The effect of dietary estrogens and acupuncture combined with exercise or exercise alone on bone mineral density in adolescent female athletes suffering from the female athlete triad: A randomized clinical trial

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ABSTRACT

The aim of this study was to investigate the effect of dietary estrogens and acupuncture combined with exercise or exercise alone on bone mineral density in adolescent female athletes suffering from the female athlete triad. A single blind randomized controlled trial was carried out. A total of 44 girls (in adolescence age) diagnosed with low BMD (bone mineral density) resulting from female athlete triad participated in this study. They were divided into three equal groups (group A, B and C). Group A (n = 15) received estrogen from dietary origin in the form of soy milk or soybeans daily in the breakfast combined with weight bearing (WB) exercises. Group B (n = 15) received acupuncture therapy on the acupoints responsible for bone density according to Chinese meridians combined with WB exercises, while group C (n = 14) received WB exercises only. The program was given for 45-60 min/session, three sessions per week for 24 weeks. Z score by Dual-energy X-ray absorptiometry (DEXA) for bone mineral density (BMD) assessment and estradiol 2 (E2) for measuring serum...
estrogen in blood were measured before treatment and after 24 weeks of treatment. The study groups were comparable on all outcome measures. Within-group comparison showed statistically significant improvements in all measured variables (p < 0.05). Further, between-groups comparison revealed statistically significantly higher improvements in the measured variables, in favor of group B (p < 0.05). The acupuncture sessions combined with WB exercises may be effective in improving bone mineral density in female athlete triad, rather than diet modification by adding soy phytoestrogen in meals beside exercise or exercise alone.

**KEYWORDS**

Acupuncture; Bone Mineral Density; Female Athlete Triad; Soy; Weight Bearing Exercises

**1. INTRODUCTION**

Bone density disorders affect a large percentage of the population, and are most common in female athletes (MacKnight, 2017). The female athlete triad is a condition that includes eating disorders, amenorrhea, and osteoporosis. Female athletes with low energy reserves are more likely to develop the remaining components of the triad. Inadequate caloric intake induced by pathologic caloric restriction or increased energy expenditure may result in low energy availability, especially when body mass index (BMI) of the female athlete is less than 17.5 kg/m2 (Matzkin et al., 2015).

Athletic amenorrhea results from an unstable release of gonadotropin-releasing hormone (GnRH). Sudden loss of weight and excessive training can influence the release of GnRH which directly affects luteinizing hormone (LH) while follicle stimulating hormone (FSH) release from the pituitary gland leading to disruption of the hypothalamic pituitary ovarian axis causing amenorrhea. Estrogen is involved in the physiology of bone mineral density (BMD) and bone development in a complex way as it prevents bone resorption, allowing bone development to increase and improve so that, BMD is reduced in an estrogen-deficient state, increasing the risk of fragility fractures (Garg & Berga, 2020).

Decreased BMD and fatigue fractures result in about 50% of the total tibia fractures in runners complaining anterior leg pain, in addition to 30% of total fractures of navicular bone due to focused force on the central third of the bone generated from the compression between cuneiform and talus during heel strike and 10% to 20% of total fractures of metatarsals especially second and third ones (Harrast & Colonno, 2010).
Soy consuming is one from the basics of traditional Asian traditions among their population, thus the soybeans are the main ingredient in the traditional Asian recipes as it contains high percentages of phytoestrogens, polyphenols with a molecular structure identical to endogenous estrogens (Rizzo & Baroni, 2018). Isoflavones are phytoestrogens that have a similar structure and function to estradiol are found in soybeans and other plants. Isoflavones found in soy include genistein, daidzein, and glycitein and they have been proposed to have protective effects against many hormonal dysfunctions. Soy has high isoflavone content and these isoflavones binds to estrogen receptors (ERs) and have estrogenic-like effects (Messina, 2014).

In traditional Chinese medicine (TCM), bone loss is related to a disruption of qi function in the zang-fu organs, as well as an imbalance between yin and yang, leading in osteoporosis. Deficiency of qi is a syndrome in which the functions of the zang-fu viscera (TCM internal organ systems) are diminished because of weakness and/or excessive usage of the internal organs' energies. Acupuncture can help females with osteoporosis not only relieve pain, but it can also prevent osteoporosis by regulating endocrine hormone levels in the body, increasing bone density, and treating improper bone metabolism with minimal side effects (Ren et al., 2020; Xu et al., 2005). Acupuncture can be applied in different manners (Guo et al., 2016).

Exercise training has many advantages, such as improving mechanical properties of bone by changing its composition, promoting osteogenesis thus, preventing bone loss. Weight-bearing exercises are most popular among children, adolescents, adults, and postmenopausal women as they generate the highest mechanical load on bones (Shanb & Youssef, 2014). Weight bearing exercises that are characterized by high axial compressive forces have been identified as having the most positive influence on BMD. Weight bearing are generally more osteogenic than non-weight bearing ones as well as positive associations between muscle strength and BMD in children and adults (Duncan et al., 2002).

Adolescents and adults who participate in endurance sports, such as running, often have lower BMD than athletes participating in ball and power sports. This is confirmed by some cross-sectional studies in female adolescent and collegiate runners that have demonstrated increased rates of low BMD over time which needs a modification in their exercise to include weight bearing training (Scofield & Hecht, 2012). The aim of this study is to investigate the effect of dietary estrogens and acupuncture combined with exercise or exercise alone on bone mineral density in adolescent female athletes suffering from female athlete triad.
2. METHODS

2.1. Design and Setting

A single blind randomized controlled trial of three equal groups of participants enrolled in a prospective outcome registry of individuals with decreased bone mineral density at the outpatient clinic of faculty of physical therapy, Badr University in Cairo, and Sport Medicine Specialized Center in Nasr City. Patients with decreased bone mineral density of female athlete triad due to estrogen deficiency in adolescence, were referred from the gynecology clinic of the National Research Center in Cairo (2020-2021).

This registry was approved by the Ethical Review Committee of the Faculty of Physical Therapy, Cairo University. P.T. REC/012/003070, conducted according to the ethical guidelines of the Declaration of Helsinki 1964 and Declaration of Tokyo 1975 and registered in clinical trial. Gov with ID number NCT04748250. It was conducted transparently and presented in accordance with CONSORT guidelines. All participants or their parents who were able to independently complete the informed written consent process did so after eligibility criteria for the registry were confirmed.

2.2. Participants

Sixty girls diagnosed with low BMD resulting from female athlete triad were assessed for eligibility to participate in this study. Seven participants did not meet the inclusion criteria and five participants refused to participate. Out of the 60 girls who participated in the current study, only 44 females completed the study and were divided into 3 groups: 15 participants in groups A and B, and 14 participants in group C. The experimental design is shown as a flow diagram in Figure 1.

Patients were included if they are participated in running sport and were diagnosed with female athlete triad, their age was between fourteen to seventeen years old, their body mass index was less than 17.5 kg/m², their Z-score on DEXA was between -1.0 and -2.5, their serum estradiol 2 E2 level was less than 15 pg/ml and they were able to follow instructions (Hariani et al., 2016). All registry participants had amenorrhea (0–3 cycles/year for at least the last 18 months). Examination of the patient confirmed the primary diagnosis of female athlete triad.

The evaluating physician employed a combination of patient history and subjective reports of symptoms, clinical examination results, lab investigations, and radiographic imaging using DEXA of the involved limb to make the diagnosis. Patients who were taking any hormonal treatment, had a current or history of respiratory disease, diabetes, metabolic bone disorders, rheumatoid arthritis, thyroid or parathyroid disease, malignancy, cardiac, renal, or inflammatory bowel disease, or who
were taking oral or inhaled steroids or receiving any other form of traditional physical therapy modalities were excluded from the study (Gibson et al., 2000).

Figure 1. Consort flow diagram
2.3. Randomization

The study participants (forty-four runner female athlete participants at adolescence age and diagnosed with low bone mineral density resulting from female athlete triad), were randomly divided into three equal groups (A, B and C). Following the baseline examination, concealed allocation was performed using a computer-generated randomized table of letters created prior to the start of data collection by a researcher not involved in the recruitment and/or treatment of patients. Individual and sequentially lettered index cards were used to randomly assign participants to the treatment groups. The index cards were folded and placed in sealed, opaque envelopes. A second therapist, blinded to baseline examination findings, opened the envelope, and proceeded with treatment according to the group assignment. Each participant received a sealed envelope containing one of the letters A, B and C. Researchers responsible for evaluations were blinded to participants’ allocation.

2.4. Outcome Measures

2.4.1. Serum Estradiol 2 E2

The IMMULITE 1000 immunoassay analyzer was used to identify the levels of these hormones in the blood before and after treatment. Estradiol has a detection limit of 18 pmol/L in adolescence, thus any number less than 18 is termed estrogen insufficiency and measurements could not be taken during a specific stage of the menstrual cycle (Gibson et al., 2000; Sehested et al., 2000).

2.4.2. Dual-Energy X-Ray Absorptiometry Scanning (DEXA)

DEXA was used to measure the bone mineral density (BMD, grams per square centimeter) of the shin of tibia, navicular, and metatarsals in each patient using a Hologic QDR1000W densitometer (Hologic, Waltham, MA). Scan analysis was carried out by trained technicians who were blinded of the athletes' menstruation and activity state (Gibson et al., 2000).

Serial BMD examinations by DEXA may be necessary for females who have obtained a lower-than-desirable bone mass because of external causes during the critical developmental period of adolescence (Faulkner, 2005; Brown-Taylor et al., 2021).

2.5. Intervention

2.5.1. Group A (Soy Group)

Group A consisted of fifteen participants, who received soy phytoestrogen in the form of cup of soy milk in the breakfast daily for 24 weeks. One serving of a traditional soy food such as a cup of
soy milk (250 ml) contains about 30 mg of isoflavones. Every bottle of original soy milk contains 300 milliliters (Messina & Messina, 2000; Setchell et al., 2002).

In addition to weight bearing exercise program with duration 45-60 min/session, three sessions/week for 24 weeks included double leg press, quarter squats up to right angle knee flexion, wide stance mini-squat, quadruped position and step-up exercises and standing on one limb with arm support. The patients had a warming up routine of a brisk walk and gentle stretch of arms, knee flexors, calf muscles, lower back muscles for 10 min, finishing with cooling down in the form of a brisk walk for 5 min (Shanb & Youssef, 2014).

2.5.2. Group B (Acupuncture Group)

Group B consisted of fifteen participants, who received acupuncture sessions for thirty minutes, three times per week for 24 weeks. Alcohol was used to map and disinfect the acupuncture points. All patients had the identical acupuncture points selected. Acupuncture sites in the abdomen have the same somatic innervations of the ovaries and uterus, and needles were placed into these points perpendicularly to a depth of 15-35 mm. Points includes conception vessel (CV) 3, 4, 6 & 10, stomach (ST) 25, 28 & 29, bladder (BL) 11 and 23 and Gallbladder (GB) 39. The acupuncture point was palpated with the left hand, and the skin was stretched or squeezed to form a fold. With the right hand, the needle was quickly inserted; twisting it to the desired depth till the De Qi sensation was obtained. The following methods were employed: twisting the needle, lifting, and thrusting the needle, a combination of lifting, thrusting, and twisting, tapping, and waving the needle.[20][21][22] In addition to weight bearing exercise program with the same prescription as in group A.

2.5.3. Group C (Exercise group)

Group C consisted of fourteen participants, who received weight bearing exercise program with the same prescription as in group A.

Adherence rate to the treatment program was assessed at two intervals. The first interval was after 12 weeks of the beginning of the treatment program, while the second one was at the end of the treatment program (after 24 weeks).

2.6. Sample Size

A prior sample size calculation for the study was carried out by conducting statistical tests with the G Power software (version 3.1.9.2) and taking the conventional effect size values recommended by Cohen (1992) into account. Accordingly, the required sample size was calculated as at least 30
(assumptions of $\alpha = 0.05$, $\beta = 0.82$ and large effect size=0.46). Then, 44 children were recruited to account for the possible dropout rates.

2.7. Statistical Analysis

All statistical analyses were conducted through the Statistical Package for Social Studies (SPSS) version 24 for Windows (IBM, SPSS, Chicago, IL, USA). Descriptive analyses, including mean and standard deviation, were performed for all variables. ANOVA was used to compare baseline characteristics between groups. Normal distribution of data was checked using the Shapiro-Wilk test for all variables. Adherence rate was compared between 3 and 6 months between groups using ANOVA test.

The mean values of the Z score and Estrogen level were compared across the three groups using a mixed model MANOVA, as well as between pretest and posttest in each group using a within-group comparison. The effect size was calculated using partial eta squared. For subsequent multiple comparisons, post-hoc tests using the Bonferroni correction were used. A p-value of < 0.05 was considered statistically significant.

3. RESULTS

3.1. Subject Data

We compared the subjects’ data (age, weight, height, and BMI) between the three groups and the results show that there are no statistically significant differences between the three groups ($p > 0.05$) (Table 1).

<table>
<thead>
<tr>
<th>Table 1. Baseline characteristics of participants</th>
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<td></td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Height (cm²)</td>
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<tr>
<td>Weight (kg)</td>
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<tr>
<td>BMI (kg/m²)</td>
</tr>
</tbody>
</table>
3.2. Adherence Rate

Adherence rate was calculated as (actual minutes in treatment/scheduled minutes) *100. The mean ± SD adherence rate in the first 3 months was 96.2 ± 9.42% and that for the second 3 months was 93.98 ± 11.64%. There was no statistically significant difference in adherence between the first and second 3 months (p = 0.35). Treatment and time had a statistically significant interaction (Wilks’ Lambda = 0.37; F =12.48, p = 0.001, $\eta^2 = 0.38$). Time had a statistically significant main impact (Wilks’ Lambda = 0.53; F =359.96, p = 0.001, $\eta^2 =0.94$). Treatment had a statistically significant main impact (Wilks’ Lambda = 0.49; F =8.40, p = 0.001, $\eta^2 = 0.29$).

3.3. Effect of treatment on DEXA and Serum Estradiol 2 (E2)

3.3.1. Within-group comparison

There was a statistically significant increase in DEXA and E2 at post-treatment in comparison to pre-treatment at three groups (A, B and C), the percentages of improvement of DEXA were (47.09, 76.96%, and 60.10 %) with a p value of 0.001, while the percentages of improvement of Serum Estradiol 2 were (47.70 %, 90.98% and 49.06%) respectively with a p value of 0.001 (Table 2).

3.3.2. Between-groups comparison

Pre-treatment comparisons found no statistically significant differences in any of the measured variables between the three groups (p > 0.05). A post-treatment comparison of the groups demonstrated a statistically significant improvement in the measured variables in group B compared to group A and C (p < 0.001). Between the groups A and C, there was no statistically significant difference in any of the measured variables (p > 0.05) (Table 2).
Table 2. Mean values of DEXA and E2 before and after treatment of all groups

<table>
<thead>
<tr>
<th>Group</th>
<th>DEXA</th>
<th>Serum Estradiol 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ±SD</td>
<td>A vs B</td>
</tr>
<tr>
<td>Group A</td>
<td>Pre-treatment</td>
<td>-1.72±0.41</td>
</tr>
<tr>
<td></td>
<td>Post-treatment</td>
<td>-0.91±0.26</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>P=0.001</td>
</tr>
<tr>
<td>Group B</td>
<td>% of Change</td>
<td>47.09%</td>
</tr>
<tr>
<td>Group C</td>
<td>% of Change</td>
<td>76.96%</td>
</tr>
<tr>
<td>Group C</td>
<td>% of Change</td>
<td>60.10%</td>
</tr>
</tbody>
</table>

4. DISCUSSION

The main outcomes of our study indicated that the acupuncture sessions combined with weight bearing exercises may be more effective in improving bone mineral density in female athlete triad at adolescence age rather than diet modification by adding soy phytoestrogen in meals beside exercise or exercise alone.

Laboratory and radiological examination showed a statistically significant improvement from baseline to post-treatment in the three groups, with improvement in the measured variables in the group B compared to group A and C (acupuncture + WB) (p < 0.001), while between the groups A and C, there was no statistically significant difference in any of the measured variables. The results of the current study might prove that acupuncture + WB intervention have greater effective strategies in providing laboratory and radiological improvements in female athlete triad.

The results of this study came in agreement with the work of Pan et al. (2018) who concluded that acupuncture therapy in the form of warm acupuncture, electroacupuncture and needling may aid patients with low BMD. Our study is also in agreement with findings of previous studies that reported bilateral acupuncture of SP6 and ST36 acupoints could have a significant effect on LH, FSH, E2 and prolactin hormones in females and this may be linked to long term changes in gene expression due to its effect on endorphin levels which affect the release on GnRH and thus
influencing the release of FSH, LH surge, E2, normalizing the hypothalamic pituitary ovarian axis and ovarian function, which is the primary cause of female athletes' low bone mineral density (Chen, 1997; Chang et al., 2002; Zhu et al., 2019).

In an Australian university osteoporosis clinic, a study was conducted on 202 participants to find the proportion of selecting acupuncture as a treatment modality. The results showed that about fifth of the participants have used acupuncture as a complementary medicine method, which indicates the progression in using acupuncture in managing bone density disorders, which support the results of the current study (Mak, 2015). On the other hand, our results agree with the finding of many experimental studies conducted to manage osteopenia in animals by using acupuncture revealed that acupuncture increases bone mineral density (Zhang et al., 2004; Zhou et al., 2011; Atsushi et al., 2018).

On the contrary to the results of this current study, Fu et al. (2018) concluded that acupuncture decreases the levels of serum estradiol 2 hormone during ovulation time and so it has no benefit on bone mass, while Thakur et al. (2016) reported that estrogen medication may be more beneficial than acupuncture in decreasing hypoestrogenism symptoms in women, although acupuncture therapy had fewer adverse effects than estrogen.

The results of the current study came in line with a study which reported that patients with low BMD would benefit from weight bearing exercise because muscular strength is the main output of exercises enhancing BMD, because the force exerted by muscle to pull bones while muscular contraction has a strong osteogenic stimulus (Kerr et al., 2001; Vincent & Braith, 2002). Different studies reported that the effect of exercise programs on bone is load dependent. And affect positively the intrinsic and extrinsic mechanical properties of bone confirming the results of present study (Huang et al., 2003).

A previous study conducted on children and adolescents that have decreased bone mineral density due to muscle weakness resulting from neural defect, and the results concluded that weight bearing exercise has a significant effect on improving BMD of the femur, this supporting the results of present study (Kim et al., 2017). Our results are also supported by a systematic review which reported that weight bearing exercise as jumping interventions during childhood and adolescence improve bone mineral density and structural properties without side effects (Gómez-Bruton et al., 2017).

A previous study was carried out on adolescents who were given 50 mg isoflavone equivalents per day for 6 weeks, and they measured bone turnover. The results showed that adolescent girls between 14-16 years show an optimization of the peak bone mass in response to the
same servings of soy milk which support and came in parallel with the results of our study (Coxam, 2008).

According to Asian research females in Shanghai (China), who ate high quantities of soy foods, were one-third less likely to get bone disorders than Chinese females who ate the low quantities of soy foods, which supports the results of our study (Song et al., 2007).

In consistency with the results of the group A in this study, a previous study was conducted to compare between bone mineral density of Japanese and American women. The study found that the Japanese women have lower rates of bone fractures than American women despite having nearly the same BMD values. These results were assumed to be caused primarily by dietary variables and consuming soy-based meals. Researchers conducted epidemiological and interventional investigations with soy isoflavones in various ethnic groups (Atmaca et al., 2008).

Other studies found that isoflavones have a significant effect on bone mineral density especially at lumber spine rather than isoflavones supplements and there is a strong relation between soy protein consumption and bone density and have significant effect on serum estrogen levels, enhancing the general function of reproduction, having a positive effect in hypoestrogenism cases as female athlete triad which supports and confirms the results of present study (Ricci et al., 2010; Ho et al., 2003; Emara et al., 2019; Albert et al., 2002).

On the other hand, a previous study concluded that after 7 months of increased soy ingestion of 32 mg isoflavones/d, there was a reduction in luteal estrogen level; that might consider the ethnicity aspect that could have an impact of soy on health in general (Jefferson, 2010).

5. LIMITATIONS

A limitation of this study was the lack of long-term follow-up of the girls to verify maintenance of improvement over time. The participants were all adolescent girls. Most of the data were from Chinese and English women, so further studies are recommended to evaluate the long-term effects of this protocol on bone mineral density in the female athlete triad in girls and women of different nationalities and different age groups, and to evaluate pain levels and functional performance.

6. CONCLUSIONS

The combination between acupuncture and weight bearing exercises was the most effective intervention to enhance safely bone mineral density in female athlete triad at adolescence age, rather
than the combination between diet modification by adding soy phytoestrogen in meals in addition to weight bearing exercises or weight bearing exercises alone.

7. REFERENCES


**AUTHOR CONTRIBUTIONS**

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

**CONFLICTS OF INTEREST**

The authors have no conflicts that are directly relevant to the content of this study.

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