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## Aerobic exercise training in healthy postmenopausal women: effects of hormone therapy

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**Abstract:** Objective: We investigated the influence of hormone therapy (HT) on submaximal central and peripheral function in healthy postmenopausal women (PMW) after 12 weeks of endurance training. Design: A randomized double-blind placebo-controlled study in a research and clinical facility was conducted. All subjects (n=23) underwent 12 weeks of aerobic exercise training (walking 5d/week at 70-80% peak heart rate). Eleven subjects received HT, 12 received placebo. HT consisted of daily 17 beta-estradiol (1 mg) with cyclic micronized progesterone (200 mg) or placebo for 10 days per month. Subjects were tested pre- and post- exercise training. Primary outcome measures were submaximal stroke volume (SV), cardiac output, and total peripheral resistance (TPR) measured during cycling at 30W, 45W, and 60W. Secondary outcome measures were ventilatory threshold (VT), peak oxygen uptake (VO<sub>2</sub> peak), and resting and peak-ischemic calf blood flow. Results: At baseline, HT and placebo groups were similar (P>0.05) in age (57 ± 1 years; mean ± SEM), height (162 ± 2 cm), weight (72 ± 4 kg), VO<sub>2</sub> peak (21.5 ± 1.4 ml\*kg<sup>-1</sup>\*min<sup>-1</sup>),

and all cardiovascular measures. Post-training oxygen consumption and heart rate decreased ( $P < 0.05$ ) within groups during each submaximal exercise workload. SV, cardiac output and TPR remained unaltered ( $P > 0.05$ ).  $\text{VO}_2$  peak and oxygen consumption at the VT increased ( $P < 0.05$ ) within groups. Resting and post-ischemic blood flow were unaltered. HT did not influence any of the cardiovascular responses. Conclusions: These findings suggest that in healthy PMW, 12 weeks of aerobic training is effective in eliciting favorable cardiovascular adaptations, regardless of the presence of short-term HT.

## **Aerobic Exercise Training in Healthy Postmenopausal Women: Effects of Hormone Therapy**

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## ABSTRACT

**Objective:** We investigated the influence of hormone therapy (HT) on submaximal central and peripheral function in healthy postmenopausal women (PMW) after 12 weeks of endurance training. **Design:** A randomized double-blind placebo-controlled study in a research and clinical facility was conducted. All subjects (n=23) underwent 12 weeks of aerobic exercise training (walking 5d/week at 70-80% peak heart rate). Eleven subjects received HT, 12 received placebo. HT consisted of daily 17 beta-estradiol (1 mg) with cyclic micronized progesterone (200 mg) or placebo for 10 days per month. Subjects were tested pre- and post- exercise training. Primary outcome measures were submaximal stroke volume (SV), cardiac output, and total peripheral resistance (TPR) measured during cycling at 30W, 45W, and 60W. Secondary outcome measures were ventilatory threshold (VT), peak oxygen uptake ( $\text{VO}_2$  peak), and resting and peak-ischemic calf blood flow. **Results:** At baseline, HT and placebo groups were similar ( $P>0.05$ ) in age ( $57 \pm 1$  years; mean  $\pm$  SEM), height ( $162 \pm 2$  cm), weight ( $72 \pm 4$  kg),  $\text{VO}_2$  peak ( $21.5 \pm 1.4 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ), and all cardiovascular measures. Post-training oxygen consumption and heart rate decreased ( $P<0.05$ ) within groups during each submaximal exercise workload. SV, cardiac output and TPR remained unaltered ( $P>0.05$ ).  $\text{VO}_2$  peak and oxygen consumption at the VT increased ( $P<0.05$ ) within groups. Resting and post-ischemic blood flow were unaltered. HT did not influence any of the cardiovascular responses. **Conclusions:** These findings suggest that in healthy PMW, 12 weeks of aerobic training is effective in eliciting favorable cardiovascular adaptations, regardless of the presence of short-term HT.

**Keywords:** cardiovascular, estrogen, stroke volume, cardiac output, blood flow.

## INTRODUCTION

Exercise training is strongly recommended in post-menopausal women (PMW) to reduce coronary artery disease (CAD) risk<sup>1, 2</sup> to counter the effects of osteoporosis<sup>3, 4</sup> and to improve body composition and overall health.<sup>5</sup> Endurance-trained women have a significantly lower risk for all-cause mortality and nonfatal cardiovascular events.<sup>6</sup> In addition, regular aerobic exercise is linked to enhanced vascular function, including decreased arterial stiffness<sup>7-9</sup> and improved endothelial function.<sup>10</sup> Increased arterial stiffness and endothelial dysfunction are independently and in combination linked with increased cardiovascular disease risk.<sup>7-10</sup> Similar to the benefits of exercise, estrogen administration in healthy PMW is also associated with improved endothelial function,<sup>11</sup> and decreased arterial stiffness.<sup>7</sup> Despite early reports of estrogen administration reducing the risk of coronary heart disease,<sup>12</sup> recent randomized placebo-controlled trials reported either no benefit or an increased rate of cardiac events with hormone therapy (HT).<sup>13, 14</sup> Discordance between findings from observational studies and randomized control trials are suggested to be related to the type and timing of HT relative to the onset of menopause.<sup>15, 16</sup> In support of this 'timing hypothesis',<sup>16</sup> recent re-analysis of data from the WHI<sup>14</sup> has shown that younger and recently PMW (50-59 years of age) have lower absolute risks for adverse outcomes from HT initiation than do older and long after PMW.<sup>17</sup> Randomized clinical trials are currently underway to specifically investigate the cardiovascular effects of HT and 17 beta-estradiol in recently PMW.<sup>18, 19</sup> Meanwhile, the administration of HT in women for the relief of menopausal and uro-genital symptoms to avoid fractures and atrophy of connective tissue continues to be advocated.<sup>20</sup>

In light of the recommendation for PMW to participate in regular aerobic exercise to elicit numerous health benefits, and that approximately 28% of PMW aged 50-74 years are still prescribed HT in the US alone,<sup>21</sup> potential cardiovascular interactions between these interventions are of clinical relevance. Knowledge of such interactions would help guide practitioners and patients toward appropriate HT and exercise decisions. However, the interaction between HT and cardiovascular hemodynamics in response to exercise training is not well understood in general, in particular whether cardiovascular responses and adaptations to exercise training differ among users and non-users of HT. Although improvements in peak and submaximal cardiovascular parameters, such as stroke volume and cardiac output, in response to short-term endurance training have previously been shown to be independent of HT status in health<sup>22</sup> and disease,<sup>23</sup> no controlled studies have been reported. As such, the purpose of this study was to examine the influence of HT on central and peripheral function before and after 12 weeks of aerobic exercise training using a randomized, placebo control design in healthy PMW. Considering the well known benefits of estrogen on vascular function,<sup>7, 11, 24</sup> it was hypothesized that HT would confer greater improvements to central and peripheral measures of cardiovascular function compared with non-users.

## **METHODS**

*Study Design:* A randomized, double-blind, placebo-controlled, exercise-training study was conducted. Following medical screening, subjects were randomly assigned to either HT or placebo control group (N-HT), with physiological assessments made at baseline and after 12 weeks of aerobic training. The Human Research and Ethics Committee at



the University of Toronto approved the study protocol and written consent was obtained from all subjects following the principles of the Helsinki Declaration.

*Study Population:* Subjects included PMW who had undergone natural or surgical menopause and were amenorrheic for at least one year. Subjects were recruited through gynecologist physician referral, and through local newspaper advertisements. The number of years since menopause was not recorded. A total of 37 PMW were recruited over a one year period. A total of 14 (38%) subjects either dropped out of the study or were excluded due to non-compliance to the exercise training for various reasons, including time commitment (n=6), illness (n=6), and orthopaedic conditions that limited their ability to walk (n=2). There were no drop-outs associated with the HT. A total of 23 subjects completed the study, 11 of which received HT and 12 received placebo (N-HT). Since we have previously demonstrated unaltered central cardiovascular function during submaximal aerobic exercise in response to 12 weeks of HT alone in healthy PMW,<sup>24</sup> we did not examine the independent effects of HT in the current study.

*Eligibility Criteria:* Serum estradiol (E<sub>2</sub>) and follicle-stimulating hormone (FSH) levels confirmed postmenopausal status (FSH>40 IU/L; E<sub>2</sub> <80 pmol/L).<sup>24</sup> Eligible subjects were healthy, sedentary and had not performed regular exercise for at least 1 year. Exclusion criteria included diagnosed diabetes mellitus, use of any cardiac medications or any orthopaedic conditions likely to impede the ability to participate in exercise training and testing.

*Randomization:* Subjects were randomized to either treatment or placebo groups and performed baseline measures prior to drug assignment, after which subjects were given their package of pills for the study, consisting of either 1mg oral 17 $\beta$ -estradiol or placebo. 200mg of micronized progesterone, or placebo, were administered for those with and without an intact uterus, respectively, for a period of 10 days/month. This medication phase was positioned at approximately 3 weeks, after each estradiol treatment, for a total of three months. To minimize the effects of the progesterone, post-HT cardiovascular measures were obtained during the very last week of estradiol treatment. Subjects were instructed to talk to the study physician, who was readily available, should they wish to discuss any symptoms or ask any questions regarding the HT treatment, and not to share this information with the research team.

*Anthropometry:* Adiposity measures included body mass index (BMI), and waist and hip circumference. The sum of skinfolds (mm; John Bull, British Indicators) were obtained from three sites: suprailiac, triceps, and subscapular regions with percent body fat estimations calculated using standard equations.<sup>25</sup>

*Blood Analysis:* Blood samples, performed at the same time of day (08:00 am – 12:00 pm) during each visit to the laboratory, were drawn from the antecubital vein using standard venipuncture techniques. Serum E<sub>2</sub>, total testosterone and progesterone were assessed in duplicate using competitive immunoassay methods (Ortho-Clinical Diagnostics Inc., Vitros Immunodiagnostic Products, Rochester, NY). Sex-hormone binding globulin (SHBG) was assessed using chemiluminescent immunometric assay

methods (Immulite, Diagnostics Products Corporation, Los Angeles, CA). Serum was stored at  $-20^{\circ}\text{C}$ , and all samples were run in the same assays to eliminate contributions from inter-assay assay variance. All intra-assay CVs were  $<8\%$ . Assay sensitivities were 10 pmol/L for  $\text{E}_2$ , 0.25 nmol/L for progesterone, less than 0.03 nmol/L for testosterone, and 0.02 nmol/L for SHBG. Free androgen index (FAI) was calculated using the ratio of total testosterone to the concentration of SHBG.<sup>26</sup>

*Graded Exercise Testing:* Graded exercise to maximal effort was performed on an electrically braked cycle ergometer (Ergoline Ergometrics 800S) using standard open-spirometry techniques. Protocol included a 1-min warm-up at zero load, with work rate increased by 15 W/min until exhaustion. An automated sphygmomanometer was used to record blood pressure each minute (Tango, Sun Tech Medical Instruments). Measures of peak oxygen uptake ( $\text{VO}_2$  peak), minute ventilation, ventilatory threshold (VT), peak work rate, and peak heart rate were obtained as described previously by our group.<sup>24</sup> The VT was determined using the break point in the ventilatory equivalent.<sup>27</sup>

*Cardiac Output and Total Peripheral Resistance:* A second exercise test was used to determine cardiac output (Q) during exercise using the equilibrium  $\text{CO}_2$  rebreathing technique.<sup>28</sup> Three submaximal workloads of 30, 45, and 60 W (corresponding to ~40, 50, and 60% of peak work rate, respectively) were assessed. Stroke volume (SV; ml) was determined as Q divided by heart rate (HR; beats/min). Total peripheral resistance (dynes/sec/cm<sup>-5</sup>) was calculated as (mean arterial pressure[mmHg]/Q[L/min])  $\times$  80.

*Skeletal Muscle Blood Flow:* Resting calf blood flow (BF) and peak flow-mediated vasodilatory capacity were measured in the supine position using venous occlusion strain-gauge plethysmography (Vasculab SPG16, Medasonics, Mountain View, CA) as described previously.<sup>24, 29</sup> Briefly, an indium-gallium strain gauge was used with an occlusion cuff excluding BF to the foot. To produce maximal flow-mediated peripheral vasodilation both cuffs were inflated to 200 mmHg for 3-mins, followed by ischemic plantar flexion exercise to fatigue on a specialized ergometer. BF was measured from the slope of the time-leg volume curve. Vascular conductance (G) was calculated as BF/mean arterial pressure (MAP). Test-retest reliability for BF measures in our laboratory yielded an intra-class correlation alpha =.91 for peak BF and 0.90 for resting BF. HR, MAP, and blood pressure were recorded from the middle finger on a beat-to-beat basis using the Finapres method (Finapres, Ohmeda, Englewood, CO).

*Exercise Training:* Exercise Training consisted of walking five days per week, with at least one supervised session per week. Each exercise session composed of a 10-minute warm-up, a 45 minute aerobic conditioning (walking) session, and a 10-minute cool down. For the first two weeks, subjects were prescribed a walking intensity of 70% peak HR (~55% VO<sub>2</sub> peak), with training intensities increased to 75-80% peak HR (65-75% VO<sub>2</sub> peak).<sup>30</sup>

*Compliance:* Treatment compliance was confirmed via pill counts at each testing time point and cross-referenced to serum hormone blood values. Exercise training compliance was confirmed by individual weekly training diaries, including completed distance

(number of laps), total aerobic conditioning time, exercise HR, and subjective feelings. Failure to maintain 75% of the weekly prescription resulted in exclusion from the study sample.

*Data Analysis:* The data were analyzed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL), version 11. The following calculation was used to determine the number of observations needed to provide an 80% probability of finding differences of 1.5 standard deviations in two-sample tests confirming the effects of HT and exercise at a significance level of 0.05:<sup>31</sup>

$$n = 2 * (t_{\alpha=0.05}^{df=21} + t_{\beta=0.20}^{df=21})^2 * (SD/d)^2 = 2 * (2.08 + 1.32)^2 * (1/1.5)^2 = 10$$

Paired *t*-tests were used for overall effects, and independent *t*-tests for the differences between groups (HT and N-HT) at baseline. Effects of exercise were calculated as post-exercise training measures vs. baseline measures. Independent *t*-tests were used to detect differences in effects of exercise training between groups (HT and N-HT). The Bonferroni correction was applied for multiple comparisons. All data are reported as means  $\pm$  standard error of the mean (SEM), and are considered statistically significant at  $P < 0.05$ .

## RESULTS

*Subject Characteristics:* Baseline subject characteristics are presented in Table 1. Groups were similar ( $P > 0.05$ ) in age, height, weight, percent body fat, lean body mass, total fat mass, and body mass index. Significant reductions ( $P < 0.05$ ) in waist circumference,

percent body fat, and total fat mass were observed within, but not between, groups post-exercise training. Body mass was not altered ( $P>0.05$ ) after 12 weeks of exercise training.

*Serum Hormones:* There were no significant differences in serum hormone measures between the groups at baseline (see Table 2). As expected, serum  $E_2$  concentration increased significantly ( $P<0.05$ ) from  $43.6\pm3.5$  pmol/L to  $581.4\pm65.3$  pmol/L after three months in the HT group, but did not increase in the placebo group. Progesterone and testosterone levels did not increase in either group. FAI (arbitrary units) decreased significantly from  $1.49\pm0.2$  to  $0.72\pm0.1$  in the HT group ( $P<0.05$ ), reflecting the significant changes found in SHBG ( $37.9\pm0.4$  to  $77.8\pm4.7$  nmol/L) with HT. SHBG did not change in the placebo group.

*Compliance:* Pill counts confirmed compliance (100%) and were confirmed against hormone levels. The HT and placebo groups had a similar training HR (beats/min) at baseline ( $119\pm3$  vs.  $114\pm2$ ), after two months ( $122\pm3$  vs.  $116\pm2$ ), and after three months ( $123\pm2$  vs.  $117\pm2$ ), as assessed during each training session. Total weekly distance (km) walked was also similar between groups after one, two and three months of training, respectively ( $4.3\pm0.2$  vs.  $4.4\pm0.1$ ,  $4.7\pm0.2$  vs.  $4.7\pm0.1$ , and  $5.0\pm0.2$  vs.  $5.2\pm0.2$ , for HT vs. N-HT, respectively). Training speed (km/hr) ranged between 4.6-4.8 for HT users, and between 4.6-4.7 for non-users ( $P>0.05$ ). Frequency of training sessions did not differ between groups ( $P>0.05$ ), with both groups attaining ~85-95% of prescribed distances ( $P>0.05$ ).

*Submaximal Cardiovascular Responses:* Data for Q, SV, HR and total peripheral resistance (TPR) during 30W and 60W workloads are presented in Table 3. There were no differences in physiological responses between groups, nor did HT influence any of the examined variables. In the sample as a whole, there was a significant training-induced lowering of HR at each of the 3 workloads (30W,  $P=0.002$ ; 45W,  $P=0.002$  [data not shown]; 60W,  $P=0.001$ ), along with a modest decrease in  $\text{VO}_2$  at 30W ( $P=0.014$ ) and 60W ( $P=0.040$ ) in the group as a whole after training. There were no significant ( $P>0.05$ ) changes in Q or calculated SV at any submaximal workload in either group.

*Peak Oxygen Uptake and Ventilatory Threshold:*  $\text{VO}_2$  peak and VT increased similarly between HT and N-HT groups (Figure 1). Mean  $\text{VO}_2$  peak ( $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) at baseline ( $22.01\pm1.12$ ) increased by ~9% after 12 weeks ( $24.34\pm1.36$ ) in the HT group. Similarly, N-HT women increased  $\text{VO}_2$  peak ~14% after 12 weeks from  $21.06\pm1.72$  to  $23.45\pm1.48$ , matching the HT group's rate of increase. Similar changes were observed for the VT ( $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ), which increased significantly ( $P<0.05$ ) within, but not between, groups. The HT group ( $13.10\pm0.46$ ) increased ~9% after 12 weeks ( $14.96\pm0.97$ ), with corresponding VT values for the N-HT group being  $11.90\pm1.31$  to  $12.17\pm1.09$ , representing an ~5% increase. These increases were independent of HT status.

Peak HR, peak MAP, and VT HR were not significantly different ( $P>0.05$ ) within or between groups at baseline or after 12 weeks of training. Peak and VT workload (watts) were also similar ( $P>0.05$ ) at baseline and after 12 weeks of training.

*Peripheral Vascular Function:* Resting and peak-ischemic BF responses were similar ( $P>0.05$ ) at baseline. Increases in peak-ischemic BF failed to reach statistical significance after exercise training ( $P=0.09$ ) and was not influenced by HT ( $P=0.43$ ). Similarly, both groups demonstrated comparable ( $P>0.05$ ) resting MAP and G at baseline, without any alteration after training. Baseline peak-ischemic vascular resistance was also similar ( $P>0.05$ ) between groups, with peak-ischemic G increasing significantly ( $P<0.05$ ) within groups after training ( $0.39\pm0.03$  to  $0.46\pm0.03$  in HT, and  $0.37\pm0.03$  to  $0.39\pm0.04$  N-HT; Figure 2). Using pooled data from both groups, improvements in peak-ischemic G showed a significant effect of exercise ( $P=0.032$ ), but not for HT ( $P=0.213$ ).

## DISCUSSION

In the current study we demonstrate that 12 weeks of moderate aerobic exercise training is equally effective in conferring beneficial central and peripheral cardiovascular adaptations in users and non-users of short-term HT in healthy PMW. Post training we observed decreased oxygen consumption and HR during each submaximal exercise workload, but SV, cardiac output and TPR were unaltered in both groups.  $\text{VO}_2$  peak and oxygen consumption at the VT were also increased in both groups. The gains in  $\text{VO}_2$  peak occurred in the absence of central (cardiac) adaptation(s), although modest improvements in peripheral hemodynamics were observed. Collectively, our findings suggest that a short-term endurance exercise intervention in healthy PMW elicits similarly favorable cardiovascular health gains in users and non-users of short-term HT.

Increased  $\text{VO}_2$  peak in response to three months of aerobic training is consistent with the widely-known physiologic adaptations seen with regular endurance exercise.



Despite the well documented vasoregulatory effects of estrogen, HT did not confer a greater response. This is, in part, in contrast with evidence supporting an effect of HT on aerobic capacity in sedentary PMW.<sup>32</sup> It is possible, however, that the net effect of exercise training may be sufficient to overwhelm any subtle effects conferred by HT. Our observed exercise training-dependent increase in  $\text{VO}_2$  peak ( $\sim 9\%$ ) was similar to that reported by Lindheim and colleagues<sup>33</sup> after 6 months of endurance training in PMW (range = 42-59 years) with and without HT, training 3 days a week for 30-mins at 70% HR peak. Others have also reported no effect of varying hormonal preparations and dosages of HT on  $\text{VO}_2$  peak after endurance training.<sup>22, 34-36</sup> Similarly, studies demonstrate no effect of short-term HT alone on peak oxygen uptake in healthy PMW not undertaking exercise training.<sup>24, 37, 38</sup> Collectively, these data and ours support little or no effect of HT on peak oxygen uptake, irrespective of exercise training.

Our findings indicate that HT does not influence measures of SV, Q, TPR or calculated arterio-venous oxygen difference during submaximal exercise in healthy PMW undergoing short-term endurance training. These findings are consistent with cross-sectional data reporting that long-term ( $>2$  years) HT fails to influence SV, Q, HR, TPR and arterio-venous oxygen difference across a range (40, 60 and 80%  $\text{VO}_2$  peak) of submaximal exercise intensities among age-matched older ( $\sim 63$  years) sedentary, regularly physically active and athletic PMW.<sup>36</sup> Similarly, others demonstrate that long-term ( $>1$  year) HT is not associated with alterations in submaximal (60% of  $\text{VO}_2$  peak) SV, Q, and arterio-venous oxygen difference after 20 weeks of aerobic training (55-75% of  $\text{VO}_2$  peak) in healthy young ( $\sim 55$  years) PMW.<sup>22</sup> These data, and ours, suggest that

HT both short- and long-term, does not alter central hemodynamic responses during submaximal exercise in aerobically trained and untrained PMW.

Given our failure to detect any influence of HT on central hemodynamic responses, we collapsed the data to compare the exercise training-dependent effects with other studies in PMW. Consistent with an effect of regular aerobic training, we observed a decrease in  $\text{VO}_2$  and HR at each exercise intensity workload after 12 weeks of training. However, we did not observe changes in Q, SV, MAP, TPR or arterio-venous difference. These changes are both consistent<sup>39, 40</sup> and inconsistent<sup>22, 36</sup> with previous studies. For example, Spina and colleagues<sup>40</sup> reported no change in submaximal SV in conjunction with an increase in arterio-venous content difference in healthy older PMW ( $63 \pm 4$  years) after nine months of endurance training. Conversely, after 20 weeks of endurance training in younger ( $\sim 55$  years) PMW, Green et al.,<sup>22</sup> observed a significant increase in SV reported at 60% of  $\text{VO}_2$  peak and at 50W. This group<sup>22</sup> also reported significant decreases in submaximal  $\text{VO}_2$ , HR and Q. In athletic, but not recreationally physically active PMW, McCole and colleagues<sup>36</sup> reported exercise training dependent gains in  $\text{VO}_2$ , Q, and SV. While the interaction between exercise training duration and exercise intensity on submaximal central adaptations are unclear, these studies suggest that improvements in central hemodynamic responses during exercise are greatly effected by exercise intensity, with higher intensity mediating greater improvements.

Despite evidence that HT has been shown to restore peripheral BF,<sup>24, 41</sup> vascular conductance,<sup>24, 41</sup> endothelium-dependent vasodilation<sup>11</sup> and total peripheral resistance<sup>42, 43</sup> in estrogen deficient PMW, we detected only modest non-significant gains in resting and peak-ischemic calf blood flow, and TPR. It is possible that our discordant findings

may be due to the attenuation of estrogen-mediated vasodilation by progesterone.<sup>44</sup>

Indeed, this could be an important factor in other studies reporting little or no effect of HT on submaximal hemodynamics. However, since we positioned testing at the farthest possible time-point from the progesterone administration (3 weeks), it is unlikely to have impacted our results.

The effects of aerobic exercise training on peripheral BF in PMW are unclear. We observed no significant effect of exercise training on BF. This is, in part, comparable with studies reporting no change in resting BF after 31 weeks of a walk/jog program at 70-90% of  $\text{VO}_2$  peak for 45 minutes, 3-5 days per week in older ( $65 \pm 3$  years) healthy PMW.<sup>45</sup> Conversely, peak ischemic blood flow has been shown to increase  $\sim 18\%$ <sup>45</sup> and  $\sim 40\%$ <sup>46</sup> in response to 32 weeks of endurance training. The greater increases in peak-ischemic blood flow reported by Martin et al.,<sup>45</sup> compared to that found in the current study is likely due to the longer and more intense training program.

### *Limitations*

Our sample size is small, and as such, we may be underpowered to detect subtle differences between groups. Consequently, our findings should be interpreted with caution. The exclusion of an HT-only group in the present study also meant we could not examine the independent effects of short-term HT alone on central and peripheral function. It is possible our low-dose hormone regimen may not have been sufficient to confer improvement to central and peripheral function. Alternative hormonal preparations, dosages and/or duration of treatment may have impacted our parameters of interest differently. Of interest, recent studies have also indicated underlying vascular

changes among women reporting hot flashes.<sup>47</sup> Since we did not document whether our PMW were experiencing vaso-motor symptoms we were not able to determine the effects of hot flashes on our cardiovascular measures. Finally, although frequency, distance walked and exercise intensity were not reported to be different between the groups, absence of full supervision of the exercise training sessions may have also have affected our findings.

### *Conclusions*

This study demonstrates that 12 weeks of aerobic training at a moderate intensity is equally effective in conferring increases to  $\text{VO}_2$  peak and  $\text{VO}_2$  at VT in users and non-users of short-term HT in healthy PMW. This finding suggests that in the absence of disease, short-term HT does not influence peripheral or central cardiovascular adaptations. Further randomized controlled studies examining the long-term effects of HT and endurance training in women who have recently undergone menopause compared with those who are long-after the menopausal transition will help clarify the potentially independent and combined effects of these interventions in health and disease.

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## FIGURE LEGENDS

**Figure 1.** Oxygen uptake at VT (a) and VO<sub>2</sub> peak (b) in the study groups before and after three months of aerobic training. \* $P<0.05$  from baseline within groups. No significant differences were detected between groups for VT and VO<sub>2</sub> peak pre- and post-exercise training.

**Figure 2.** Peak ischemic vascular conductance (a) and calf blood flow (b) in the study groups before and after three months of aerobic training. \*  $P<0.05$  from baseline within both groups. §  $P=0.056$  within the group. No differences were observed between groups.



**Table 1.** Baseline characteristics of the study groups.

	<b>HT</b>	<b>N-HT</b>
	<b>n = 11</b>	<b>n = 12</b>
<b>Age (years)</b>	55 ± 1	58 ± 2
<b>Height (cm)</b>	162.6 ± 2.5	161.5 ± 1.3
<b>Weight (kg)</b>	72.7 ± 4.7	71.8 ± 4.2
<b>Waist (cm)</b>	84.6 ± 3.1	87.8 ± 3.2
<b>Body Fat (%)</b>	37.6 ± 1.9	38.7 ± 1.2
<b>LBM (kg)</b>	44.6 ± 1.9	43.5 ± 1.9
<b>FM (kg)</b>	28.2 ± 2.9	28.2 ± 2.4
<b>BMI (kg/m<sup>2</sup>)</b>	27.5 ± 1.7	27.5 ± 1.5

Values are mean ± SEM.

LBM, lean body mass; FM, fat mass; BMI, body mass index.

**Table 2.** Baseline serum hormone levels of the study groups.

	<b>HT</b>	<b>N-HT</b>
	<b>n = 11</b>	<b>n = 12</b>
<b>E<sub>2</sub> (pmol/L)</b>	43.6 ± 3.5	40.1 ± 0.7
<b>Progesterone (nmol/L)</b>	0.3 ± 0.1	0.2 ± 0.1
<b>SHBG (nmol/L)</b>	37.9 ± 3.6	46.9 ± 5.9
<b>Testosterone (nmol/L)</b>	0.5 ± 0.1	0.6 ± 0.1
<b>Free Androgen Index</b>	1.5 ± 0.2	1.4 ± 0.2
<b>FSH (IU/L)</b>	83.4 ± 4.1	91.9 ± 4.7

Values are mean ± SEM.

E<sub>2</sub>, estradiol; SHBG, sex-hormone binding globulin; FSH, follicle stimulating hormone.

**Table 3.** Submaximal cardiovascular responses to two different cycle ergometer workloads for HT and N-HT at baseline and after 3 months of aerobic exercise training.

	HT (n=11)		N-HT (n=12)	
	Baseline	Post-Training	Baseline	Post-Training
<b>30W</b>				
<b>VO<sub>2</sub> (L/min)</b>	0.69 ± 0.03	0.63 ± 0.02*	0.67 ± 0.03	0.65 ± 0.03*
<b>Cardiac Output (L/min)</b>	6.52 ± 0.40	6.27 ± 0.33	6.60 ± 0.22	6.59 ± 0.44
<b>HR (beats/min)</b>	109 ± 4	103 ± 3*	106 ± 4	102 ± 3*
<b>SV (ml)</b>	61.00 ± 5.66	61.69 ± 3.98	63.08 ± 2.63	64.88 ± 3.65
<b>MAP (mmHg)</b>	100 ± 3	96 ± 4	102 ± 3	103 ± 4
<b>TPR (dynes/s/cm<sup>-5</sup>)</b>	15.89 ± 1.03	15.68 ± 0.73	15.55 ± 0.67	16.12 ± 0.91
<b>a-v O<sub>2</sub> difference</b>	108.07 ± 5.53	101.50 ± 3.04	102.38 ± 3.87	100.31 ± 3.36
<b>60W</b>				
<b>VO<sub>2</sub> (L/min)</b>	1.01 ± 0.04	0.95 ± 0.03*	0.99 ± 0.04	0.96 ± 0.04*
<b>Cardiac Output (L/min)</b>	8.31 ± 0.35	8.16 ± 0.28	8.62 ± 0.29	8.84 ± 0.47
<b>HR (beats/min)</b>	128 ± 4	121 ± 4*	124 ± 3	118 ± 3*
<b>SV (ml)</b>	65.97 ± 4.23	68.36 ± 3.35	70.32 ± 3.13	74.41 ± 2.75
<b>MAP (mmHg)</b>	112 ± 4	112 ± 3	120 ± 3	118 ± 4
<b>TPR (dynes/s/cm<sup>-5</sup>)</b>	13.59 ± 0.49	13.82 ± 0.53	14.12 ± 0.50	13.59 ± 0.48
<b>a-v O<sub>2</sub> difference</b>	121.74 ± 3.27	117.25 ± 3.71	115.15 ± 3.57	110.70 ± 5.24

All values are mean ± SEM.

VO<sub>2</sub>, oxygen consumption; HR, heart rate; SV, stroke volume; MAP, mean arterial pressure; TPR, total peripheral resistance. \*  $P < 0.05$  compared to baseline within groups. HT did not influence any cardiovascular measure. There were no differences between groups.

Figure 1

Figure 1.

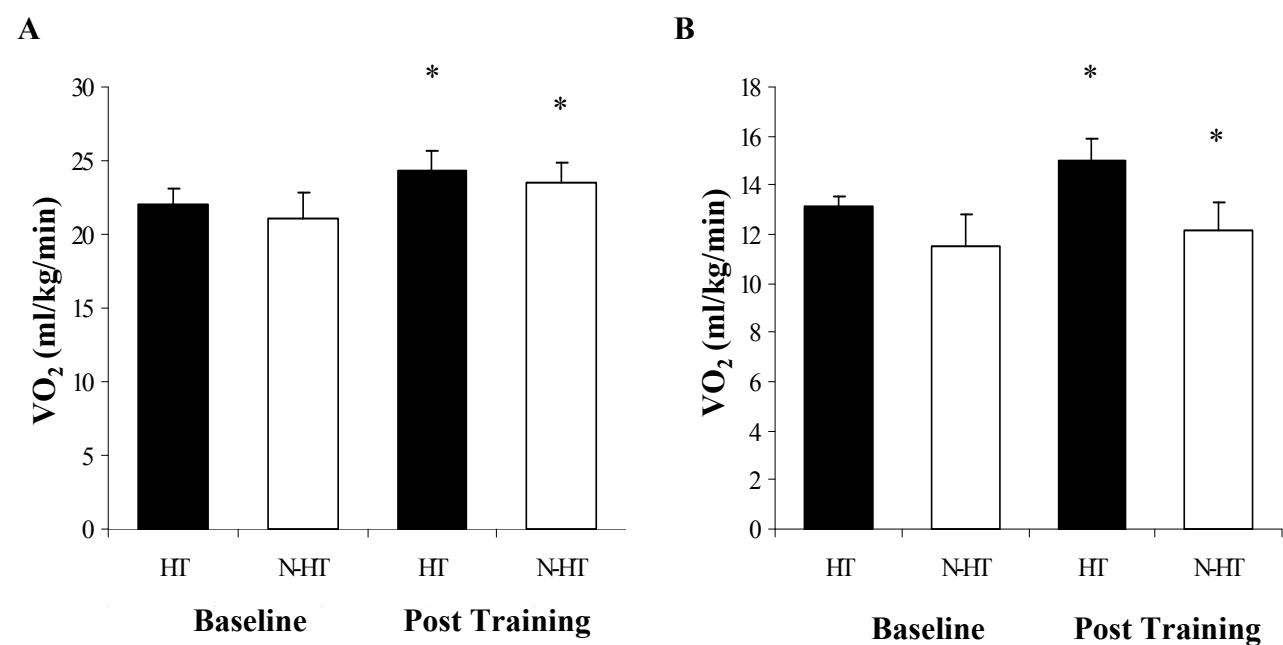
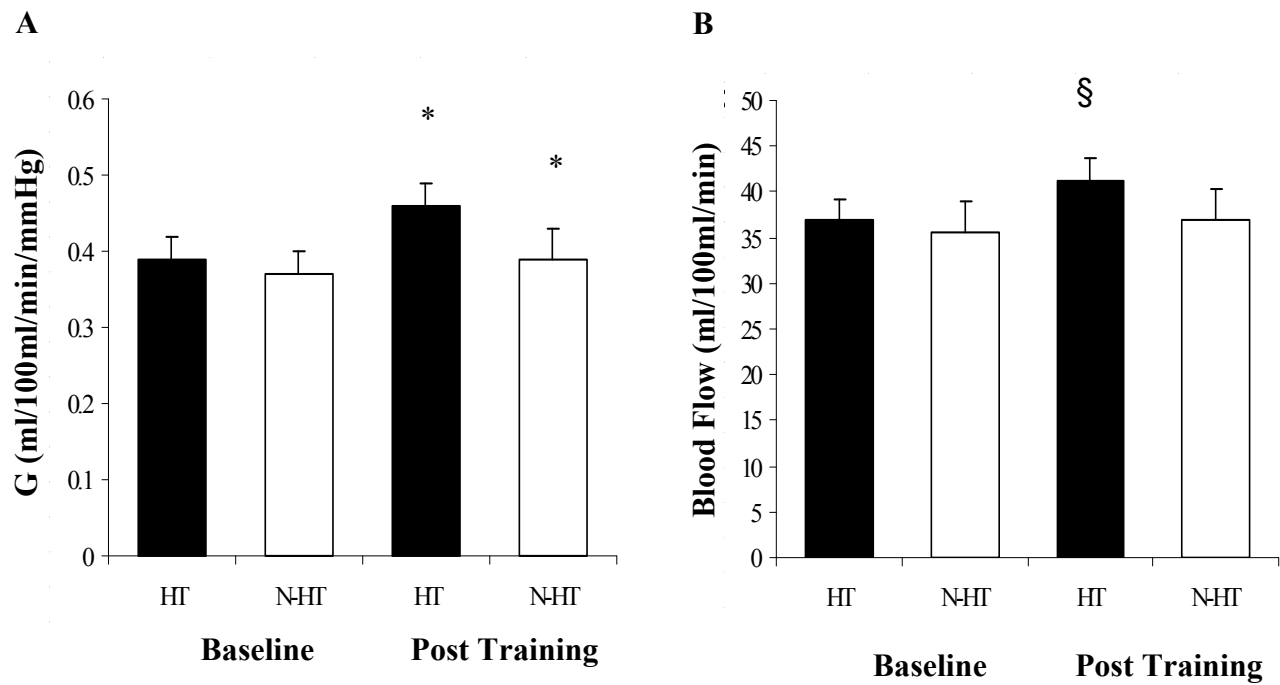


Figure 2

Figure 2.



Isaac Schiff, MD  
*Menopause* Editor-in-Chief  
Chief, Vincent Memorial Obstetrics and Gynecology Service  
The Women's Care Division  
Massachusetts General Hospital  
55 Fruit Street, VBK-113  
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November 30, 2008

Dear Dr Schiff:

Thank for your email and reviewer comments regarding our paper 'Aerobic Exercise Training in Healthy Postmenopausal Women: Effects of Hormone Therapy'. We have attempted to address each concern individually and have amended the manuscript accordingly. Please see below our responses to each reviewer. We look forward to receiving your feedback.

Yours sincerely,  
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### **Reviewer 1**

#### ***General Comments to Authors***

The study by O'Donnell et al compared a small group of PMW who underwent aerobic training for 12 weeks with another small group who were randomly put on HT as well. The investigators measured or calculated a long list of parameters related to exercise and cardio-respiratory function. It seems that the study was carefully planned and executed and the results are of interest. However, my philosophy is that an article should serve the readers rather than the investigators. If this article would have been submitted to a cardiology journal, the following comment would not have to be made. However, the format and phrasing of a manuscript sent to a journal designated for a general readership should be adapted accordingly. Most of Menopause readership would find such article too long, too detailed, and perhaps too difficult to understand.

#### ***Authors Response to General Comments***

We thank the reviewer for his/her comments. In order to facilitate greater understanding for the readership of Menopause, we have modified the methods and relevant aspects of the discussion to amplify the message regarding cardiovascular function and health. Specifically, the length, phrasing and formatting of the manuscript have been adjusted for

the general readership as the reviewer suggests. We trust this now reflects a balanced and relevant approach.

### ***Comments to Author***

1) there is a growing body of evidence suggesting that the cardio-vascular reactions to HT may differ among flushers and non-flushers. In a small group of examinees, this might be a limitation since there is no information on the percentage of flushers. Additionally, there might be a difference between women with an intact uterus and those who had surgical menopause (eliminating also a major source of androgen production).

### ***Authors Response***

At the time of data collection, the clinical significance of hot flashes was, and remains today, related largely to their impact on quality of life rather than an association with physical health risk (Thurston et al., 2008). As such, we did not deem collection of such data from our participants necessary in order to answer our research hypotheses. More recently, however, research indicates underlying vascular changes among women reporting hot flashes. Using data from the WHI study, Rossouw and colleagues (2007) report that the greatest coronary heart disease risk associated with HT was concentrated among older women at baseline reporting moderate to severe vasomotor symptoms. As such, we appreciate that hot flashes may have impacted our findings, however, we are unable to address this concern in the manuscript beyond acknowledging this as a limitation. As such, the following text has been added to p17, under Limitations: 'Recent studies indicate underlying vascular changes among women reporting hot flashes.<sup>18</sup> Since we did not document whether our PMW were experiencing vaso-motor symptoms we were not able to determine the effects of hot flashes on our cardiovascular measures.'

In response to the comment regarding differences in cardiovascular responses between women with an intact uterus and those who had surgical menopause, we acknowledge that this may also have impacted our results. Indeed, in naturally menopausal women, ovarian hormone biosynthesis declines progressively, providing low circulating levels of estrogens and androgens. In surgically menopausal woman, estrogen and androgen levels are rapidly and significantly reduced. However, since we demonstrate similarly low levels of estrogen, progesterone, testosterone, and SHBG at baseline in both groups of women, it is reasonable to postulate that the effects of natural versus surgical menopause had little or no effect on our findings.

Thurston RC, Bromberger JT, Joffe H, Avis NE, Hess R, Crandall CJ, Chang Y, Green R, Matthews KA. Beyond frequency: who is most bothered by vasomotor symptoms? Menopause. 2008 Sep-Oct;15(5):841-7

Rossouw JE, Prentice RL, Manson JE, Wu L, Barad D, Barnabei VM, Ko M, LaCroix AZ, Margolis KL, Stefanick ML. Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. JAMA. 2007; 297: 1465-1477

### ***Comments to Author***

2) Dosage of estrogen and estrogen-progestagen combination may be important. 1 mg estradiol was used, which is a low dose, and some of the previous studies quoted

probably used other hormonal preparations or other dosages. This should be mentioned in the Discussion. In page 7, upper sentence, the time relation between the use of progestagen and the performance of exercise is not so clear (in the Discussion it is explained in a better way [page 16]). By the way, what is "micropogesterone"? is it micronized progesterone?

#### *Authors Response*

The reviewer is correct in identifying that comparable previous studies looking at similar cardiovascular outcomes did indeed use varying hormonal preparations at differing doses compared to that used in the current study. To highlight this, in the Discussion, under the section entitled 'HT and Exercise Training Influences on Maximal Oxygen Uptake', we have amended our manuscript to read: 'Others have also reported no effect of varying hormonal preparations and dosages of HT on VO<sub>2</sub> peak after endurance training.<sup>23, 33-35,</sup> We have also added the following to the Limitations section (p17): 'It is possible our chosen hormone regimen may not have conferred optimal benefit to central and peripheral function. Alternative hormonal preparations, dosages and/or duration of treatment may have impacted our parameters of interest differently.'

In page 7, the time relation between use of progestin and exercise testing has been reworded to clarify this to the reader. In the Methods section, under 'Randomization' (p7), the manuscript has been amended to read: 'This medication phase was positioned at approximately 3 weeks, after each estradiol treatment, for a total of three months. To minimize the effects of the progesterone, post-HT cardiovascular measures were obtained during the very last week of estradiol treatment.'

The progesterone used in the current study was indeed a micronized progesterone formula (Prometrium). We have amended the term 'micropogesterone' to read 'micronized progesterone'.

#### *Comments to Author*

3) If one looks at the baseline characteristics of the study group, the standard deviations seem very narrow for a small group. Same goes for the cardiovascular reactions. There should have been a higher variance in age, height, BMI, etc if women were randomly selected.

#### *Authors Response*

The variance reported in the baseline characteristics of the study groups are standard error of the mean rather than standard deviation. As such, the variance appears low. For example, age of the N-HT study group is reported as  $58 \pm 2$  yrs (mean  $\pm$  SEM). If reported as mean  $\pm$  SD, the values would appear as  $58 \pm 6$  yrs.



### ***Comments to Author***

4) Results show only the comparison of baseline versus post-training values at 30W, 45W, 60W. It would be interesting to see the resting results as well.

### ***Authors Response***

The authors thank the reviewer for their comment. However, since the novelty of the study is centred around the effects of HT on submaximal cardiovascular hemodynamics, resting data for the cardiovascular measures were not reported. Further, we (Kirwan et al., 2004) and others (Snabes et al., 1997) have previously demonstrated no effect of short-term HT on resting indices of cardiovascular function in healthy postmenopausal women. As such, resting data was deemed unnecessarily repetitive and inconsistent with the focus of the current research hypotheses, and respectfully suggest its inclusion would not really add to the message.

Kirwan LD, MacLusky NJ, Shapiro HM, Abramson BL, Thomas SG, Goodman JM. Acute and chronic effects of hormone replacement therapy on the cardiovascular system in healthy postmenopausal women. *J Clin Endocrinol Metab.* 2004 Apr;89(4):1618-29

Snabes MC, Payne JP, Kopelen HA, Dunn JK, Young RL, Zoghbi WA. Physiologic estradiol replacement therapy and cardiac structure and function in normal postmenopausal women: a randomized, double-blind, placebo-controlled, crossover trial. *Obstet Gynecol.* 1997 Mar;89(3):332-9

### ***Comments to Author***

5) Following the comments made by the investigators in the Limitations section, perhaps the consequences of acute and chronic hormone use on exercise parameters should be mentioned too.

### ***Authors Response***

We have added the following to the Limitations section so as to mention the potential consequences of acute versus chronic HT on exercise parameters: 'It is also important to note that different hormonal preparations, dosages and/or duration of treatment may have impacted our exercise parameters differently to that observed in the current study.'

### ***Comments to Author***

6) A minor comment: authors should carefully check the references: for example, ref. 20 is not completed; ref 1+22 - JAMA, rather than Jama. Page 13, Discussion, first line should read aerobic exercise is equally .

### ***Authors Response***

We thank the reviewer for reporting these referencing errors. The references have been checked and have been amended where necessary.

In response to the Discussion comment, the authors believe it is important to be specific and state the type and duration of training that affected our results.

## **Reviewer 2**

### ***General Comments to Author***

The authors compared the effects of a 12-week endurance training program (walking 5 days per week at 70-80 % peak heart rate) on submaximal cardiovascular responses in healthy overweight and sedentary postmenopausal women being on vs. off hormone therapy (HT). The main focus of this paper was to verify whether HT users confers better improvements to central and peripheral measures of cardiovascular function compared to non users. Results show i) decreases in oxygen consumption and heart rate but unaltered stroke volume, cardiac output and total peripheral resistance, and ii) increases in peak oxygen uptake and oxygen consumption at the ventilatory threshold, during submaximal exercise performed on cycle ergometer, in both groups. The main conclusion of the present study is that short term HT use does not seem to influence cardiovascular responses after 12 weeks of aerobic training. Data presented in this manuscript are of great interest in the field of cardiovascular adaptations to physical exercise.

### ***Authors Response to General Comments***

We thank the reviewer for their thoughtful comments.

### ***Comments to Author***

Although stated as a limit of the study (p 17), this reviewer has some concerns about the lack of a non exercising group on HT which will allow the authors to conclude on the potential effects of HT use (even at short term) alone on the cardiovascular function of postmenopausal women.

### ***Authors Response***

Indeed, as acknowledged in the Limitations section of the manuscript, in the absence of a HT group only we were not able to determine whether submaximal cardiovascular hemodynamics were impacted independent of exercise training. Our decision to exclude this group in the current study was based on our previous work demonstrating unaltered central cardiovascular function in response to 12 weeks of HT in healthy postmenopausal women (Kirwan et al., 2004). To further explain our reason for not including a HT-only group in the current study, we have added the following to the end of the Study Population section: 'Since we have previously demonstrated unaltered central cardiovascular function during submaximal aerobic exercise in response to 12 weeks of HT alone in healthy PMW,<sup>12</sup> we did not examine the independent effects of HT in the current study.'

Kirwan LD, MacLusky NJ, Shapiro HM, Abramson BL, Thomas SG, Goodman JM. Acute and chronic effects of hormone replacement therapy on the cardiovascular system in healthy postmenopausal women. J Clin Endocrinol Metab. 2004 Apr;89(4):1618-29

### ***Comments to Author***

According to this referee, data recorded at 45 W are not necessary (Table 3) as they did not add substantial information on the outcome variables of this study.

#### *Authors Response*

Data recorded at 45W have been removed from Table 3.

#### ***Comments to Author***

This reviewer has also some concerns concerning compliance to the aerobic training program as it was apparently not under supervision (p 10). This issue should be discussed as another limit of the study.

#### *Authors Response*

Exercise training sessions were supervised (at least) once per week only. To address this concern the following has been added to the Limitations section of the manuscript:

‘Although frequency, distance walked and exercise intensity were not reported to be different between the groups, absence of full supervision of the exercise training sessions may also have affected our findings.’

#### **Reviewer 3**

#### ***Comments to Author***

Statistical review: In a randomized trial, the appropriate analyses are intention to treat. The 14 subjects who dropped out of the study should be included in at least some analyses. Also, of the 14 subjects who dropped out, please state how many were on HT and how many on placebo.

#### *Authors Response*

Whilst the divide between HT and placebo dropouts were similar (n= 8, n=6, respectively), we followed conventional methods used in most exercise-intervention studies which does not use the intention-to-treat principle. We certainly agree that an intent-to-treat design is a powerful approach when, for example, an effective treatment arrests progression of disease during its administration. Thus, a patient benefits long after the patient becomes noncompliant or the treatment is terminated. Therefore, in this example, the observations from the last patient evaluation are advantageous. However, our primary question was whether or not HT influenced the exercise training response, with exercise training being the primary intervention. Since most dropouts from training studies (and ours) are not due to the intervention *per se*, there is little bias contributed from a dropout, mostly due to the fact that the primary endpoints of ‘exercise adaptation’ are not realized (e.g. increased VO2max) without it. Moreover, in the present study, dropouts occurred within the first 1/3 of the study and were related to lack of time/commitment (n=6 exercise, n=5 placebo) and injury unrelated to exercise (n=2 in exercise group, n=1 placebo group), further limiting any possible carry-over effect. Given that the dropouts were a) similar in number and cause in both groups, b) occurred very early in the training program, and c) a failure to exercise would preclude the primary treatment effect, we are confident our statistical analysis did not introduce unwarranted bias in general, and increase the chance of committing a type 1 error, in particular.

### ***Comments to Author***

Please include in the Methods a description of how the appropriate sample size was chosen, and the statistical power to detect effects.

### ***Authors Response***

A description of how the appropriate sample size was chosen and the statistical power to detect effects has been added to the Methods section, as follows: ‘The following calculation was used to determine the number of observations needed to provide an 80% probability of finding differences of 1.5 standard deviations in two-sample tests confirming the effects of HT and exercise at a significance level of 0.05 level (Diamond, 1981):

$$n = 2 * (t_{\alpha=0.05}^{df=21} + t_{\beta=0.20}^{df=21})^2 * (SD/d)^2 = 2 * (2.08 + 1.32)^2 * (1/1.5)^2 = 10'$$

**Diamond WJ** 1981 Practical experiment designs for engineers and scientists. Belmont, CA: Lifetime Learning Publications

### ***Comments to Author***

A repeated measures ANOVA would provide more power than the t-tests used in the analyses presented.

### ***Authors Response***

The authors acknowledge that a repeated measures ANOVA would provide more power than multiple t-tests using Bonferroni correction. However, we had decided that that in light of this being an interventional study, particularly a drug interventional study, we were advised to use a more conservative method of analyses. As such, *t*-tests using a Bonferroni correction was chosen.

### ***Comments to Author***

The authors appropriately discuss the small sample size as a study limitation. However, their conclusions are fairly definitive, considering the small sample size. Please revise the study conclusions to be much more cautious, and please do not make any clinical recommendations based on this small study.

### ***Authors Response***

The study conclusion has been amended accordingly to be more cautious and to remove any clinical recommendations. Incidentally, training studies examining dose-response with, or without control groups are commonly reported using samples of this size based upon the aforementioned sample-size determination. Although larger samples are obviously preferred, we and many others would suggest that training studies are more challenging to complete than drug-intervention studies given recruitment and compliance issues.

**\*Copyright Transfer Form**

This piece of the submission is being sent via mail.