Effect of aerobic training on depression and thyroid function in treated hypothyroid women

Efeito do treinamento aeróbico na depressão e função tireoidiana em mulheres com hipotireoidismo tratado

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ABSTRACT | INTRODUCTION: Hypothyroidism is commonly associated with depression and a slowing in mental activity. OBJECTIVES: The current investigation aimed to investigate the effect of aerobic training on depression and thyroid function in treated hypothyroid females. METHODS AND MATERIALS: Thirty women diagnosed with primary hypothyroidism that was regulated and accompanied by mild to moderate levels of depression completed a 12-week randomized controlled exercise trial involving two equal groups: aerobic (n = 15) and control (n = 15) groups. The exercising group performed three sessions of aerobic exercise per week at low to moderate intensity for 12 consecutive weeks while the control group performed no exercise intervention during the same duration. At baseline and post-intervention, Beck Depression Inventory score (BDIS), thyroid stimulating hormone (TSH) and free thyroxin (T4) were assessed. RESULTS: The exercising group showed significant improvements in BDIS, TSH and free T4 compared to baseline (p < 0.05) with negligible change in the control group (p > 0.05). As compared to the control, all measured items differed significantly in favor to the aerobic training group. CONCLUSION: In women with treated hypothyroidism, aerobic training can minimize depressive symptoms and enhance thyroid function. Clinical Trial Registry Number: PACTR202305810673587

KEYWORDS: Hypothyroidism. Depressive Symptoms. Exercise

RESUMO | INTRODUÇÃO: O hipotireoidismo é comumente associado à depressão e à lentidão da atividade mental. OBJETIVOS: A presente investigação teve como objetivo investigar o efeito do treinamento aeróbico na depressão e na função tireoidiana em mulheres com hipotireoidismo tratado. MÉTODOS E MATERIAIS: Trinta mulheres diagnosticadas com hipotireoidismo primário que foi regulado e acompanhado por níveis leves a moderados de depressão completaram um teste de exercício controlado randomizado de 12 semanas envolvendo dois grupos iguais: aeróbico (n = 15) e controle (n = 15). O grupo de exercício realizou três sessões de exercício aeróbico por semana em intensidade baixa a moderada por 12 semanas consecutivas, enquanto o grupo de controle não realizou nenhuma intervenção de exercício durante a mesma duração. No início e pós-intervenção, o escore do Inventário de Depressão de Beck (BDIS), hormônio estimulante da tireoide (TSH) e tiroxina livre (T4) foram avaliados. RESULTADOS: O grupo de exercícios apresentou melhorias significativas em BDIS, TSH e T4 livre em comparação com a linha de base (p < 0,05) com alteração insignificante no grupo de controle (p > 0,05). Comparando o controle, todos os itens medidos diferiram significativamente em favor do grupo de treinamento aeróbico. CONCLUSÃO: Em mulheres com hipotireoidismo tratado, o treinamento aeróbico pode minimizar os sintomas depressivos e melhorar a função da tireoide. Número de Registro do Ensaio Clínico: PACTR202305810673587


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Introduction

Hypothyroidism is a pathological condition typified by inadequate synthesis or functionality of thyroid hormones. The clinical manifestations of this condition encompass a spectrum that ranges from elevated levels of thyroid stimulating hormone (TSH) in asymptomatic individuals to severe hypothyroidism that may lead to multisystem organ failure and coma (known as myxedema coma) on rare occasions. Elevated levels of thyroid-stimulating hormone (TSH) are commonly observed in cases of overt or clinical hypothyroidism, in conjunction with decreased levels of free thyroxine (T4) and triiodothyronine (T3).

Hypothyroidism prevails among approximately 5% of the overall population, whilst an additional 5% of cases are expected to go undiagnosed. The prevalence of hypothyroidism increases with advancing age, exhibiting a maximal incidence between the age range of 30-50 years. Furthermore, the affliction of this condition is notably more prevalent among the female population, with a frequency exceeding that of the male population by 10 times.

There exists a distinct correlation between hypothyroidism and depression. In 1825, Parry first established a connection between hypothyroidism and depression by observing an augmentation in "nerve strokes" in patients with thyroid disorders. Depression is the predominant psychiatric manifestation commonly reported among female individuals with hypothyroidism. In 1995, a study was conducted by Cleare et al. The study conducted on a sample of 20 participants showed that a noteworthy proportion (40%) of individuals diagnosed with hypothyroidism exhibit indications of clinical depression. The occurrence of thyroid hormone disorders is intricately linked with alterations in the physiological and structural functions of the brain, as well as adverse effects on neurocognitive behavior. Correcting the thyroid imbalance can partially alleviate mood disorders including anxiety and depression that can be caused by either a rise or drop of the thyroid hormones. It is believed that one of the etiological factors contributing to the manifestation of depression in hypothyroidism is lowered thyroid hormones metabolic processes in the brain. Also, serotonin, dopamine, and neurotrophic brain-derived factor levels in the pre-frontal brain and hippocampus were shown to be lower in hypothyroidism-related depression-like responses.

Through the prescription of levothyroxine at levels that normalize the serum TSH, the conventional treatment goal in hypothyroidism is to establish biochemical euthyroidism. Levothyroxine treatment does, however, lessen depressive symptoms in hypothyroid women, and these patients nevertheless show a significant higher incidence of depression compared to normal.

According to studies currently available, women who participate in aerobic exercises can not only boost mood and retain a happy outlook, but they may also control their depressive and anxious symptoms. Also, aerobic exercises showed a considerable rise in the levels of T4 & T3 and a decrease in TSH in hypothyroid individuals.

Based on this, we hypothesized that aerobic training could be beneficial for minimizing depressive symptoms and enhancing thyroid function in hypothyroid women. As such, it presented an intriguing opportunity to investigate the potential effects of aerobic exercise as a non-pharmacological intervention on the manifestation of depression associated with hypothyroidism, as well as its effect on thyroid function, in treated hypothyroid women.
Methods

Study design and settings

The present trial is an interventional study that is characterized by being randomized, controlled, and executed in parallel groups. Between January 2022 and May 2023, the present study was conducted under the auspices of an approved research protocol (P.T.REC/012/003424). Approval was obtained from the Ethics Committee of Human Scientific Research, and the study adhered to the principles of the Helsinki Declaration. Each patient signed a consent form indicating their acceptance to take part in the trial.

Randomization and allocation

In the present study, a computer software program was employed to design a randomization table for the purpose of simple randomization. The allocation ratio used was 1:1. The allocation sequence was obfuscated by a series of sequentially numbered envelopes that were sealed in an opaque manner, thus ensuring that neither the researcher nor the participant were privy to the approaching assignment.

Sample size calculation

The determination of the necessary sample size for the study groups in relation to the primary outcome of the current investigation, namely the Beck Depression Inventory score (BDIS), was conducted using the G*POWER statistical tool (version 3.1). This determination was based on a previous study, which indicated a significant decrease in the post-intervention scores of BDIS in the adjunctive exercise condition compared with the control condition and aimed to ensure adequate statistical power for the present investigation. The present investigation was conducted with a predetermined power level of 0.95 and a level of significance set at a p-value of 0.05, ultimately resulting in a sample size of 14 subjects for each group. However, a total of 15 participants were enrolled in each group, with consideration given to potential dropouts and the need to obtain results with greater precision.

Subjects

Thirty female patients with controlled primary hypothyroidism and associated clinical depression (mild to moderate) were recruited from El Mahalla general hospital's outpatient endocrine unit in Egypt. Depression was diagnosed by a psychiatrist using BDIS. Women with a history of primary hypothyroidism (high TSH and low thyroid hormone levels), controlled (TSH between 0.4-5 mIU/L and free T4 between 0.7 to 1.78 ng/dL) for at least 6 months, ages from 30 to 50 years old, BMI between 20 and 30 kg/m$^2$, and on thyroid hormone replacement therapy (Levothyroxine) were eligible. Exclusion criteria included secondary hypothyroidism, subclinical hypothyroidism (high TSH, normal thyroid hormone level), pituitary disease (low TSH, low thyroid hormone level), individuals with a past medical record of psychiatric disorders, encompassing addiction and the use of antipsychotic medications, unstable cardiovascular problems such as arrhythmia and heart failure, diabetes, chronic chest disease, patients on medications affecting muscle power such as steroids or thyroid function such as biotin, musculoskeletal diseases that may interfere with physical activity, pregnancy & lactation women, patients with hypovitaminosis D or hypocalcemia and graded exercise test contraindications.

The female participants were randomly allocated into two equivalent groups including: aerobic training group (n=15, 40.3±5.1 years, 25.5±2.4 kg/m$^2$) and control group (n=15, 41.3±5.6 years, 26.2±2.4 kg/m$^2$). All included participants completed the study with no losses and also performed all the proposed exercise sessions regularly. The follow of participants in all groups is shown in Figure 1.
Evaluations

All evaluations were conducted by blinded assessors who were not told which study group is.

Clinical examination and history taking

These tests were conducted by a skilled endocrinologist with expertise in the field. The study included a comprehensive report on various clinical features, such as the age and weight of patients, their hypothyroidism histories, and co-morbidities. A Health digital scale (model BYH01) manufactured in China was employed to establish the initial measurements of body weight and height. For each women, their BMI was calculated by dividing their weight (in kilograms) by their height (in meter squared) using a formula: BMI=Weight (Kg) /Height (m²).21

Depression

Before and after the procedures, depression was evaluated using the Arabic version of the BDIS. The BDIS is a standardized self-report tool comprising of 21 items designed to evaluate the presence and severity of depressive symptoms.22 A cumulative score is computed through the summation of the individual items, and higher scores denote greater degrees of depression.22

Each part receives a score between 0 and 3. As a result, each person’s overall score ranges from 0 to 63. The categorization of depression severity is based on a scoring system that assigns scores within a range of 0 to 13 to no depression, 10 to 15 to mild depression, 16 to 23 to moderate depression, 24 to 36 to severe depression, and a score of 37 or higher indicates very severe depression.22 According to Fawzi et al., the Arabic version of the BDIS has outstanding reliability and a high validity.22
Thyroid function

TSH and T4 were tested at baseline and at the study's completion to evaluate thyroid function. Prior to administering the daily dose of Levothyroxine, venous blood samples of 5 ml capacity were procured from participants' left hand vestibule within the premises of the laboratory situated at the general hospital of El Mahalla, during the time window of 8 to 10 a.m. Whilst in a state of repose and relaxation, they were seated comfortably. Using the iChroma II immunofluorescence analyzer (Boditech Med Incorporated. ichroma™, Republic of Korea) and RADIM kits (Italy), the enzyme-linked immunosorbent assay (ELISA) methodology was employed to quantify the concentrations of TSH and free T4.

Intensity of Physical Activity

A graded exercise test was conducted pre-intervention, under the supervision of a physiotherapist, to ascertain the maximal heart rate (HRmax) and determine the intensity of training. The equipment utilized in this test was an electrically automated treadmill, specifically the Phantom AC6069m model which was manufactured in Taiwan. The treadmill was equipped with a digital display and a set of control buttons that allowed for alterations in both the inclination angle and the speed of the apparatus during the testing procedure. The digital display featured the recording of time elapsed and distance traversed throughout the testing procedure. The graded exercise test on the treadmill was conducted in congruence with the Bruce protocol consisting of seven progressive stages. The subject underwent testing until her volitional exhaustion threshold was reached. Since heart rate was tracked using a pulse oximeter (Granzia, Pulsox-304, Italy) during this test, the HRmax was ascertained through evaluation of the highest recorded value within the last 30 seconds prior to the termination of the respective test. The HRmax was utilized to establish the level of exercise intensity.

Interventions

Usual medical treatment

Thyroid hormone replacement treatment (Levothyroxine) was administered to all patients in both groups at varying dosages as indicated by the endocrinologist as listed in Table 1.

Aerobic Training

For the exercising group, an individualized aerobic training regimen was devised utilizing the FITT principle, encompassing the variables of frequency, intensity, time, and type. Prior to commencing each training session, a 10-minute session of exercises with low intensity was undertaken as a physiological preparation measure. Over a period of 12 consecutive weeks, the aerobic training intervention involved engaging in three sessions per week on the treadmill for a duration of 30 to 45 minutes each, performed at a low to moderate intensity level corresponding to 50% to 70% of the HRmax. During the course of the session, the heart rate was recorded continuously via a pulse oximeter. HRmax was computed utilizing a graded exercise test, adhering to established protocols and practices within the realm of empirical research. After the training session, a period of ten minutes was allocated for cool-down activities in the form of stretching exercises. During the course of the study, the aerobic exercise protocol was methodically escalated in accordance with patient tolerance, as gauged by the Borg scale. Initially, the endeavor was carried out by elongating the duration of the exercise session, whilst ensuring the constancy of the exercise intensity. Subsequently, the exercise intensity was augmented as part of the process.

The occurrence of any clinical manifestations indicative of postural hypotension, physical activity intolerance, or inadequate central and peripheral perfusion, such as vertigo, impaired vision, cognitive deterioration, excessive weariness, thoracic discomfort, palpitations, cramps, diaphoresis, pallor, saturation reduction exceeding 4%, cyanosis, or hyperactive cardiac response, served as the rationale for cessation of all workout within the exercising cohort.
Statistical Analysis

To perform the statistical examination, the IBM Statistical Package for the Social Sciences (SPSS) for Windows version 22, developed by SPSS, Inc., Chicago, IL, was utilized. Before conducting the final analysis, the data were inspected for the presence of outliers or extreme scores and assessed for normality. The present investigation constituted a preliminary step toward the computation of parametric assessments pertaining to the evaluation of divergence. According to the results of the Shapiro-Wilk test, it was determined that the observed data followed a normal distribution, with a significance level greater than 0.05. For the identical cohort, the antecedent and consequent intercession facts were subjected to evaluation employing a paired t-test. The differentiation between the two groups was evaluated via an unpaired t-test. To conduct an examination of the categorical variables among various groups, an application of the Chi-square test was executed. All significant differences were identified through statistical analysis conducted with a confidence interval of 95%, resulting in a significance level of $P < 0.05$.

Results

Baseline

Initially, no significant disparities were observed at baseline between both groups in the patients’ demographic, anthropometric measures and levothyroxine dose ($p > 0.05$) (Table 1). Moreover, all the indicators of results, encompassing BDIS, TSH and free T4, indicated non-significant disparities between the two groups ($p > 0.05$) at baseline (Table 2).

Depression

The present study found that the implementation of aerobic training resulted in a notable improvement in depressive symptoms among the participants, demonstrated by a statistically significant reduction of the BDIS in comparison to the baseline ($p = 0.001$). Concomitantly, the control group did not demonstrate any significant changes in depressive symptoms ($p = 0.44$), as illustrated in Table 2. There were significant differences in the post-study mean scores of BDIS between groups, showing a preference for the exercise group ($p = 0.001$), as illustrated in Table 2.

Thyroid function

When compared to the baseline, the female participants who underwent aerobic training exhibited noteworthy advancements in their thyroid function. Specifically, there was a significant reduction in TSH levels ($p = 0.005$) and a significant increase in free T4 levels ($p = 0.009$). No significant alterations were observed in the levels of TSH ($p = 0.67$) or free T4 ($p = 0.55$) within the control group as indicated in Table 2. Post-tests comparison of the two groups using unpaired t-test revealed a statistically significant decrease in TSH values ($p = 0.001$) and a statistically significant increase in free T4 levels ($p = 0.002$) in the group engaged in aerobic training. These findings are presented in Table 2 and demonstrate significant differences between the aerobic training group and the control group.
Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Aerobic training</th>
<th>Control</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>40.3±5.1</td>
<td>41.3±5.6</td>
<td>0.61</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.5±2.4</td>
<td>26.2±2.4</td>
<td>0.44</td>
</tr>
<tr>
<td>Hypothyroidism duration (years)</td>
<td>3.66±2</td>
<td>4.44±2.7</td>
<td>0.39</td>
</tr>
<tr>
<td>Constipation</td>
<td>6 (40%)</td>
<td>6 (40%)</td>
<td>0.46</td>
</tr>
<tr>
<td>Dry skin</td>
<td>7 (60%)</td>
<td>5 (33.3%)</td>
<td>0.45</td>
</tr>
<tr>
<td>Hair loss</td>
<td>7 (46.6%)</td>
<td>5 (33.3%)</td>
<td>0.45</td>
</tr>
<tr>
<td>Levothyroxine dose (mcg/day)</td>
<td>113.3±53.3</td>
<td>96.6±42.1</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Notes: The data are presented in the manner of Means ± SD, with statistical significant level assessed through both unpaired and paired t-tests denoted by *p-value* and **p-value**, respectively; a p value less than 0.05 is considered to be statistically significant. BDIS: Beck Depression Inventory scale; MD: mean difference; TSH: thyroid stimulating hormone; mIU/L: milli-international units per liter; T4: thyroxine; ng/dL: nanograms per deciliter.

Source: the authors (2023).

Table 2. Outcome measures in both groups before and after the interventions

<table>
<thead>
<tr>
<th>Variable</th>
<th>Aerobic training</th>
<th>Control</th>
<th>P-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDIS</td>
<td>baseline</td>
<td>19.2±5.1</td>
<td>18.8±4.3</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>10.1±4.7</td>
<td>18.2±4.4</td>
</tr>
<tr>
<td></td>
<td>MD</td>
<td>-9.1</td>
<td>-0.6</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>0.001*</td>
<td>0.44</td>
</tr>
<tr>
<td>TSH (mIU/L)</td>
<td>baseline</td>
<td>33±0.89</td>
<td>33±0.53</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>2.3±0.47</td>
<td>3.3±0.46</td>
</tr>
<tr>
<td></td>
<td>MD</td>
<td>-0.7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>0.006*</td>
<td>0.67</td>
</tr>
<tr>
<td>FreeT4 (ng/dL)</td>
<td>baseline</td>
<td>1.24±0.21</td>
<td>1.26±0.25</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>1.47±0.15</td>
<td>1.20±0.26</td>
</tr>
<tr>
<td></td>
<td>MD</td>
<td>0.23</td>
<td>-0.06</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>0.009*</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Notes: The data are presented in the manner of Means ± SD, with statistical significant level assessed through both unpaired and paired t-tests denoted by *p-value* and **p-value**, respectively; a p value less than 0.05 is considered to be statistically significant. BDIS: Beck Depression Inventory scale; MD: mean difference; TSH: thyroid stimulating hormone; mIU/L: milli-international units per liter; T4: thyroxine; ng/dL: nanograms per deciliter.

Source: the authors (2023).
Discussion

Engaging in regular aerobic exercise has the capacity to enhance thyroid function and lessen the severity of symptoms connected to hypothyroidism, such as depression.

In the current investigation, it was clear that aerobic training induced significant improvements regarding depression and thyroid functions. The current investigation endeavors to elucidate the potential benefits of aerobic exercise on both depressive symptoms and thyroid function in treated hypothyroid women.

Depression

As compared to the baseline, the exercising group exhibited substantial improvements in depressive symptoms as shown on BDIS, while the control group showed no discernible change from baseline. Yet in contrast to the control group, aerobic training showed more significant improvements in BDIS.

Based on the research conducted by Rahman and Ali, it is suggested that the presence of hypothyroidism may result in a depressive state due to the potential impairment of the thyroid hormones’ ability to enhance the central adrenergic system. It is postulated that this system plays a key role in controlling an individual’s emotional state and overall mood, or by the reduction in the serotonin precursor levels and limited accessibility of the serotonin transporter as induced by hypothyroidism.

The antidepressant efficacy of moderate aerobic training may be ascribed to the capacity of aerobic exercise to elevate levels of brain serotonin. Furthermore, it is notable that serotonin amplifies hippocampal synaptic plasticity which could potentially contribute to the mitigation of symptoms associated with depression. Furthermore, the release of neurotrophic brain-derived factor may potentially be associated with the impacts of physical exercise. The authors Sigwalt et al. according to the findings, a tri-weekly swimming regimen resulted in an upsurge in hippocampal mRNA expression levels of brain-derived neurotrophic factor.

An Indian pilot research investigated the influence of yoga on depression among a cohort of 38 hypothyroid women, and yielded findings that were consistent with our own research. Following the yoga instruction, a considerable reduction in levels of both BDIS and TSH was observed. In a rat model, Ezzat and Abd-El Hamid set out to investigate if aerobic exercise may alleviate adult mice’s depression caused by hypothyroidism. The findings indicated that moderate aerobic exercise has the potential to alleviate depression that is triggered by hypothyroidism. This effect is attributed to the elevation of neurotrophic brain-derived factor levels, which consequently enhances the activity of the serotonergic system, stimulates neurogenesis in the hippocampus, and reduces neuronal cell apoptosis. These results were observed after a four-week period of regular exercise.

Also, the benefits of exercise on depression in teenagers were examined in a recent systematic review and meta-analysis involving a sample size of 1331, examining fifteen scholarly articles and 19 comparisons. It was concluded that exercise confers mental health benefits to individuals with depression. The findings of the analysis revealed that the performance of aerobic exercise elicited significant effects on the demographic of adolescents exhibiting symptoms of depression. Another research investigated the effect of aerobic exercise on depressive symptoms, anxiety levels, self-esteem, and overall quality of life among a sample of 80 individuals who were experiencing depression. Findings from the study indicated that engaging in aerobic exercise proved to be an effective and beneficial intervention for reducing symptoms of depression and anxiety, as well as for enhancing self-esteem and improving the overall quality of life among those with depressive symptoms. Consequently, patients were counseled to incorporate aerobic exercise along with medication as a pragmatic, economical, and routine approach to managing their depressive symptoms.

Thyroid function

As compared to baseline, the exercising group exhibited substantial improvements in thyroid function including TSH and free T4, while the control group showed no discernible change from baseline. Yet in contrast to the control group, aerobic training showed more significant improvements in TSH and free T4.
Our research yielded results that were comparable to those of Altaye et al., in accordance with the findings presented in their study. According to the report, after 16 weeks of implementing an aerobic exercise intervention, a considerable alteration in the response of TSH and thyroid hormones (T3 and T4), was observed, as compared to the subjects in the control group. Also, over the course of a 3-month regimen of aerobic exercise, pregnant women diagnosed with hypothyroidism experienced a statistically significant increase in T4 and decrease in TSH levels when compared to both their baseline measurements and a control group.

Moreover, over a duration of eight weeks, individuals belonging to the experimental cohort undertook aerobic exercise for 30-60 minutes at 65-75% of their HRmax thrice weekly demonstrated significant increases in T4, T3 and thyrotropin releasing hormone levels after the eight-week exercise program, but TSH levels declined significantly.

This study's findings agree with earlier researches that also showed with aerobic exercise intervention, serum TSH was found to be considerably decreased and T3 and T4 significantly increased. This showed that doing aerobic workouts can increase thyroid hormones in the blood. In contradiction to present research findings, other preceding studies revealed that aerobic exercise has no discernible effect on thyroid hormone levels. These discrepancies might be attributed to the women's distinct characteristics.

There are various clinical implications to this study. In an effort to explore the findings of past studies in hypothyroid patients complaining of depression, this study was done as a contribution to the area of exercise training in hypothyroidism. This study provides healthcare professionals who manage depression associated with hypothyroidism with additional knowledge regarding the effectiveness of the aerobic exercise form. The lack of awareness about the advantages of exercise in such individuals may be one of the explanations for the poor referral of such patients to physiotherapy. Furthermore, aerobic exercise form appears to be a cost-effective intervention that requires little equipment. Aerobic training may not only improve the effect of the hormonal replacing drugs, but also alleviate depressive symptoms in hypothyroid women.

This study was limited by the fact that HRmax could not be measured through direct means, such as cardiopulmonary exercise testing, owing to the unattainability of the necessary equipment. Furthermore, a dearth of male participants could potentially undermine the extrapolation of findings to the broader population.

### Conclusion

The present study indicates that, in women with treated hypothyroidism, an eight-week aerobic training program can effectively reduce depressive symptoms and enhance thyroid function.

### Acknowledgement

The author expresses his sincere gratitude to all participants for their invaluable contributions to this study.

### Conflicts of interest

No financial, legal, or political conflicts involving third parties (government, private companies, and foundations, etc.) were declared for any aspect of the submitted work (including but not limited to grants and funding, advisory board participation, study design, manuscript preparation, statistical analysis, etc.).

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### References


