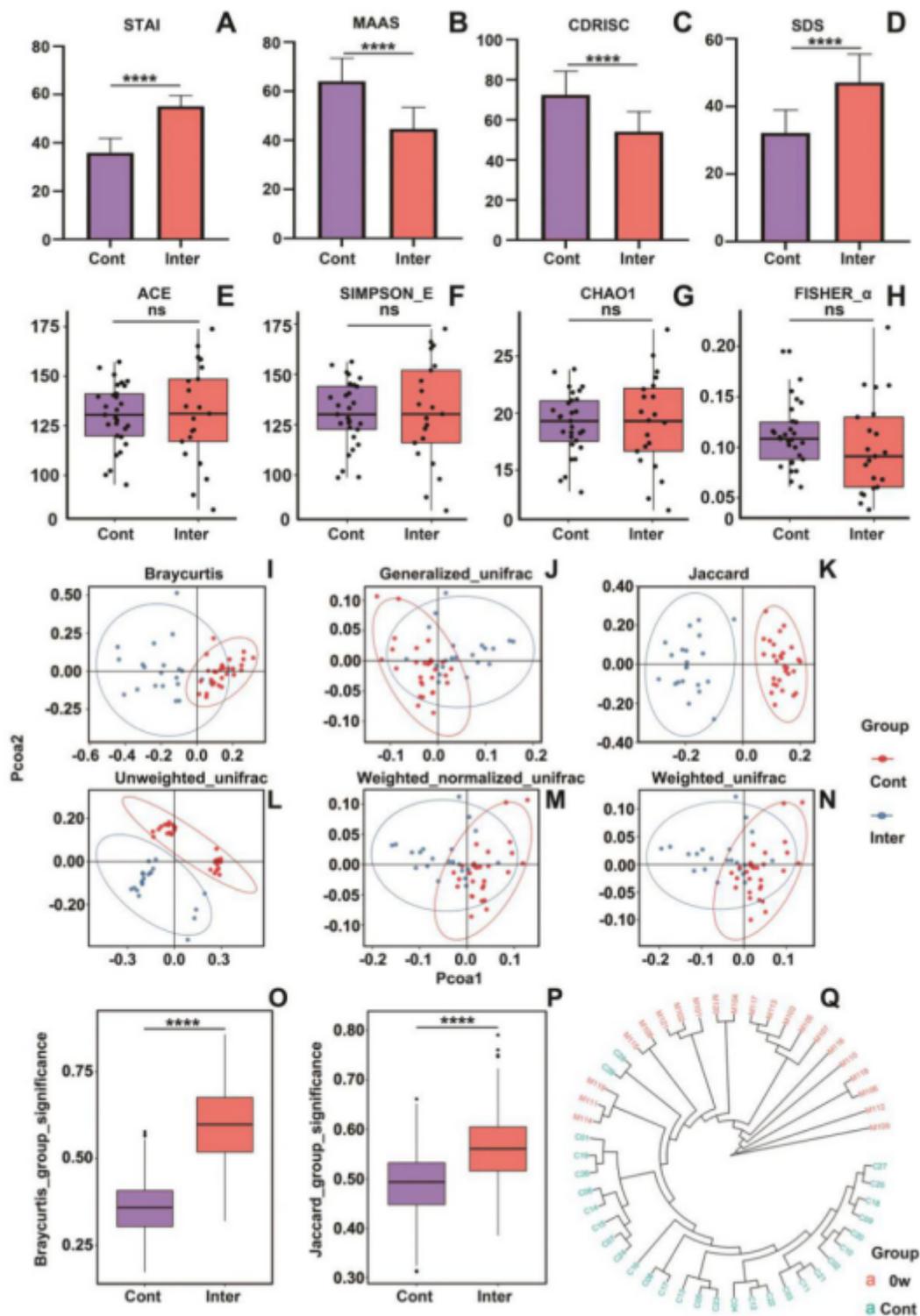


Figure 1

Comparison of psychometric indicators, diversity indices, and PCoA between intervention group (high trait-anxiety) and healthy control group. The mean scores were compared on the scales of STAI (A), MAAS (B), CDRISC (C), and SDS (D). The α -diversity indices included Ace Index (E), Simpson_E Index (F), Chao1 (G) and Fisher_ α (H). The β -diversity indices were illustrated by different algorithm (I-N). Clustering

of fecal bacterial communities according to PCoA analysis by different algorithms: the distance indices (O-P) and cluster tree (Q).

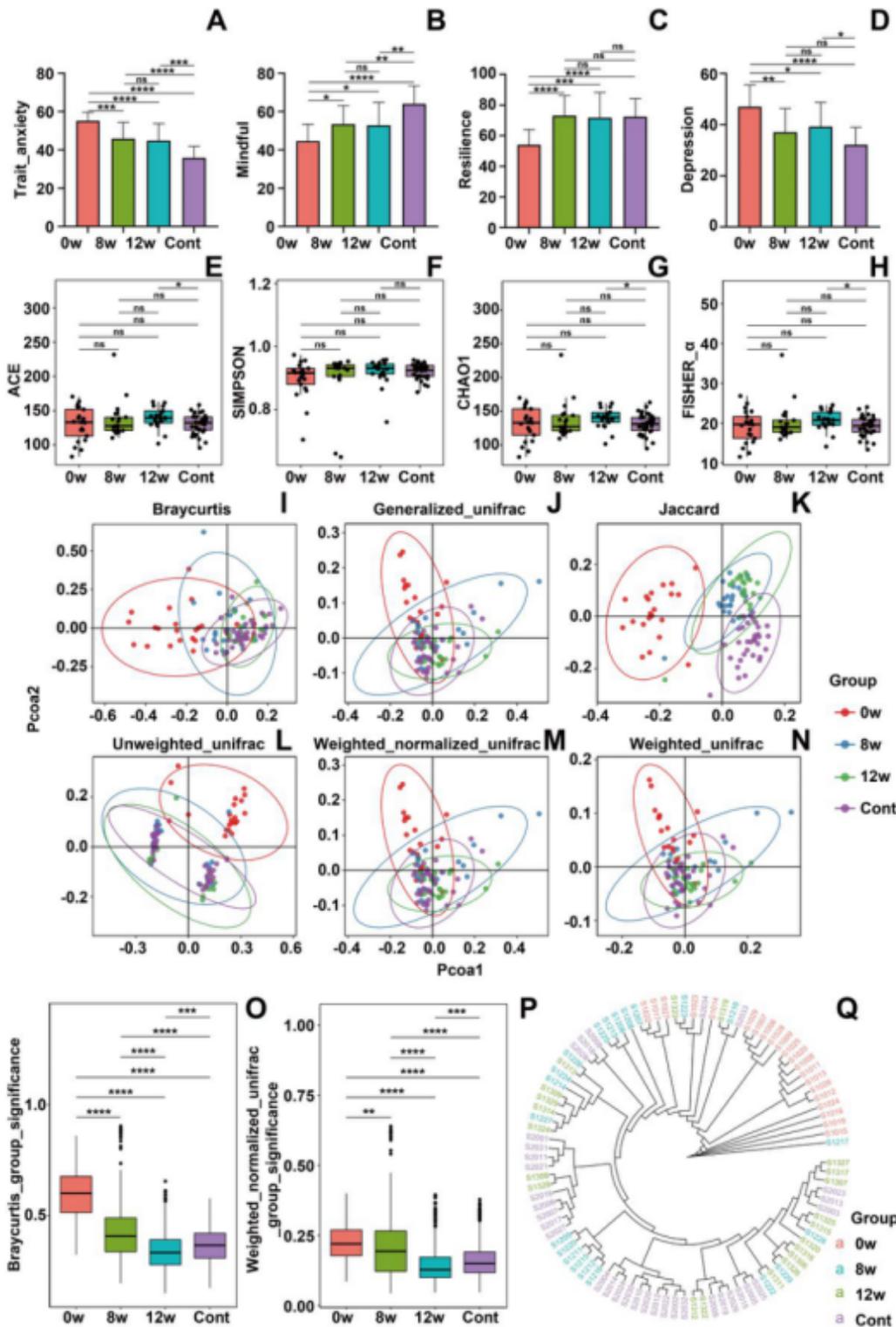


Figure 2

Comparison of psychometric indicators, diversity indices, and PCoA in different time points before and after MBCT intervention (at baseline, at the end of intervention, and at the 4-week following the end of intervention). The scores were compared on scales of STAI (A), MAAS (B), CDRISC (C), and SDS (D). The

α -diversity indices included Ace Index (E), Simpson_E Index (F), Chao1 (G) and Fisher_ α (H). The β -diversity indices were illustrated according to PCoA analysis by different algorithms(I-N). Clustering of fecal bacterial communities: the distance indices (O-P) and cluster tree (L).

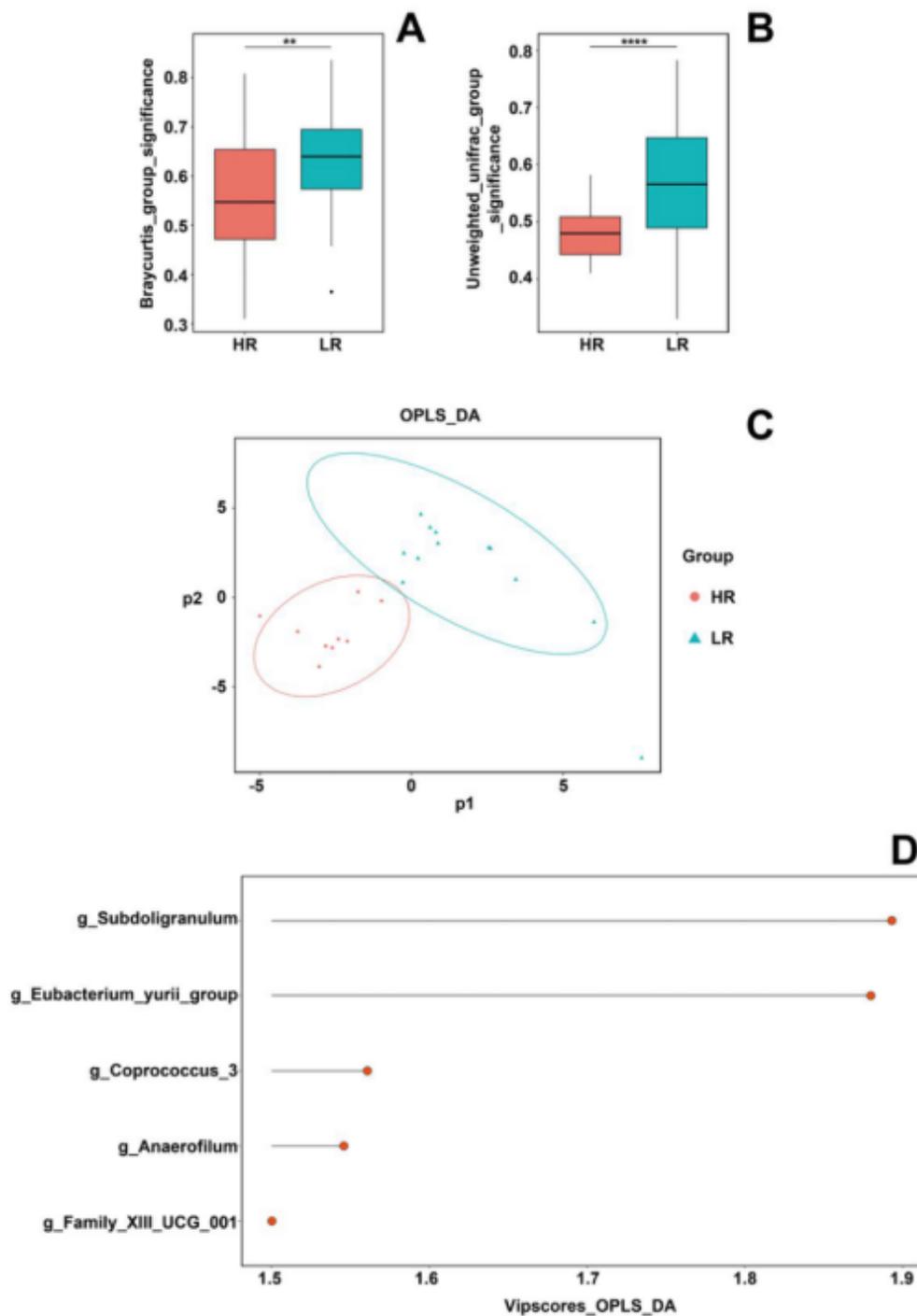


Figure 3

Significant genera that contributed to response strength to mindfulness intervention. (A) Bray-Curtis distance. (B) Unweighted_unifrac distance. (C) PLS-DA analysis. (D) Significant genera with vipscore ≥ 2

in PLS_DA analysis.

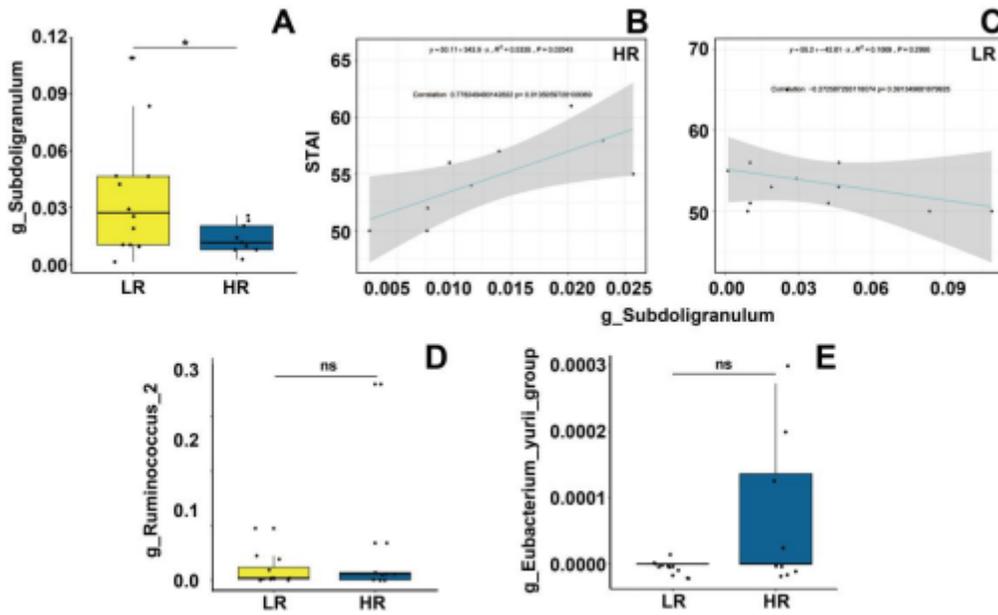


Figure 4

Difference on relative abundance of significant genera between high responders and low responders: Subdoligranulum (A), Ruminococcus (D) and Eubacterium_yurii_group (E). The linear correlation between the relative abundance of the genera Subdoligranulum and STAI scores were shown in (B-C).

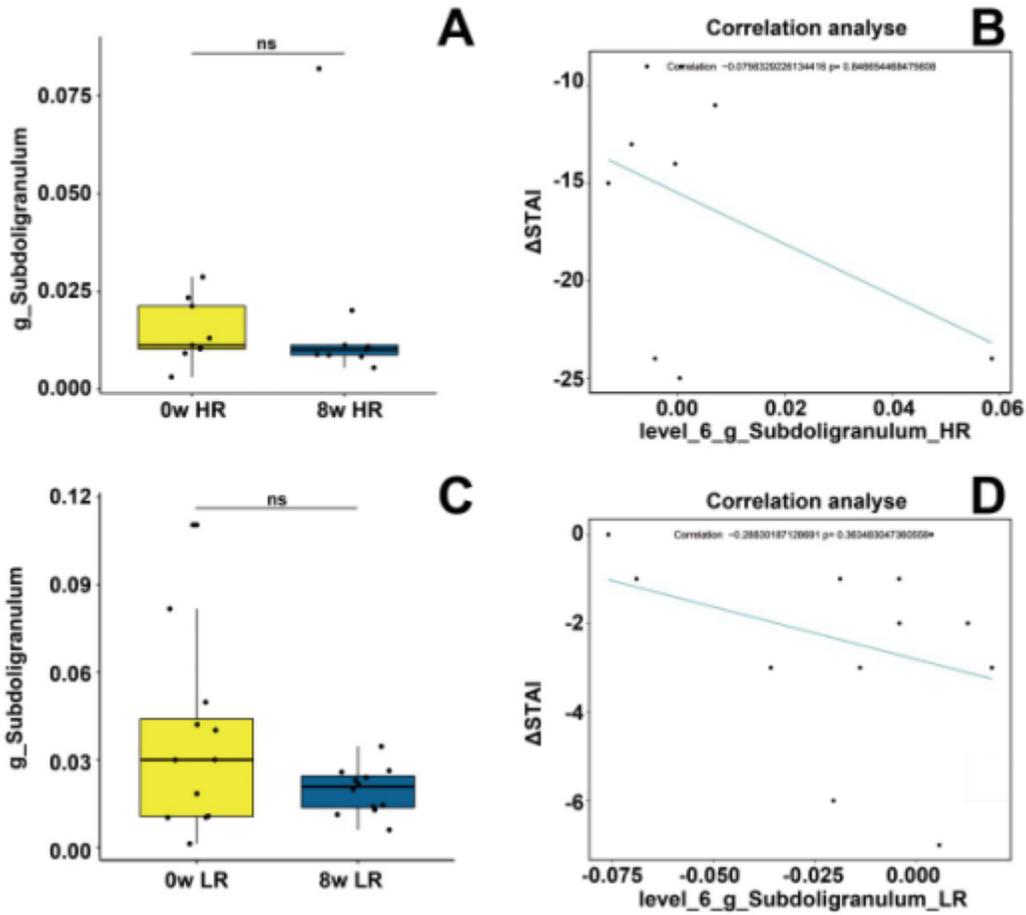


Figure 5

Difference on relative abundance of Subdoligranulum genera before and after the intervention among high responders (A) and low responders (C), respectively. The linear correlation between δ Subdoligranulum and δ STAI score among high responders (B) and low responders (D), respectively.

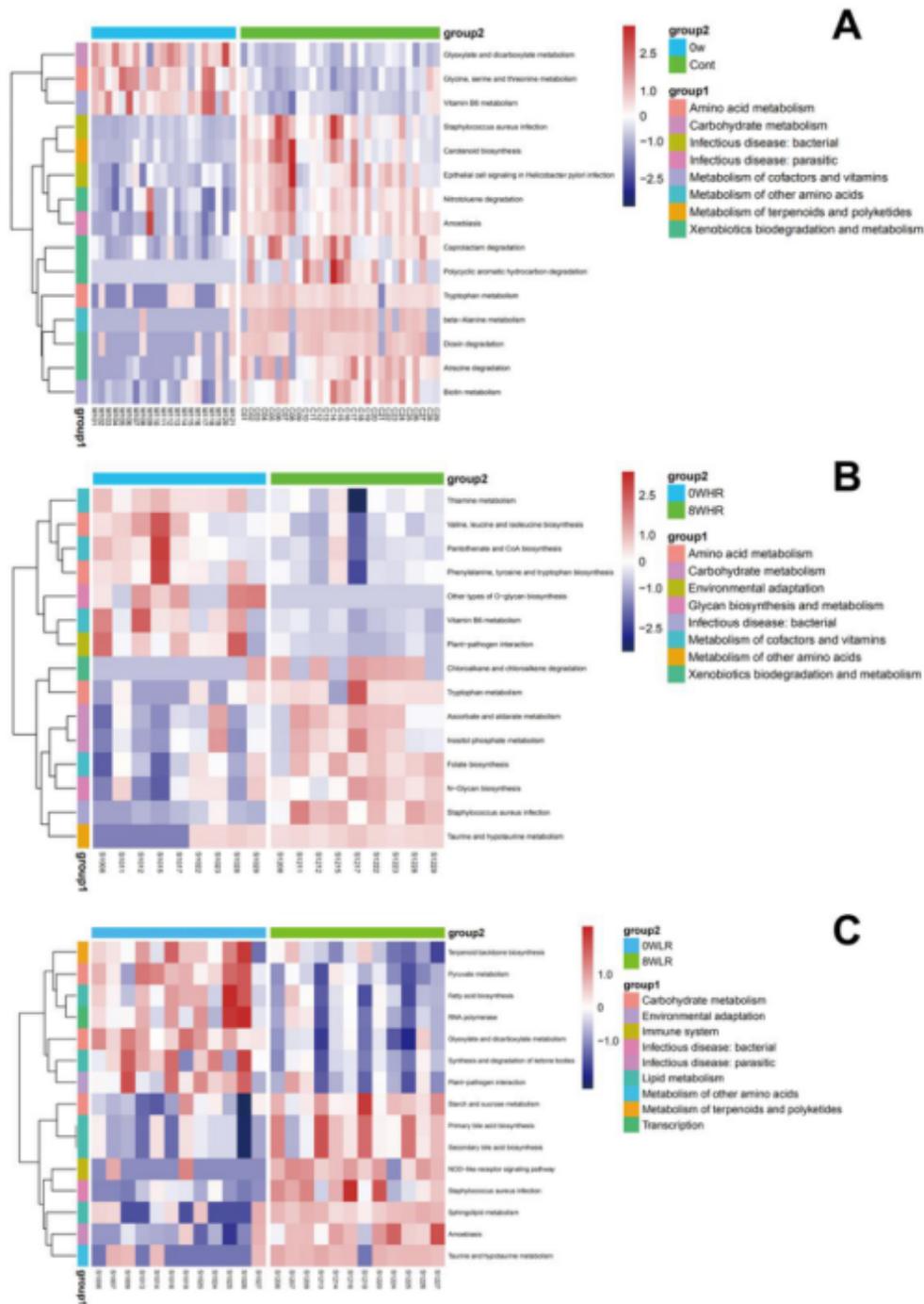


Figure 6

Heat maps of selected significant metabolites between the high trait-anxiety group and healthy control group (A) , before and after intervention among high responders (B) and among low responders (C).

Supplementary Files

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