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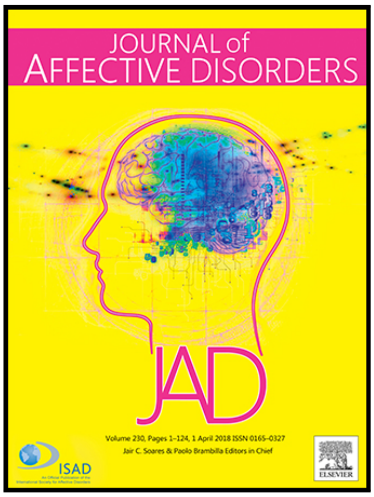


# Amplitude of low-frequency fluctuation (ALFF) alterations in adults with subthreshold depression after physical exercise: A resting-state fMRI study

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**Highlights**

- Subthreshold depression (StD) is found to be a threatening precursor to major depressive disorder, presenting two to four criterion depressive symptoms for 2 weeks or longer with at least one of the core symptoms (depressed mood or anhedonia) but does not meet diagnostic criteria of a full-blown MDD;
- physical exercise is a promising self-supportive adjunctive intervention and an effective measure which can reduce depressive symptoms;
- Despite initial evidence that physical exercise may be useful in reducing the risk of major depression in StD individuals, a prominent question regarding the effects of physical exercise is how it affects neural changes.
- To our knowledge, amplitude of low-frequency fluctuations(ALFF) analysis of exercise intervention in StD population has not been reported yet.
- We observed that physical exercise can not only change the patterns of spontaneous brain activity in both StD and HC groups, but also significantly reduce the depressive symptoms of the StD group.

**Amplitude of low-frequency fluctuation (ALFF) alterations in adults with subthreshold depression after physical exercise: A resting-state fMRI study**

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ABSTRACT

Background: Physical exercise has been proved to reduce the risk of major depression in Subthreshold depression (StD) individuals effectively, yet little is known about the spontaneous brain activity changes associated with physical exercise.

Methods: A total of 70 adult subjects, including 38 StD and 32 healthy control (HC) subjects, underwent a resting-state functional magnetic resonance imaging (rs-fMRI) before and after eight-week aerobic exercise respectively. Then, the amplitude of low-frequency fluctuation (ALFF) alterations between the two groups were quantitatively analyzed.

Results: Before exercise intervention, the rs-fMRI data showed increased ALFF of the right putamen in the StD group compared with HC group. After exercise intervention, there was no significant ALFF change observed between the StD and HC groups. The longitudinal ALFF differences from pre- to post- exercise intervention showed significantly decreased ALFF in the right middle and inferior occipital gyrus, right middle and inferior temporal gyrus, right fusiform gyrus (FG), while increased ALFF in the right middle cingulate, right superior parietal louble, right inferior parietal lobule (IPL)（inferior parietal gyrus and supramarginal gyrus), and bilateral precuneus in the StD group. As for HC group, the results showed that decreased ALFF in the right FG and right parahippocampus, while increased ALFF in the right precuneus, right middle cingulate, right supplementary motor area, right superior parietal lobule and right paracentral lobule in the HC group. No significant correlation between changes of ALFF and clinical scale scores in the StD group.

Limitations: The definitions of StD are varied in terms of different studies, the final sample size was relatively small, and the age range of the subjects in this study was narrow. Meanwhile, the exercise intervention trial was short-term.

Conclusions: These results further support the standpoint that physical exercise has the potential to reshape the abnormal patterns of spontaneous brain activity in adults with StD.

Keywords: Subthreshold Depression; Prevention; Exercise; Neuroimaging; Resting-state.

[1] Abbreviations: StD, Subthreshold depression; HC, healthy control; rs-fMRI, resting-state functional magnetic resonance imaging; ALFF, amplitude of low-frequency fluctuation; FG, fusiform gyrus; IPL, inferior parietal lobule

## 1. Introduction

Major depressive disorder (MDD) is a highly prevalent disorder that severely limits psychosocial functioning with substantial economic costs (GBD 2017 Disease and Injury Incidence and Prevalence Collaborators, 2018), and has been projected as the most debilitating disorder worldwide by 2030 (WHO, 2017). However, only about one-third of the disease burden attributable to MDD could be alleviated, even under the most optimistic assumption that adequate resources for evidence-based treatments were provided for all patients with MDD(Andrews et al., 2004). Therefore, attention has increasingly been focused on the early identification of high-risk groups and timely preventive measures (Rotenstein et al., 2016).

Subthreshold depression (StD)/subsyndromal depression is found to be a threatening precursor to MDD (Tuithof et al., 2018), presenting two to four criterion depressive symptoms for 2 weeks or longer with at least one of the core symptoms (depressed mood or anhedonia) (Rodríguez et al., 2012), but does not meet diagnostic criteria of a full-blown MDD (Pincus et al., 1999). In addition, previous studies have shown that many StD individuals have persistent symptoms of depression at 12-month follow-up. Nearly fifty percent of those turn into moderate functional impairment, and at least ten percent to twenty percent develop into major depression (Cuijpers and Smit, 2004; Ludvigsson et al., 2019). Accordingly, confirmation of effective interventions for people with StD has significant meaning in preventing the onset of major depression.



Considerable empirical researches have demonstrated that physical exercise is a promising self-supportive adjunctive intervention and an effective measure which can reduce depressive symptoms. Therefore, physical exercise is highly recommended in a variety of national clinical guidelines (Choi et al., 2019; Gartlehner et al., 2016;

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Ravindran et al., 2016). Besides, recent studies suggest that it is possible to prevent the onset of MDD by targeting StD individuals with physical exercise (Kok and Reynolds, 2017; Rebar et al., 2015). Such as, a Meta-analytic data from randomized clinical trials has reported that physical exercise can evidently reduce depressive symptoms in StD populations (Gordon et al., 2018), and Yen KT et al. (Yen et al., 2020) has demonstrated that jogging has a significant contribution to nonclinical depressive prodrome among adolescents.

Despite initial evidence indicates that physical exercise may be useful in reducing the risk of major depression in StD individuals, a prominent question regarding the effects of physical exercise is how it affects neural changes. Resting state functional magnetic resonance imaging (rs-fMRI) is a simple but promising imaging sequence to investigate the brain function and neurobiological mechanisms and has been widely used to clarify the essential changes of brain function in clinical trials (Biswal et al., 1995; Lang et al., 2014). Amplitude of low-frequency fluctuations (ALFF) is a stable feature proposed to characterize the local properties of the rs-fMRI signal (Zang et al., 2007; Zuo et al., 2010), which has been conducted on participants with StD in previous exploratory trials (Li et al., 2016; Li et al., 2014). Some studies on ALFF analysis suggested that the StD group demonstrated a normalization of abnormal brain activity in the striatum, compared to healthy controls (Qiu et al., 2020; Stringaris et al., 2015). To our knowledge, however, ALFF analysis of exercise intervention in StD population has not been reported yet.

Given the above considerations, the purpose of this study was to conduct a rs-fMRI ALFF analysis to explore alterations in brain spontaneous activity in adults with StD before and after eight weeks of moderate-intensity aerobic exercise. We hypothesized that there were differences in spontaneous neuronal activity between individuals with StD and healthy controls before exercise intervention, and a further hypothesis is that physical exercise training would reduce individuals' depressive symptoms to a great extent and neural changes would be observed in corresponding brain regions over time.

## 2. Methods

### 2.1 Trial design

The experiment is a prospective, two-arm, parallel-group, controlled 2×2 experimental design. Specifically, all participants were informed about the longitudinal design of the study at the beginning, meanwhile, they all received two clinical scale scores and scanned by rs-fMRI at baseline and within one week after completing an eight-week of exercise follow-up. Anxiety often coexists with depression (Tiller, 2013), therefore, the Patient Health Questionnaire-9 (PHQ-9) and Zung Self-Rating Anxiety Scale (SAS) were used as clinical scales to assess depressive and anxiety symptoms respectively (Spitzer et al., 1999; Zung, 1971). All participants were instructed to avoid physical exercise for 24 hours prior to each scanning so as to minimize the acute disturbance of exercise. Return for follow-up was encouraged through appropriate remuneration.

The study protocol was approved by the Ethics Committee of Xuzhou Medical University (2020-KY-006). Volunteers were mainly recruited through advertisements in this area. Verbal and written consent were obtained from all volunteers before an extensive screening procedure which includes touchscreen questionnaires, in-person interviews, and physical exercise training.

### 2.2 Recruitment and eligibility criteria

A total of 86 adult volunteers were invited to participate in the trial. The primary inclusion criteria were as follows: (1) Han nationality, ages 18–50 years, and right-handed; (2) senior high school level of education or above; (3) had not received any psychotherapy within 6 months; (4) no organic disease or other serious substance addiction (such as tobacco, alcohol, or drugs); (5) did not endorse a noticeable suicidal ideation ( the Sub-item 9 score of PHQ-9 > 1); (6) no MRI scanning contraindication (such as claustrophobia or metal implants); (7) nonpregnancy or lactation, or women planning to be pregnant；(8) had no contraindications for medical maximal exercise (e.g. hypertension, cardiovascular disease, recent injury) screened with the Physical Activity Readiness Questionnaire (PARQ) (Thomas et al., 1992); (9) had engaged only in irregular exercise or low-moderate level habitual physical activity based on the Chinese version of the International Physical Activity Questionnaire-Short Form (IPAQ-SF) (Craig et al., 2003; Lee et al., 2011).

The volunteers were initially divided into two groups: the StD group (PHQ-9 score ≥ 5 with at least 1 of the core symptoms ( Kroenke et al., 2017; Gilbody et al., 2017; Morgan et al., 2012), n = 50) and the health control (HC) group (PHQ-9 score < 5, n = 36). Then, within 1-2 weeks, all potential participants were scheduled for a structured clinical interview based on the Diagnostic Statistical Manual of Mental Disorders Axis Disorders (DSM-5) conducted by an experienced psychiatrist so as to exclude MDD and other psychiatric disorders, such as bipolar disorder, schizophrenia, personality disorder and mental retardation, within the past one year. In addition, the symptoms resulted from recent adjustment reactions of adverse life events (such as the death of relatives, or changes in the work environment) would be excluded.

Study endpoints criteria of the exercise training: (1) absence or not reaching the exercise prescription for more than two weeks; (2) any new psychological or physical symptoms during the intervention; (3) any major life events that may affect subject's health and wellbeing.



## 2.3 Exercise paradigm

Participants engaged in exercise sessions three to four times a week for 8 weeks. Each session proceeds moderate-intensity aerobic exercise for approximately 45 minutes, which contains a gradual ramp-up period, as well as an adaptive 5-10 min warm-up (light aerobic exercise at 30% of heart rate reserve) and ended with 5 min of cool-down period consisting mainly of relaxation exercise. Moderate-intensity was equivalent to continuous heart rate monitoring and maintaining 60%-75% of age-predicted maximal heart rate (Karvonen et al., 1957) (i.e., 220-individual's age (in years)). The exercise paradigm was based on the recommendations of the public health recommendations by American College of Sports Medicine (Department of Health and Human Services Website, 2008) and American College of Sports Medicine position stand (Garber et al., 2011), and it has been confirmed to be the effective mode in decreasing depressive symptoms (Brush et al., 2020).

Individualized, supervised aerobic exercise sessions were practiced on nonconsecutive day by closed-skill exercise (Voss et al., 2010), such as outdoor fast-walking, slow-running, or on either the treadmill or stationary bike, which were the same as those used in Toups's research (Toups et al., 2016). During the study, each participant wore a bracelet device around the wrist that is used to measure heart rate, exercise time, frequency and intensity. Data of the exercise paradigm was uploaded to the online diary applet after each session. Considering the effectiveness of intervention and the higher degree of compliance for initiating and maintaining exercise (Firth et al., 2019), the subjects were monitored in small training groups by a regular staff on a weekly basis in the duration of the study. Exercise sessions were practiced alone on nonconsecutive day. If the subjects who failed to attend this program or achieved the targeted rang, they were instructed to encouraged to continue the exercise intervention or to increase the speed.

## 2.4 MRI data acquisition

Eligible participants completed MRI scanning on a 3.0T GE Discovery750W MRI scanner (General Electric Medical Systems, Waukesha, WI, USA) equipped with a 16-channel head-neck united array coil (GE Healthcare), using the same acquisition parameters. The resting state refers to the supine position during the scan, in which the participants were instructed to stay awake, breath peacefully and evenly, without thinking about anything in particular. Foam cushions were used to limit head motion, and malleable ear plugs were used to reduce noise from the scanner. The resting-state blood oxygen level-dependent (BOLD) imaging was performed by using T2-weighted single shot gradient- echo-planar imaging (EPI), with repetition time = 2000 ms, echo time = 30 ms, flip angle = 90 °, field of view = 240 mm × 240 mm, matrix = 64 × 64, slice thickness / spacing = 3.6 mm / 0.2 mm, 33 slices in total; (2) The sagittal T1WI structure image was acquired on the Bravo sequence with repetition time = 8.464 ms, echo time = 3.248 ms, flip angle=12°, field of view = 256 mm × 256 mm, matrix = 256 × 256, slice thickness / spacing = 1 mm / 0 mm. All images were quality controlled by a board-certified radiologist and subsequently anonymized the participants' identities.

2.5 Processing of rs-fMRI data and ALFF analysis

Data preprocessing was manipulated with SPM12 (Statistical Parametric Mapping, UK) and RESTplus v1.24 (Jia et al., 2019) (http://www.restfmri.net/forum/REST) toolkits within Matlab 2014a (The MathWorks Inc., MA). The procedure was processed as follows: 1) The first 10 time points were removed to avoid non-equilibrium effects of magnetization and to allow subjects to adjust to the noise of the scanner. 2) Slice timing and correction of head-motion were then performed to exclude the subjects whose head movements exceeded 3° rotation or shifted 3 mm in any direction (Friston et al., 1996). 3) The structural images were co-registered with the average function image after motion correction by linear transformation. 4) New segmentation algorithm was carried out to segment the transformed structural image into gray matter, white matter, cerebrospinal fluid, skull, extra brain and soft tissue. 5) The motion-corrected functional imaged were spatially normalized to Montreal Neurologic Institute (MNI) space and resampled to 3mm×3mm×3mm voxels. 6) A standardization smoothing (Gaussian kernel: full width at half maximum FWHM = 4 mm) was performed to reduce the influence of spatial noise and the differences in brain structure between subjects. 7) Linear detrending processing and individual-level regression analysis were performed respectively to remove the linear signal drift and minimize the influence of head motion (Friston et al., 1996) (Friston-24 model), white matter signal noise, and cerebrospinal fluid signal noise.

ALFF was computed based on Fast Fourier transform (FFT) and the time series of each voxel was transformed to frequency domain without band-pass filtering. The square root was firstly calculated at each frequency of the power spectrum, and then the mean square root was obtained across 0.01–0.08 Hz band for each voxel. The final step was to divide the ALFF of each voxel by the global mean of ALFF values (mALFF) (Zang et al., 2007) .

2.6 Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences SPSS23 (SPSS Inc., Chicago, IL, United States). Differences of demographic variables were calculated using Student's t-test. or ChiSquare-tests appropriate at baseline. Paired sample t-tests were performed to investigate changes of clinical states such as depressive and anxious symptoms between baseline and follow-up in each group respectively. Data of demographic variables and clinical states were presented as the range of minimum-maximum (mean ± SD). DPABI software V4.0 (Yan et al., 2016) (http://restfmri.net/forum/DPABI) was used to conduct two sample t-test for horizontal ALFF differences between the StD and HC groups at baseline and follow-up, and paired t-test for longitudinal ALFF differences in the StD and HC groups respectively. In addition to the multiple comparison correction (GRF, voxel level $P < 0.01$, clustering level $P < 0.05$, two tailed) based on Gaussian random field theory, the mean frame displacement was taken as the covariate of all t-tests to avoid the influence of confounding variables (Jenkinson et al., 2002). In order to confirm the influence of the threshold on the results, we have additionally provided the results with other thresholds in supplementary materials (individual voxel $P < 0.005$ and 0.001, cluster size > 10 voxels). To be specific, based on each metric map, we extracted metrics values of each spherical ROI with the center at the peak point of each cluster of corrected T map and a radius of 6 mm respectively. A Spearman rank correlation analysis was examined between the signals of StD group and depression and anxiety scale scores before and after intervention. The statistical threshold for these correlation analyses were set at $P < 0.05$.

## 3. Result

### 3.1 Baseline Characteristics

The original sample size was 86, of which 6 subjects were excluded from the clinical interviews (5 persons with MDD, 1 person with mania); 6 subjects withdrew from the project before completing the program because of personal scheduling conflicts; 2 subjects were excluded because of suprathreshold movement over the course of rs-fMRI scanning at either pre- or post- intervention and 2 subjects were excluded because of image conversion failure or different slices, resulting in a subsample of 70 participants (StD group, n = 38; HC group, n = 32)，ranged from 18 to 48 (mean age 29.06 ± 8.18, 43 female). No negative effects of the interventions caused during the trial.

At baseline, there were no significant differences between the two groups with respect to age, gender, educational attainment or Body Mass Index (BMI). Participants were also balanced at screening for active versus nonactive physical activity using the IPAQ-SF at baseline (all $P > 0.05$). See Table 1. Attendance was recorded and both groups completed more than 80% (StD group: 91%, HC group: 93%) of the effective exercise adherence.

**Table1.** Baseline demographics of all subjects.

| Variables | StD group (n =38) | HC group (n = 32) | $t/x^2$ value | *P* value |
|---|---|---|---|---|
| Age (years) | 29.84±6.83 | 28.13±9.68 | -0.39 | 0.167[a] |
| Gender (f/m) | 21/17 | 21/11 | 1.33 | 0.248[b] |
| Education years | 16.13±2.04 | 15.09±2.36 | -1.97 | 0.068[a] |
| BMI ($kg/m^2$) | 22.06±3.06 | 22.04±3.08 | -0.02 | 0.974[a] |
| IPAQ-SF, n (%) | | | | |

| | | | | |
|---|---|---|---|---|
| Low-level | 34(89.47%) | 26(81.25%) | 0.96 | 0.327[b] |
| Moderate-lever | 4(10.53%) | 6(18.75%) | | |

BMI = Body Mass Index; IPAQ-SF = International Physical Activity Questionnaire-Short Form

[a] The *P* value was obtained by Student's t-test; [b] The *P* value was obtained by two-tailed Pearson chi-square t test; Low-level, no physical activity reported or some activity reported but not meet the Moderate-lever criteria; Moderate-lever, 3 or more days of vigorous activity lasts at least 20 minutes/day, 5 or more days of moderate- or low-intensity activity lasts at least 30 minutes/day, or 5 or more days of any combination of low-, moderate-intensity or vigorous activity of at least energy expenditure 600 MET· min/week.



### 3.2 The effect of physical exercise on symptom changes

After aerobic exercise intervention, the PHQ-9 and SAS scale scores were significantly improved in the StD group; the SAS scale scores were significantly improved but there was no obvious change of the PHQ-9 scale scores in the HC group (Table 2).

**Table 2.** The results of clinical scores at baseline and post-intervention.

| Groups | PHQ-9 | | *P* value | SAS | | *P* value |
|---|---|---|---|---|---|---|
| | At baseline | Post-intervention | | At baseline | Post-intervention | |
| StD | 9.37 (2.75) | 4.92 (3.40) | 0.000 | 46.13 (7.45) | 37.13 (6.29) | 0.001 |
| HC | 2.97 (2.35) | 2.31 (2.04) | 0.237 | 37.13(5.89) | 33.16 (5.39) | 0.007 |

### 3.3 Resting-state fMRI data

#### 3.3.1 The effect of StD on ALFF

At baseline, an initial group analysis was conducted on ALFF maps to explore the differences in regional activity between the StD and HC groups. The results indicated that StD subjects showed increased ALFF in the right putamen compared with HCs (Table3, Fig.1).

**Table 3.** Regions showing ALFF differences between the StD and HC groups at baseline

| Location<br>Cluster（AAL） | BA | Number of voxels | Peak Location | Peak *t* value | MNI coordinates | | |
|---|---|---|---|---|---|---|---|
| | | | | | X | Y | Z |
| Cluster 1 | N/A | 71 | Putamen_R | 4.08 | 27 | 6 | -3 |
| Putamen_R | | 49 | | | | | |

AAL = Anatomical Automatic Labeling; BA = Brodmann Aarea. MNI = Montreal Neurological Institute.

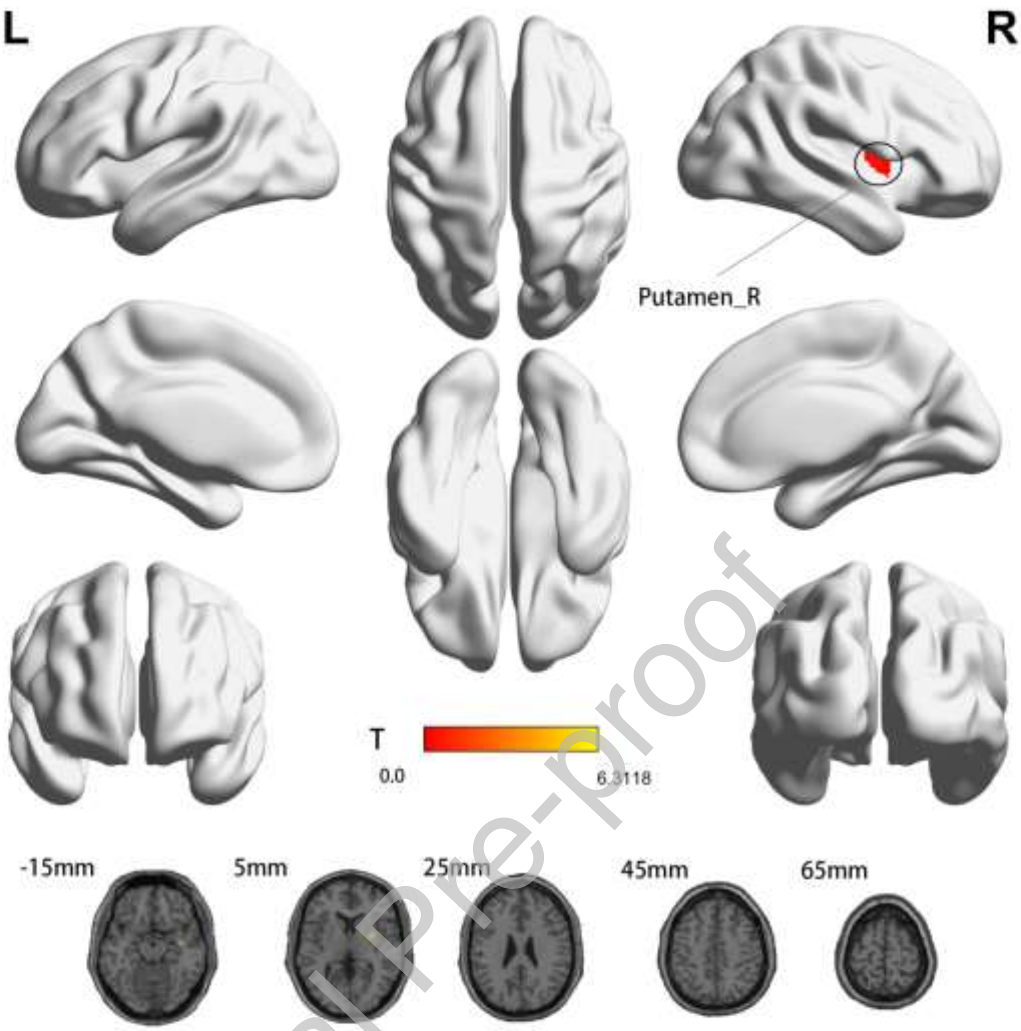


**Fig. 1.** The horizontal ALFF differences in the right putamen between the StD and HC groups at baseline. Warn (red-yellow) colors represent increased ALFF between-group.



3.3.2 The effect of physical exercise on regional ALFF

After aerobic exercise intervention, there was no significant ALFF change observed between the StD and HC groups. The longitudinal ALFF differences from pre- to post- exercise intervention showed significantly decreased ALFF in the right middle and inferior occipital gyrus, right middle and inferior temporal gyrus, right fusiform gyrus (FG), while increased ALFF in the right middle cingulate, right superior parietal louble, right inferior parietal lobule (IPL)（inferior parietal gyrus and supramarginal gyrus), and bilateral precuneus in the StD group (Table 4, Fig.2). As for

HC group, the results showed that decreased ALFF in the right FG and right parahippocampus, while increased ALFF in the right precuneus, right middle cingulate, right supplementary motor area, right superior parietal lobule and right paracentral lobule in the HC group (Table 5, Fig.3).

**Table 4.** Regions showing ALFF differences in the StD group from pre- to post- exercise intervention

| Location<br>Cluster（AAL） | BA | Number of voxels | Peak Location | Peak *t* value | MNI coordinates | | |
|---|---|---|---|---|---|---|---|
| | | | | | X | Y | Z |
| Cluster 1 | 20 | 52 | Temporal_Inf_R | -4.33 | 48 | -9 | -33 |
| Temporal_Inf_R | | 40 | | | | | |
| Cluster 2 | 39 | 331 | Occipital_Mid_R | -5.29 | 54 | -72 | 30 |
| Occipital_Inf_R | | 80 | | | | | |
| Occipital_Mid_R | | 67 | | | | | |
| Temporal_Mid_R | | 37 | | | | | |
| Fusiform_R | | 30 | | | | | |
| Cluster 3 | 31 | 62 | Cingulum_Mid_R | 4.00 | 6 | -33 | 45 |
| Cingulum_Mid_R | | 44 | | | | | |
| Cluster 4 | 7 | | Precuneus_R | 4.67 | 6 | -72 | 42 |
| Precuneus_R | | 59 | | | | | |
| Parietal_Sup_R | | 12 | | | | | |
| Cluster 5 | N/A | 131 | Parietal_Inf_R | 4.98 | 51 | -36 | 45 |
| Parietal_Inf_R | | 59 | | | | | |
| SupraMarginal_R | | 41 | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Cluster 6 | N/A | 189 | Precuneus_R | 5.10 | 12 | -54 | 60 |
| Precuneus_R | | 89 | | | | | |
| Precuneus_L | | 46 | | | | | |
| Parietal_Sup_R | | 28 | | | | | |

**Table 5.** Regions showing ALFF differences in the HC group from pre- to post- exercise intervention

| Location<br>Cluster（AAL） | BA | Number of voxels | Peak Location | Peak *t* value | MNI coordinates | | |
|---|---|---|---|---|---|---|---|
| | | | | | X | Y | Z |
| Cluster1 | 37 | 87 | Fusiform_R | -5.80 | 36 | -42 | -12 |
| Fusiform_R | | 48 | | | | | |
| ParaHippocampal_R | | 18 | | | | | |
| Cluster2 | 32 | 62 | Supp_Motor_Area_R | 6.31 | 3 | 6 | 45 |
| Cingulum_Mid_R | | 44 | | | | | |
| Supp_Motor_Area_R | | 7 | | | | | |
| Cluster3 | 7 | 173 | Precuneus_R | 5.66 | 6 | -51 | 63 |
| Precuneus_R | | 90 | | | | | |
| Parietal_Sup_R | | 27 | | | | | |
| Paracentral_Lobule_R | | 24 | | | | | |

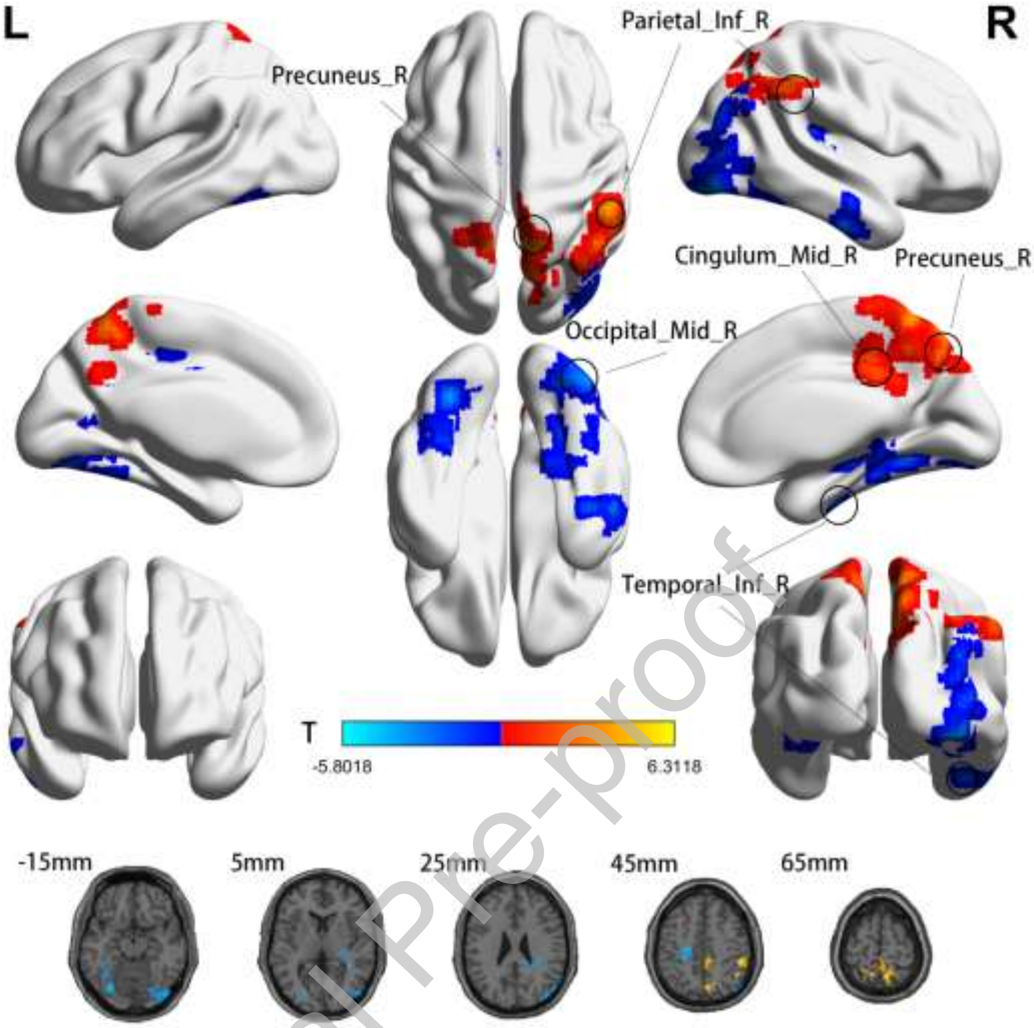


**Fig. 2.** The longitudinal ALFF differences in the StD group from pre- to post- exercise intervention. Cool (teal-blue) colors represent decreased ALFF, whereas warn (red-yellow) colors represent increased ALFF.

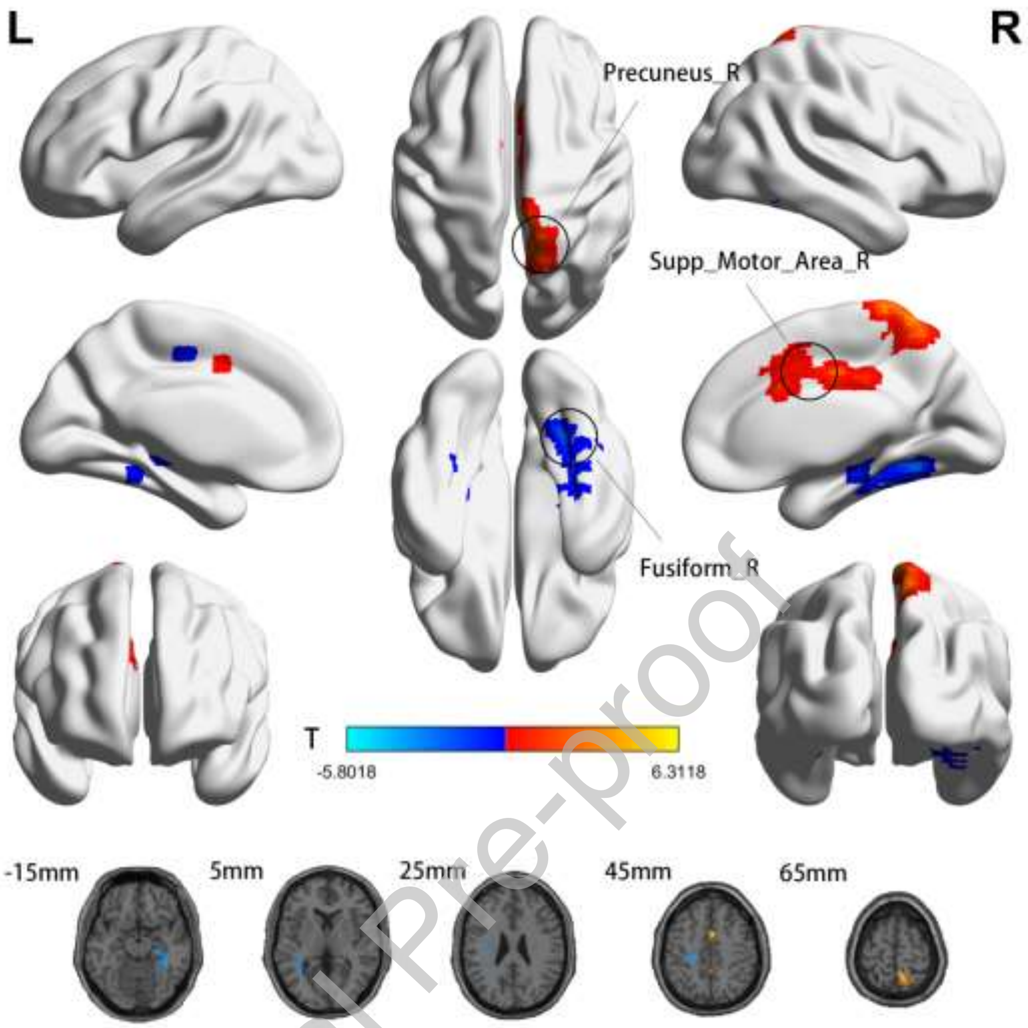


**Fig. 3.** The longitudinal ALFF differences within HC group from pre- to post- exercise intervention. The pseudocolor scheme is similar to that in Fig. 2.

3.4 Brain-behavior correlation analyses

Despite significant changes in ALFF have been found in several regions in the StD group from pre- to post- exercise, ALFF were not associated with depression or anxiety scale scores ($P > 0.05$). The exploratory analysis of the subitems of PHQ-9 in the StD group showed that the right inferior temporal gyrus is positively correlated with the item6 (guilt, self-blame, or worthlessness) score before intervention ($r = 0.433$, $P = 0.007$), and the right precuneus is negatively correlated with the item1 (anhedonia) score after intervention ($r = -0.472$, $P = 0.003$). No significant correlation between abnormal brain regions and other subitems score observed in StD group before and after intervention.

**4. Discussion**

To the best of our knowledge, this is the first study that discovers a significant spontaneous brain regional activity changes by using rs-fMRI ALFF analysis focused on individuals with StD corresponding to reduced depressive symptoms and anxiety symptoms after an 8-week aerobic exercise. More importantly, it highlights that physical exercise may be an implementable efficacious method for protecting against the development of subsequent depression in individuals. We also found that the depression scale score of the HC group also decreased after physical exercise. Notably, the StD and HC groups both showed an excellent attendance rate of exercise training (91% versus 93%). This result may indicate that the somatic symptoms of StD individuals were not significantly impaired, while people with MDD tend to lack motivation to initiate and maintain an exercise routine (Stanton and Reaburn, 2014).

At baseline, compared with HCs, analysis of the rs-fMRI data revealed increased ALFF in the individuals with StD in the right putamen, which manifested increased spontaneous neuronal activity within the local brain region. As a part of the striatum of the basal ganglia, the putamen has physiological functions which may not be only related to the control of autonomous movement, the regulation of detailed conscious activities and motor responses (Jackson et al., 2019), but also participates in high-level cognitive functions such as memory, emotion and reward learning (Kreitzer and Malenka, 2008; Tao et al., 2013). Subdued reward processes may be biological markers for the increased risk of depression over time (Sachs-Ericsson et al., 2018). He et al., (2019) found that, compared to HCs, individuals with SD showed the less response in the putamen during consumption of social gain. Our preliminary findings indicated that abnormal ALFF in the right putamen may be a trait-like variant in people with StD and as the target for neurofeedback.

After eight weeks of aerobic exercise proceeded in the StD and HC groups respectively, we did not find any difference in brain regions between the StD and HC groups. The results showed that regularity of exercise intervention was helpful to the convergence of the differences between the two groups. Further investigation in our study demonstrated the changes of brain activity in both groups from pre- to post-exercise intervention. We found that the changes of spontaneous brain regional activity were all involved in the right precuneus, right FG, right middle cingulate, right SPL in both StD and HC groups, which are involved in a broad variety of high-level cognitive functions. The activity of the precuneus increased in both StD and HC groups, and the activity of the IPL (inferior parietal gyrus and supramarginal gyrus) increased in the StD group, both of which fall within default mode network that believed to be involved in self-referential processing, affective cognition, and emotion regulation (Wong et al., 2012; Davey et al., 2016). Additionally, the precuneus may precede the occurrence of depressive episodes and influence the development of MDD (Frey and McCabe, 2020). The FG, a crucial structure of the visual recognition circuit (e.g., middle/inferior temporal gyrus, middle/inferior occipital gyrus and FG), is considered as a participating medium in functionally-specialized computations of high-level vision, such as face perception, object recognition, and reading (Guo et al., 2012; McDermott et al., 2020). Besides, our study showed decreased ALLF in the right middle and inferior occipital gyrus, and right middle and inferior temporal gyrus in the StD group, which might suggested residual somatosensory alterations in the post-exercise individuals with StD. The cingulate cortex serves the functions of inhibitory controls, behavioral corrections, as well as involved in the regulation of emotional processing, the middle of which mainly for responsing selection and feedback-guided decision making (Etkin and Schatzberg 2011; O'Neill et al., 2012). A recent fMRI study found that the occipito-parieto-cingulate circuit was involved in emotional regulation, emotional processing, somatosensory processing and self-referential processing (Pannekoek et al. 2013). Xu et al., (2019) found a significant increased activity in the SPL in young healthy individuals after moderate intensity aerobic exercise.

However, we did not find any correlation between ALFF and clinical score from pre- to post- intervention. The exploratory analysis of the subitems of PHQ-9 in the StD group showed that the right inferior temporal gyrus is positively correlated with the item6 (guilt, self-blame, or worthlessness) score before intervention, and the right precuneus is negatively correlated with the item1 (anhedonia) score after intervention.

Feelings of inadequacy and hopelessness were closely co-occurring with depressed mood (Zahn et al., 2015). It can be concluded that the right inferior temporal gyrus plays a prominent role in the generation of moral feelings in addition to participating in the dorsal and ventral visual pathways. Moreover, anhedonia is a hallmark symptom (American Psychiatric Association Task Force on DSM-IV, 1994). Our findings after exercise intervention may provide the evidence that the precuneus ameliorate symptom of anhedonia, and enriched our understanding of the neural basis of anhedonia in StD.

In the current research, we also found the ALFF value of the right parahippocampal gyrus decreased after aerobic exercise intervention in the HC group. The parahippocampus, which contributes to preserve memory and information processing, has been found to be structural changes in those with StD (Zhou et al., 2016). Finally, the HC group showed increased activity in the right complementary motor area and the right central paralobe at follow-up. These two brain areas both control motor and sensory innervation and play an important role in somatosensory sensation (Li et al., 2014; Liu et al., 2019).

Changes in the above brain regions during pre-/post- exercise intervention reported in our research might suggest that exercise could adjust above dysfunction related circuits, and ameliorate the improvements in memory, emotion sensory and social cognition of both StD and HC groups. In spite of its potential implications, some limitations of this study should be acknowledged. Initially, the definitions of StD are varied with respect to different studies. The assessment of StD in the present study was primarily based on the PHQ-9 scores (Crockett et al., 2020; Kang et al.,

2020) and the structured clinical interview. Subsequently, the final sample size was relatively small (n=38 in the StD group) because this study was a longitudinal trial and the subjects fell off during exercise intervention. As a consequence, it may affect the correlation analyses between changes of ALFF values in the StD group and clinical scores. Furthermore, we only take adults aged between 18 and 48 years old as our subjects and most of them are female. In this way, the findings lack generalizability and may not be suitable for adolescents or older adults. Ultimately, the exercise trial was short-term and the aerobic exercise forms are different. Future studies should be investigated that to what extent could physical exercise interventions relieve, or even help patients with MDD recover from depressive symptoms (e.g., intervention time and form).

To summarize, we observed that physical exercise can not only change the patterns of spontaneous brain activity in both StD and HC groups, but also significantly reduce the depressive symptoms of the StD group. The findings provided more potential detailed information about underlying neural biomarkers of the anti-depressant effects of exercise. Having insight in the favorable physiological stress effects of the exercise regimen could probably be helpful in alleviating and preventing depression.

## Author statement

This manuscriptis"Amplitude of low-frequency fluctuation (ALFF) alterations in adults with subthreshold depression after physical exercise: A resting-state fMRI study" was approved by all authors for publication.

I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part.

The corresponding author is responsible for ensuring that the descriptions are accurate and agreed by all authors.

**The role(s) of all authors:** Lina Huang, Qingguo Ding, Pei Liang: Conceptualization, Investigation, Writing- Original draft preparation; Guofeng Huang, Yikang Cao, Xize Jia: Methodology, Software; Jun Zhang: Subject Recruitment; Hongqiang Zhang, Wenbin Shen: Data collection: Chun hong Hu: Supervision; Qianqian Wang, Linlin Zhan: Language polishing; Wei Xing: Revision.

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**Declaration of Competing 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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