Oral estrogen therapy mitigates the effects of aerobic training on cardiorespiratory fitness in postmenopausal women: a double blinded randomized clinical trial.

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<tr>
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Oral estrogen therapy mitigates the cardiorespiratory fitness increase induced by aerobic training in healthy and hysterectomized postmenopausal women.
Londrina, March 5\textsuperscript{TH}, 2013.

Dear Editor of Menopause,

I am submitting the manuscript titled "Oral estrogen therapy mitigates the effects of aerobic training on cardiorespiratory fitness in postmenopausal women: a double blinded randomized clinical trial" for appreciation of Menopause.

In this study, we analyzed the effect of six months of estrogen therapy and/or aerobic training on maximal cardiopulmonary exercise test and the main results indicated that estrogen therapy mitigates the effects of aerobic training on cardiorespiratory fitness. We believe that these results are interesting to the lector of Menopause, mainly because this finding suggesting that in postmenopausal women taking estrogen, a greater training stimulus might be necessary to induce better cardiorespiratory fitness increases.

We declare that this manuscript is original and is not being considered elsewhere until a decision is made as to its acceptability by the Menopause Editorial Review Board. We declare no conflict of interest in the study. Furthermore, we declare that this manuscript was checked for correct English grammar by the prior submission at the Menopause.

Please let us know if anything else is necessary.

Thanks for your attention,

Sincerely,

_______________________

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**TITLE:** Oral estrogen therapy mitigates the effects of aerobic training on cardiorespiratory fitness in postmenopausal women: a double blinded randomized clinical trial.

**RUNNING TITLE:** Postmenopausal and cardiorespiratory fitness

Crivaldo G. Cardoso Jr, Ph.D.\(^1,2\), Fábio L. Medina M.D.\(^1\); Luiz G. Pinto M.D.\(^1\); Bruna Oneda Ph.D.\(^1\), Luiz A. R. Costa\(^1\), Eliana Labes\(^3\); Sandra B. Abrahão, Ph.D.\(^4\); Taís Tinucci, Ph.D.\(^1\); Décio Mion Jr, Ph.D.\(^4\); Angela M. Fonseca, Ph.D.\(^3\); Cláudia L. M. Forjaz, Ph.D.\(^1\).

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**FINANCIAL SUPPORT:** Foundation for Research Support of São Paulo, Brazil – FAPESP (01/14989-7, 06/53753-2).

No conflicts of interest, financial or otherwise, are declared by the authors.

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ABSTRACT

Objective: The aim of this study was to evaluate the isolated and associated effects of oral estrogen therapy and aerobic training on cardiorespiratory fitness of postmenopausal women.

Methods: Forty two hysterectomized healthy postmenopausal women were randomly divided (in a double-blind manner) into 4 groups: placebo–control (PLA–CO= 09), estrogen therapy–control (ET–CO= 12), placebo–aerobic training (PLA–AT= 11), and estrogen therapy–aerobic training (ET–AT= 10). The ET groups received estradiol valerate (1 mg/day) and the AT groups trained on a cycle ergometer, 3times/week at moderate intensity. Before and six months after the interventions, all the women underwent a maximal cardiopulmonary exercise test on a cycle ergometer. Results: Regardless of the hormone therapy, aerobic training increased the VO$_2$ at the anaerobic threshold (p= 0.001), the respiratory compensation point (p= 0.043) and also the VO$_2$peak (p= 0.020). The increases at RCP and peak exercise were significantly greater in the groups receiving placebo than estrogen (VO$_2$RCP, PLA-AT= +5.3±2.8 vs. ET-AT= +3.0±2.5 mL.kg$^{-1}$.min$^{-1}$, p=0.04 and VO$_2$peak, PLA-AT= +5.8±3.4 vs. ET-AT= +2.8±1.4 mL.kg$^{-1}$.min$^{-1}$, p=0.02). Conclusion: Oral estrogen therapy mitigates the cardiorespiratory fitness increase induced by aerobic training in healthy and hysterectomized postmenopausal women.

Key-words: estrogen therapy, aerobic training, maximal cardiopulmonary exercise test.
INTRODUCTION

The postmenopausal period is a very important phase of women’s lives. In addition to other changes, cardiorespiratory fitness decreases about 1% to 2% a year following menopause,\textsuperscript{1-3} and this reduction may contribute to numerous diseases and functional dependencies that can be observed during this period of life. In fact, previous studies have reported an inverse relationship between cardiorespiratory fitness and the rates of cardiovascular and all-cause mortality in postmenopausal women.\textsuperscript{1,2,4-6}

To counteract the reduction of cardiorespiratory fitness after menopause, aerobic training has been widely encouraged.\textsuperscript{4,7} Asikainen et al.\textsuperscript{4} in their review conclude that exercise training in postmenopausal women results in VO\textsubscript{2}peak improvement of 2.5 to 7 mL.kg\textsuperscript{-1}.min\textsuperscript{-1}, which represents an increase of 4% to 32%. This improvement was achieved with training regimens performed for 10 weeks to 2 years, with training sessions conducted from 2 to 5 days per week and durations lasting from 15 to 60 min (mean 33 min), and intensities ranging from 48% to 85% of VO\textsubscript{2max} (mean 65% of VO\textsubscript{2max}). However, this is not a unique finding in this population, since have a study that failed to produce improvements in VO\textsubscript{2}peak in postmenopausal women with aerobic training regimens that following the preceding characteristics\textsuperscript{8}. This controversy suggests that some factors other than the exercise-training characteristics might influence the response to aerobic training in postmenopausal women.

The use of estrogen therapy at the early phase of the postmenopausal period is a very common practice used to treat menopause-related symptoms and to prevent osteoporosis in women at high risk of fractures.\textsuperscript{9} It has been reported that estrogen therapy has important cardiovascular effects. It improves cardiac parasympathetic modulation\textsuperscript{10} and decreases heart rate,\textsuperscript{11,12} which diminishes cardiac overload. Additionally, estrogen therapy increases
endothelium dependent and independent vasodilation, which might increase oxygen delivery to the muscles.\textsuperscript{13} Taken together, these effects suggest that estrogen therapy might improve cardiovascular fitness.

Despite the aforementioned evidences, the effects of estrogen therapy on cardiorespiratory fitness are very controversial. Some observational studies have reported that VO\textsubscript{2}peak is greater in women who are receiving or who have received hormone therapy.\textsuperscript{3, 14} However, interventional studies observed no change in VO\textsubscript{2} peak when postmenopausal women received different regimens of hormone therapy.\textsuperscript{11, 15-17} Inversely, Aldrigui et al.\textsuperscript{18} verified a decrease in VO\textsubscript{2}peak and VO\textsubscript{2} at the anaerobic threshold (AT) and the respiratory compensation point (RCP) with hormone therapy.

If hormone therapy can modify VO\textsubscript{2}peak, it may also change the response of VO\textsubscript{2} peak to aerobic training. In a longitudinal assessment, Hawkins et al.\textsuperscript{19} reported that the age-related decrease in cardiorespiratory fitness of master athletes was mitigated in women who were receiving hormone therapy. However, short-term interventional studies\textsuperscript{15, 17} have failed to show any difference in VO\textsubscript{2}peak improvement promoted by aerobic training in postmenopausal women whether they were receiving hormone therapy or not. Nevertheless, in these studies interventions lasted for either 2 or 12 weeks only. So, a longer period of time might be necessary for a complete adaptation.

Based on the previous rational, we hypothesized that estrogen therapy may affect the cardiorespiratory fitness increase induced by aerobic training in postmenopausal women. Therefore, the aim of this study was to evaluate the isolated and associated effects of oral estrogen therapy and aerobic training on cardiorespiratory fitness of hysterectomized healthy postmenopausal women.
METHODS

This study was a randomized, double-blinded and placebo-controlled clinical trial, and it was conducted at the General Hospital of the University of São Paulo, Brazil, from July 2002 to July 2008. All the procedures were conducted by the same researchers with a standardized protocol. The procedures were carried out in accordance with the Declaration of Helsinki guidelines on human experimentation and approved by the Ethics Committee of the General Hospital of the University of Sao Paulo. The volunteers signed an informed consent prior to their participation. The study was registered in the Clinical Trials (NCT01120665) and followed the CONSORT Statements.²⁰

Study population

Hysterectomized and postmenopausal women ages 45 to 60 years were recruited to the study. They were nonsmokers, sedentary, normotensive (office blood pressure lower than 140/90 mmHg), non-obese (body mass index < 30 kg/m²), and without any chronic diseases, as assessed by screening tests. All the women enrolled in the study were absent of prior use of estrogen implants for the past two years, oral or transdermal estrogen for the past 3 months, and injected estrogen for the past 6 months.

Many strategies were employed to construct the sample, including advertisements in local radio stations, newspapers, and public places on the university campus. A total of 500 women volunteered for the study, but 440 did not fit all the inclusion criteria. Thus, the initial sample was composed of 60 patients. They were randomly assigned to four intervention groups:
placebo-control (PLA-CO), estrogen therapy-control (ET-CO) placebo-aerobic training (PLA-AT) and estrogen therapy-aerobic training (ET-AT). During the study, 17 women were excluded because of lack of follow-up and one because of exercise-induced asthma. Thus, 42 volunteers finished the study (Figure 1).

**Medical screening tests**

Screening tests included medical history, physical examination, blood analysis, gynecological examination, mammogram, and an exercise ECG test. Volunteers were excluded from participation if they were found to have medical problems that contraindicated estrogen therapy or exercise. Hysterectomy was diagnosed by ultrasonography, and postmenopausal status was confirmed by amenorrhea for at least 12 months associated with estradiol levels lower than 40 pg/mL, and serum-luteinizing (LH) and follicle-stimulating (FSH) hormones greater than 25 U/L and 35 U/L, respectively. Natural menopause was observed in 40 women and surgical menopause in 2.

**Experimental Groups**

Estrogen therapy consisted of 1 mg/day of estradiol valerate, while the placebo consisted of 40 mg of lactose powder, 102 mg of cornstarch, 5 mg of microcrystalline cellulose, and 3 mg of magnesium stearate. The estrogen and the placebo capsules were identical in appearance. They were prepackaged in boxes and consecutively numbered for each subject according to a randomization sequence created using Stata 9.0 (StataCorp, College Station, TX). An order
number was assigned to each woman, and each one received the capsules of the corresponding prepackaged boxes. Subjects and investigators were blinded for the therapy used by each subject.

At the beginning of the study, each participant received two boxes with 28 pills in each and was instructed to take one pill at the same time daily. Appointments with a physician were scheduled once every two months. At each appointment, two more boxes of pills were given to each subject, and adherence to the protocol was assessed by counting the number of pills not taken during the preceding two-month period. Women who took less than 80% of the pills were excluded from the study. At each physician visit, participants were submitted to a physical examination, determination of body weight, and measurement of blood pressure. Those presenting any adverse effects related to the medication were automatically excluded from the study.

The aerobic training consisted of cycling three times a week on a stationary bicycle. Training progressed in duration from 20 to 60 minutes of exercise and in intensity from the intensity corresponding to the individual AT to the RCP. This intensity was monitored by measuring heart rate (POLAR A3, Polar Electro Oy, Kempele, Finland) during the training sessions. Compliance to the exercise program was addressed, and each woman was asked to participate in at least 75% of the exercise sessions in order to be eligible to continue in the study. The control groups were advised to maintain their normal habits and not to start any physical exercise program during the study period.

**Cardiopulmonary exercise testing**
At the beginning and after six months of the interventions, a maximal cardiopulmonary exercise test was conducted in a climate-controlled laboratory. The test was conducted on a cycle ergometer (Corival Cycle), employing a protocol with 30W increments every three minutes until the subjects were unable to continue. Before each test, a 12-lead ECG was obtained (Cardio Perfect ECG System MD, St. Paul, Minnesota, USA). During the test, blood pressure was measured by the auscultatory method, using a mercury column sphygmomanometer at the end of each stage. Heart rate and ECG were continuously monitored and recorded every minute. Respiratory gases were continuously collected and oxygen uptake (VO$_2$) was analyzed at each respiratory cycle (Medical Graphics Corporation, CPX/D System, St. Paul, Minnesota, USA) during the entire test. VO$_2$ peak was determined as the greatest value of VO$_2$ achieved during the exercise in means of 30 s. Tests were considered valid if two or more of the following criteria were reached: a fatigue-limited cycle test; a plateau in VO$_2$ despite increasing work rate; a respiratory exchange ratio (RER) greater than 1.1; or respiratory rate greater than 30 incursions per minute.$^{21, 22}$

AT was visually determined using the following criteria: the intensity at which the ventilatory equivalent for oxygen (VE/VO$_2$) and end-tidal oxygen partial pressure (PETO$_2$) reached their respective minimum values and began to rise without changes in the same direction of the ventilatory equivalent for carbon dioxide (VE/VCO$_2$) and the end-tidal carbon dioxide partial pressure (PETCO$_2$).$^{23, 24}$ RCP was determined by the intensity at which VE/VCO$_2$ reached the lowest value before increasing and when PETCO$_2$ reached a maximum value and began to decrease.$^{23, 24}$ These determinations were performed separately by three experts who were blinded to which group the subjects belonged to. When there were disagreements in this first evaluation, the determination was made by a consensus.

**Data analysis**
Only data from the volunteers who effectively completed the entire protocol were analyzed. Data normality was checked by means of the Shapiro-Wilks test, using the statistical package SPSS for Windows (Statistical Package for Social Sciences, version 13.0, Chicago, IL, USA).

To compare the initial characteristics of the four groups, a one-way ANOVA was conducted. To evaluate the isolated and combined effects of therapy and aerobic training, a three-way ANOVA was employed, establishing therapy (PLA or ET) and training status (CO or AT) as the between main factors and the study phase (pre and post) as the within main factor. Post-hoc comparisons were made using the Newman-Keuls test. Values of p<0.05 were considered statistically significant, and data are reported as means and standard deviation. The software package used for these analyses was the STATISTICA (version 6.0, StatSoft Inc, Tulsa, OK, USA).

RESULTS

Of the 42 women who completed the study, 9 were in the PLA-CO group, 12 were in the ET-CO group, 11 were in the PLA-AT group, and 10 were in the ET-AT group. All the groups were similar in regard to age and to the anthropometric, cardiovascular, menopausal, and cardiorespiratory fitness parameters (Table 1). At baseline, all the volunteers had estradiol levels <40pg/mL, and 23 women presented with estradiol levels <13pg/ml, which was the minimal concentration detected by the method of analysis (6 in the PLA-CO, 8 in the ET-CO, 5 in the PLA-AT, and 4 in the ET-AT).
After 6 months, serum estradiol levels increased above the 40pg/ml in all the volunteers who were assigned to the ET groups, reaching a mean level of 54±29pg/ml. In addition, plasma FSH decreased significantly in the ET groups and did not change in the PLA groups (combined value of PLA groups, 74.8±29.1 vs. 74.8±33.6 U/L, p>0.05; ET groups, 81.5±30.8 vs. 61.0±33.3U/L, p<0.001). Plasma LH did not change in any of the groups (p>0.05).

The responses of VO\textsubscript{2} measured at rest, AT, RCP, and peak of exercise are shown in Figure 2. A significant interaction between the training status and study phase was detected for the VO\textsubscript{2}AT (p=0.001). Thus, aerobic training increased the VO\textsubscript{2}AT regardless of estrogen therapy. In addition, a significant interaction among all the main factors (hormone therapy, training status, and study phase) was detected for VO\textsubscript{2}RCP and VO\textsubscript{2}peak. Thus, after six months, VO\textsubscript{2}RCP (p=0.043) and VO\textsubscript{2}peak (p=0.020) increased in the AT groups in comparison with the pre-intervention values and also compared with the CO groups. Furthermore, the increases in VO\textsubscript{2}RCP and VO\textsubscript{2}peak were significantly greater in the PLA-AT group than in the ET-AT group (VO\textsubscript{2}RCP, PLA-AT= 5.3±2.8 vs. ET-AT= 3.0±2.5 mL.kg\textsuperscript{-1}.min\textsuperscript{-1}, p=0.04 and VO\textsubscript{2}peak, PLA-AT= 5.8±3.4 vs. ET-AT= 2.8±1.4 mL.kg\textsuperscript{-1}.min\textsuperscript{-1}, p=0.02).

Significant interactions between the factors of hormone therapy and study phase were detected for resting heart rate (PLA= 69±5 vs. 73±7; ET= 67±7 vs. 63±7, p=0.001). No change was found in the heart rate measured at AT, RCP, or peak exercise (p>0.05).

**DISCUSSION**

The new finding of the present study is that oral estrogen therapy mitigates the increase in VO\textsubscript{2} at RCP and in VO\textsubscript{2}peak promoted by aerobic training in postmenopausal women.
In the present study, aerobic training was conducted for six months, three times a week, with moderate intensity and long duration, which follows the recommendations for postmenopausal women. As expected, this kind of training increased cardiorespiratory fitness. Mean increases of 2.37±1.89, 3.80±3.22, and 4.38±3.50 mlO₂.kg⁻¹.min⁻¹ were observed for VO₂ measured at AT, RCP, and peak exercise, which corresponded, respectively, to improvements of 22%, 23%, and 22%. These results are within the range usually observed in postmenopausal women and corroborate the findings from the systematic review conducted by Asikainen et al. that found improvements in VO₂max ranging from 4% to 32%. Thus, the results show the efficiency of the training regimen employed in the present study.

As widely addressed in textbooks, the cardiorespiratory fitness increase induced by aerobic training might be attributed to several mechanisms, including functional and structural adaptations of the oxygen transport and uptake system. Briefly, aerobic training increases: i) plasma volume, venous return, internal ventricular dimension, ventricular complacency, and myocardial contractility, which results in an increased stroke volume and maximal cardiac output, leading to an increase in blood flow to the active muscles during exercise; ii) red-cell mass that increases blood capacity for carrying oxygen; iii) capillaries’ numbers and size, favoring oxygen delivery to the muscles; and iv) muscular aerobic enzymes (increasing mitochondrial content and size) and muscular glycogen storage, facilitating oxidative metabolism. Taken together, all these adaptations lead to increases in cardiorespiratory fitness.

The effects of estrogen therapy on cardiorespiratory fitness have been poorly studied and the results are controversial. In the present study, no difference was observed in VO₂AT, VO₂RCP, and VO₂peak between the PLA-CO and ET-CO groups. It is possible to conclude that a six-month period of oral estrogen therapy was unable to change the cardiorespiratory fitness of postmenopausal women. These results disagree with some previous studies that verified a
decrease\textsuperscript{18} or an increase\textsuperscript{14} in cardiorespiratory fitness with estrogen therapy. However, differences in regard to the route of administration, dose and type of hormone, and the regimen used may explain these differences and the interpretation of our results must be done carefully. Furthermore, these studies had some methodological limitations that were overcome in the present study by the employment of a randomized, double-blinded, and placebo-controlled design. In addition, the present results are in accordance with others\textsuperscript{11, 15-17} that found no difference in cardiorespiratory fitness after estrogen therapy.

Despite not changing VO\textsubscript{2}peak in the ET-CO group, estrogen therapy mitigated the increases in VO\textsubscript{2}RCP and VO\textsubscript{2}peak induced by aerobic training in the ET-AT group. Thus, ET mitigated the effect of aerobic training on cardiorespiratory fitness of postmenopausal women. To our knowledge, this is the first study to report this finding. The mechanisms by which estrogen may mitigate the effects of aerobic training on cardiorespiratory fitness are out of the scope of the present study. Previous studies suggest that estrogen therapy improves blood flow by increasing peripheral vascular conductance\textsuperscript{16, 27, 28} and endothelial-dependent vasodilation\textsuperscript{13, 16, 17, 27, 28}[Miller, 2008 #37, 29], which might improve oxygen delivery to the muscles, increasing VO\textsubscript{2}peak. However, Kirwan et al.\textsuperscript{16} verified that despite increasing peak blood flow, estrogen therapy failed to improve cardiorespiratory fitness. In addition, O’Donnell et al.\textsuperscript{15} did not find any significant effect of hormone replacement therapy on blood flow when estrogen therapy was associated with aerobic training. It is possible to suggest that as aerobic training per se increases vasodilation,\textsuperscript{29} estrogen therapy is not able to induce a further increase. This hypothesis should be addressed by future research. Estrogen therapy may also influence mitochondrial function, but this influence is not clear. Estrogen may increase mitochondrial function\textsuperscript{30} or produce mitochondrial oxidative stress\textsuperscript{30, 31}, which may affect the response to training and should be investigated in the future.
Although estrogen therapy mitigates training-induced increase on VO$_2$RCP and VO$_2$peak, it did not change the training effects on VO$_2$AT. At low intensities of exercise (i.e., AT), central cardiovascular adjustments seem to be the main determinants of VO$_2$. Previous studies have shown that estrogen has no effect on submaximal cardiovascular adjustments,\textsuperscript{3, 11, 14-18} which might explain the absence of an estrogen effect on VO$_2$AT improvement induced by training.

The present study has some limitations. Its results can be directly applied to healthy postmenopausal women; however, the extrapolation of these results to postmenopausal women with diseases commonly present at this age such as hypertension, diabetes, and others should be considered with caution. In addition, results should also be different with other training regimens, with different types of exercise, duration, and intensity. Finally, although 15 women were assigned to each experimental group, some women dropped out during the study. However, the dropout rate was similar among the groups, and significant results were obtained with the sample that finished the study.

The clinical relevance of this study lies in the fact that it confirmed that aerobic training increases cardiorespiratory fitness of health post-menopausal women regardless of estrogen therapy use. This increase may have clinical importance for postmenopausal women, since Blair et al.\textsuperscript{5} reported that low cardiorespiratory fitness (RR, 2.10; 95% CI, 1.36-3.21) is one of the most important independent predictors of cardiovascular mortality in women. However, estrogen therapy reduces the improvement in cardiorespiratory fitness, suggesting that in postmenopausal women taking estrogen, a greater training stimulus might be necessary to induce better cardiorespiratory fitness increases.
CONCLUSION

In conclusion, oral estrogen therapy mitigates cardiorespiratory fitness increase induced by aerobic training in healthy and hysterectomized postmenopausal women.

ACKNOWLEDGE

The authors want to acknowledge the volunteers of the study for their contribution. This study was promoted by the Foundation for Research Support of São Paulo, Brazil – FAPESP (01/14989-7, 06/53753-2).

REFERENCES


**TABLE AND FIGURE LEGENDS**
Table 1. Baseline characteristics of the four groups: placebo-control (PLA-CO), estrogen therapy-control (ET-CO), placebo-aerobic training (PLA-AT) and estrogen therapy-aerobic training (ET-AT).

Figure 1. - Flowchart of entry and discontinuation of subjects during the study.

Figure 2. Oxygen uptake (VO$_2$) measured at rest, anaerobic threshold (VO$_2$AT), respiratory compensation point (VO$_2$RCP), and peak exercise (VO$_2$Peak) in the four groups: placebo-control (PLA-CO), estrogen therapy-control (ET-CO), placebo-aerobic training (PLA-AT) and estrogen therapy-aerobic training (ET-AT). White bars= pre-intervention; Black bars=post-intervention; * significantly different from the pre-intervention; † significantly different from the respectively control groups; ‡ significantly different from the PLA-AT group; p<0.05.
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<th>ET-AT</th>
<th>p-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting heart rate (bat.min^-1)</td>
<td>68±4</td>
<td>69±8</td>
<td>70±8</td>
<td>65±9</td>
<td>0.53</td>
</tr>
<tr>
<td>Resting systolic BP (mmHg)</td>
<td>120±9</td>
<td>120±9</td>
<td>118±9</td>
<td>123±14</td>
<td>0.74</td>
</tr>
<tr>
<td>Resting diastolic BP (mmHg)</td>
<td>77±5</td>
<td>72±6</td>
<td>78±5</td>
<td>77±8</td>
<td>0.09</td>
</tr>
<tr>
<td>Resting mean BP (mmHg)</td>
<td>91±6</td>
<td>88±7</td>
<td>92±6</td>
<td>92±10</td>
<td>0.32</td>
</tr>
</tbody>
</table>

**Menopausal Status**

<table>
<thead>
<tr>
<th></th>
<th>PLA-CO</th>
<th>ET-CO</th>
<th>PLA-AT</th>
<th>ET-AT</th>
<th>p-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH (U/L)</td>
<td>35±18</td>
<td>43±18</td>
<td>32±15</td>
<td>33±13</td>
<td>0.42</td>
</tr>
<tr>
<td>FSH (U/L)</td>
<td>80±30</td>
<td>88±36</td>
<td>71±31</td>
<td>75±26</td>
<td>0.62</td>
</tr>
<tr>
<td>Time since hysterectomy (y)</td>
<td>8±6</td>
<td>9±7</td>
<td>9±6</td>
<td>7±5</td>
<td>0.94</td>
</tr>
</tbody>
</table>

**Cardiorespiratory fitness**

<table>
<thead>
<tr>
<th></th>
<th>PLA-CO</th>
<th>ET-CO</th>
<th>PLA-AT</th>
<th>ET-AT</th>
<th>p-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting VO_2 (ml.kg^-1.min^-1)</td>
<td>3.3±0.8</td>
<td>3.4±1.0</td>
<td>3.6±0.7</td>
<td>2.8±0.7</td>
<td>0.12</td>
</tr>
<tr>
<td>VO_2AT (ml.kg^-1.min^-1)</td>
<td>10.6±1.7</td>
<td>10.1±3.0</td>
<td>10.5±1.5</td>
<td>11.3±3.6</td>
<td>0.77</td>
</tr>
<tr>
<td>VO_2RCP (ml.kg^-1.min^-1)</td>
<td>15.5±1.9</td>
<td>15.1±3.4</td>
<td>16.8±3.8</td>
<td>15.9±3.3</td>
<td>0.65</td>
</tr>
<tr>
<td>VO_2peak (ml.kg^-1.min^-1)</td>
<td>18.8±3.3</td>
<td>18.5±3.2</td>
<td>20.4±3.0</td>
<td>18.6±3.7</td>
<td>0.49</td>
</tr>
</tbody>
</table>

BMI-body mass index; BP-blood pressure; LH-serum luteinizing hormone; FSH-follicle-stimulating hormone; VO_2-oxygen uptake; AT-anaerobic threshold; RCP-respiratory compensation point. Data are showed as mean ± SD.
Figure 1 - Flowchart of entry and discontinuation of subjects during the study.

500 Subjects analyzed for the study

440 Excluded: non-fulfillment inclusion criteria or had an exclusion criterion

60 subjects Randomized

15 subjects Placebo and control
15 subjects Estrogen therapy and control

6 dropped out no follow-up just after randomization
3 dropped out no follow-up just after randomization

12 subjects Estrogen therapy and control

6 months

15 subjects Placebo and aerobic training
15 subjects Estrogen therapy and aerobic training

4 dropped out no follow-up just after randomization

11 subjects Placebo and aerobic training
10 subjects Estrogen therapy and aerobic training

5 dropped out 4 - no follow-up just after randomization
1 - exercise-induced asthma

09 subjects Placebo and control

4 - no follow-up just after randomization
1 - exercise-induced asthma
Figure 2 – Oxygen uptake (VO$_2$) measured at rest, anaerobic threshold (VO$_2$AT), respiratory compensation point (VO$_2$RCP), and peak exercise (VO$_2$Peak) in the four groups: placebo-control (PLA-CO), estrogen therapy-control (ET-CO), placebo-aerobic training (PLA-AT) and estrogen therapy-aerobic training (ET-AT). White bars= pre-intervention; Black bars= post-intervention; * significantly different from the pre-intervention; † significantly different from the respectively control groups; ‡ significantly different from the PLA-AT group; p<0.05.
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