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The effect of short and long term aerobic training years on systemic O_2 utilization, and muscle and prefrontal cortex tissue oxygen extraction in young women

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Abstract

This study aimed to determine if systemic O_2 utilization ($\dot{V}O_2$) and tissue oxygen extraction (deoxyhemoglobin [HHb]) in the *vastus lateralis* (VL), *gastrocnemius* (GAST) and pre-frontal cortex (PFC) were different during exercise between short-term trained (STT 6 - 24 months) and long-term trained (LTT > 5 yr) young women while controlling for current training load. Thirteen STT and 13 LTT participants completed ramp incremental (RI) and square-wave constant load (SWCL) tests on a cycle ergometer. In LTT compared to STT: (i) $\dot{V}O_2$ was higher during the RI ($p = 0.024$) and SWCL ($p = 0.001$) tests; (ii) HHb in the VL ($p = 0.044$) and GAST ($p = 0.027$) was higher in the RI test; and (iii) there were significant group x intensity interactions for $\dot{V}O_2$ in the SWCL test. The additional years of aerobic training in LTT compared to STT (LTT 7.1 ± 1.9 vs STT 1.5 ± 0.4 yr) resulted in higher $\dot{V}O_2$, and HHb in the VL and GAST. These results indicate that in young women, independent of current training load, systemic $\dot{V}O_2$ and peripheral muscle O_2 extraction during exercise continues to increase beyond 24 months of aerobic training.

Key words: Peak oxygen consumption, tissue oxygenation, female.

Introduction

In adults, irrespective of age and sex, aerobic training is strongly associated with improved peak oxygen consumption ($\dot{V}O_{2peak}$) (8, 35). A strong dose-response relationship exists for improved $\dot{V}O_{2peak}$ (to a maximum level) and the volume (intensity, duration and frequency) and length (weeks) of the training intervention (23, 29). However, following initial rapid increases in $\dot{V}O_{2peak}$, the improvements plateau within 12 - 24 months of commencing aerobic training (2, 29, 32), and this usually occurs earlier in women than men (14, 28). Longitudinal studies beyond 24 months are limited to those investigating age-related decreases in $\dot{V}O_{2peak}$ (17, 43, 49). Although the $\dot{V}O_{2peak}$ of long-term trained and age matched-untrained individuals has been compared (22), the difference in $\dot{V}O_{2peak}$ between those that have gained the significant initial increases in $\dot{V}O_{2peak}$ with short-term aerobic training (12 - 24 months) and those that have been training for many years is limited to a single study of older (40 - 60 yr) women (11).

Central adaptations leading to increased maximum cardiac output (e.g. blood volume, heart function or structure) and peripheral adaptations that improve O_2 extraction and widen arterio-venous O_2 difference (e.g. blood flow distribution) are responsible for increases in $\dot{V}O_{2peak}$ from aerobic training (28, 29). The relative contribution that each component has on improving $\dot{V}O_{2peak}$ varies with age, sex and training volume (35). In young women (< 30 yr), the time course of specific mechanisms responsible for improvements in $\dot{V}O_{2peak}$ with aerobic training are poorly understood (14, 28, 36, 43, 48). There is considerable debate about the relative contribution of central (14, 48) and peripheral (36) adaptations on the initial improvements (9-12 weeks) in $\dot{V}O_{2peak.}$ With longer-term training interventions (~12 months) improvements in $\dot{V}O_{2peak}$ are reported to result primarily from peripheral adaptations alone (14) or in combination with central adaptations (28).

Peripheral adaptations resulting in improved muscle O_2 extraction are essential in improving $\dot{V}O_{2peak}$, as up to 90% of arterial O_2 is utilized at the muscle mitochondria during high intensity exercise (6, 25). Near-Infrared Spectroscopy (NIRS) provides an indirect non-invasive method to monitor changes in deoxyhemoglobin (HHb) and oxyhemoglobin (O_2Hb) in human tissue during exercise (5, 18). The HHb response is posed to reflect arterio-venous O_2 difference and thus microvascular O_2 extraction (15, 21). This parameter represents the dynamic balance between O_2 delivery and O_2 extraction at the muscle site and has previously been used to highlight the effect of aerobic training on peak muscle O_2 extraction during exercise in older women (11, 16) and young and older men (22, 37). However, in young women while proposed training induced improvements in muscle tissue oxygenation (VO_2: HHb) have been reported during the transition from low to moderate intensity exercise (39), the effect of aerobic training on peak muscle O_2 extraction is unknown. Furthermore, while studies of young men have reported changes in cerebral oxygenation (decreased O_2Hb and increased HHb) during high intensity exercise (45, 46), the cerebral oxygenation pattern in young women during high intensity exercise is unclear (41). As cerebral blood flow and oxygenation during exercise depend on cardiac output (26), which is lower in women compared to men (40), cerebral oxygen could differ between sexes.

The aims of this study were to simultaneously investigate and compare the exercise response of $\dot{V}O_2$, and HHb in the *vastus lateralis* (VL), *gastrocnemius* (GAST) and the pre-frontal cortex (PFC) between short-term aerobically trained (STT 6 - 24 months) and long-term aerobically trained (LTT > 5 years) young women matched for current training load. Participants performed ramp incremental (RI) and square-wave constant load (SWCL) (25%, 80% and 90% VT) cycling tests. It was hypothesized that: (i) $\dot{V}O_2$, and HHb in the VL and GAST would be higher in LTT compared to STT during both RI and SWCL cycling; and (ii), that there would be no difference in HHb in the PFC between the groups during the RI and SWCL tests.

Methods

Experimental Design

The study used a cross-sectional two group (STT and LTT) within subject repeated measures (exercise intensity during RI and SWCL) design. The independent variable was training status. The dependent variables were $\dot{V}O_2$, heart rate (HR), rating of perceived exertion (RPE) (Borg's 1-10 category-ratio [CR-10]) (7) and HHb in the VL, GAST and PFC. Participants' current training load was not statistically or practically different between groups (Table 1).

Each participant completed two testing sessions in a temperature controlled (20 - 23 ^{o}C) exercise physiology laboratory. Prior to testing, participants were required to abstain from alcohol and intense exercise for 24 hours and caffeine and food for four hours. As menstrual cycle phases have no significant effect on $\dot{V}O_{2peak}$ (31, 50) the timing of assessments was not aligned with the menstrual cycle.

Participants

The two participant groups of young (18 - 30 yr) Caucasian women consisted of one group of 13 short-term trained (STT 6 - 24 months) women and one group of 13 long-term trained (LTT > 5 yr) women. Participants' physical characteristics and training history are presented in Table 1. To be eligible for this study, participants were to have been regularly performing > 150 min (refer to Table 1) of moderate to vigorous aerobic training per week (mandatorily including a cycling component) for a minimum of 11 months per year (as defined from self-reported physical activity training logs). Current training status (volume and exercise intensity) is a primary factor influencing $\dot{V}O_2$ and HHb during exercise with $\dot{V}O_{2peak}$ and

HHb being higher in trained than untrained individuals (22, 29, 51). This considered, current training load (the sum of the product of each training session duration (min) and intensity [1 = low, 2 = moderate, 3 = high]) was not significantly different between LTT and STT in the current study. Furthermore, all participants reported their current training load as typical of their training during the previous six months. Pre-study screening included a Physical Activity Readiness Questionnaire (9) and a Medical Health Questionnaire. Exclusion criteria included any health or medical related issues that would affect participant safety and medications that would affect exercise capacity or O_2 extraction. This study was approved by the Human Research Ethics Committee at the University of the Sunshine Coast (S/14/676) and participants provided written informed consent.

Procedures

Session One

The aim of session one was to determine participant characteristics and $\dot{V}O_2$, HR, RPE (Table 2) and HHb in the VL, GAST and PFC during a RI (increments = 1 watt every 3 s) test to exhaustion on a Velotron cycle ergometer (Racermate, Seattle, USA). Anthropometric, pulmonary function, HR and RPE data were recorded using standard measures as previously described (11). To encourage maximum effort, participants received feedback and encouragement from the tester. Expired gas analysis (Parvo Medics, Sandy UT, USA) was used to determine $\dot{V}O_{2peak}$ and VT, with $\dot{V}O_{2peak}$ determined as the highest 15 s average $\dot{V}O_2$ value within the last minute of the RI exercise test and VT determined using the V-slope method as described by Beaver et al. (3). Briefly, this method divides the analysis of CO_2 output to O_2 uptake ($\dot{V}CO_2$ - $\dot{V}O_2$) into two linear components. The intersection of these two points is described as the V-slope VT and expressed in terms of $\dot{V}O_2$, HR or power output (W). Compared to other methods, Gaskill et al. (20) found the V-slope method to be most accurate, with stronger correlations and smaller standard deviations. The onset of VT was aligned with power output (in watts) to determine the intensity for the subsequent SWCL test. As direct comparisons were not being made between the RI and SWCL tests or between systemic $\dot{V}O_2$ to muscle O_2 extraction (HHb) (i.e. $\dot{V}O_2$ to HHb ratio), $\dot{V}O_2$ was not left shifted to accommodate for phase I- phase II $\dot{V}O_2$ lag time.

Session Two

Session two was conducted three to 28 days after session one. The aim of session two was to determine participant's $\dot{V}O_2$, HR, RPE, and HHb in the VL, GAST and PFC during a SWCL exercise test. Each participant cycled at the same calculated relative intensity of 25%, 80%, 25% and 90% of VT power output obtained from the RI test. The timing and intensities for the SWCL test were: three min at 25%, 80%, 25%, 20 min at 90% and a further three min at 25% of VT.

$\dot{V}O_2$, HR, and HHb in the VL, GAST and PFC were recorded continuously during exercise while RPE was recorded within the last 10 s of the third min of each of the three min SWCL stages, and every fourth min within the 20 min stage. To minimize cognitive stimuli, no feedback or encouragement was provided during this test.

Tissue Deoxyhemoglobin

During exercise, HHb and O_2Hb data were measured continuously and simultaneously in the left VL and GAST and in the left PFC using a single-channel NIRS system (PortaMon and Portalite, Artinis Medical Systems BV, Zetten, Netherlands). To ensure measurement consistency, optode placement was referenced to anatomical landmarks described by others (10, 24, 33). Briefly, the optodes for the VL and GAST were placed over the mid-belly of the muscle ~10 - 15 cm above and 18 - 20 cm below the knee joint, respectively. The optodes were fixed over the skin using adhesive tape and wrapped with low compression black elastic bandage. The PFC optode was placed over the skin of the left PFC ~ 3 cm left of the midline and 1 - 2 cm above the eyebrow. The PFC optode was fixed using adhesive tape and covered with a black headband.

All NIRS primary data (HHb and O_2Hb) were recorded at 10 Hz. The last 20 s of resting values were averaged to obtain baseline values. All changes were then expressed relative to these baseline values and then calculated and displayed as follows: 15 s averages for RI; 30 s averages for SWCL; total average data for the 15 s preceding 90% VT and peak exercise for RI; and total data for each exercise intensity for SWCL. Compared to O_2Hb, HHb (the primary variable for the current study) is less affected by changes in blood hemodynamics (12, 19, 21) and is thus a better indicator of O_2 extraction. Therefore, in the current study, only HHb data have been presented.

Statistical Analysis

All statistical analyses were performed using SPSS (version 24, SPSS Inc., Chicago, IL). Prior to analysis data were checked for normality and that the relevant assumptions for each test were met. To identify the presence of any significant group (STT vs LTT) and exercise intensity main effects and/or interactions, while controlling for any between group training load differences, 2-way analysis of covariance (ANCOVA) were conducted on each dependent variable within each of the tests. For the RI, 2 (group: STT and LTT) x 2 (intensity: 90% of VT and peak) ANCOVAs were performed and for the SWCL, 2 (group: STT and LTT) x 3 (intensity: 25% [first bout at 25%] 80% and 90% of VT) ANCOVAs were performed. Due to the potential substantial effect of current training load on all the dependent variables, and the expected minor variations between participants, this variable was factored into the analysis as a covariant. For all analyses, the threshold for statistical significance was set to $p < 0.05$. Partial-eta squared was used to determine the effect size as small ($\eta_P^2 > 0.01$), medium ($\eta_P^2 > 0.06$) or large ($\eta_P^2 > 0.14$) as per Cohen (13).

Results

All variables had significant ($p < 0.05$) main effects for intensity, with all variables changing as expected with increasing exercise intensity (descriptive statistics are shown in Tables 2 and Table 3). Results for group main effects and group by intensity interactions are given in Table 4. There were significant ($p < 0.05$) group main effects for $\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$) and minute ventilation (V_E) during both the RI and SWCL tests. There were significant ($p < 0.05$) group by intensity interactions for $\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$) in the SWCL test, and V_E in both the RI and SWCL tests. The group mean HHb for the VL, GAST and PFC during the RI and SWCL tests are presented in Figure 1 and Figure 2. The difference in $\dot{V}O_2$ and V_E between groups increased with increased intensity. There were significant ($p < 0.05$) group main effects for HHb in the VL and GAST in the RI test, with HHb higher in LTT (Fig. 3 and 4).

Insert Table 1 here

Insert Table 2 here

Insert Table 3 here

Insert Table 4 here

Insert Figure 1 here

Insert Figure 2 here

Insert Figure 3 here

Insert Figure 4 here

Discussion

The findings of the current study support the hypothesis in that LTT was significantly higher than STT for: (i) $\dot{V}O_2$ during RI (Table 2) and at all intensities during SWCL (Table 3); (ii) HHb in the VL during RI and (iii) HHb in the GAST during RI.

Increases in $\dot{V}O_{2peak}$ associated with aerobic training can be related to improved O_2 delivery and/or O_2 extraction due to central or peripheral adaptations, respectively. For the young women in the current study, the higher HHb in the VL and GAST in LTT indicate that longer duration (> 24 months) aerobic training improves O_2 extraction in peripheral muscles during exercise. However, as cardiac output was not measured in the current study, it is not possible to determine what extent these peripheral adaptations contribute to the higher $\dot{V}O_{2peak}$ in LTT.

Systemic Oxygen Utilization

The $\dot{V}O_{2peak}$ values for the LTT and STT are consistent with the training experience reported by the participants (34). The higher $\dot{V}O_{2peak}$ of LTT compared to STT support the suggestion that a dose-response relationship exists between increases in $\dot{V}O_{2peak}$ and aerobic training duration (yr) (29, 51) but conflict with studies that suggest maximum increases in $\dot{V}O_{2peak}$ are achieved within 24 months of aerobic training (1, 14, 28). However, these variations could be explained by differences in training duration (years) as, except for one study that estimated changes in $\dot{V}O_{2peak}$ (from sub-maximal tests) (14), there have been no reports on the effect of long-term (years) training on gains in $\dot{V}O_{2peak}$. It is also possible that irrespective of the current and recent training load not being different between the two groups, participant training volumes (duration, intensity, frequency) in the months/ years prior to this study could have influenced the current results. We are not able to exclude the possibility that genetic determinants of trainability are stronger in LTT participants and potentially contribute to the sustained training habits of this group (8).

Concomitant to the higher $\dot{V}O_{2peak}$ in LTT, $\dot{V}O_2$ at VT was also significantly higher in LTT compared to the STT. However, when expressed as a percentage of $\dot{V}O_{2peak}$ the difference is abolished indicating that improvements in VT paralleled that of $\dot{V}O_{2peak}$. These results provide additional support that the current training programs between groups were similar, as some training methods such as high exercise intensity interval training increase VT as a percentage of $\dot{V}O_{2peak}$ (42).

For $\dot{V}O_2$ during SWCL, the results supported our hypothesis with $\dot{V}O_2$ being significantly higher in LTT compared to STT and the difference between groups increasing with exercise intensity (as indicated by the ANCOVA interaction) from 0.1 $L \cdot min^{-1}$ at 25%, to 0.3 $L \cdot min^{-1}$ 80%, and 0.4 $L \cdot min^{-1}$ at 90%. Similar results have been reported following aerobic training in older men (22) and women (11, 16) indicating that, irrespective of age and sex, aerobic training improves systemic O_2 utilization while exercising at constant loads below VT.

Ventilation, Heart Rate and Rating of Perceived Exertion

A previous study between STT and LTT older women reported differences in components of ventilation (V_E = tidal volume [V_T] x breathing frequency [BF]) between groups at peak exercise and during sub-VT intensity SWCL cycling (11). In contrast to that reported in older women (11), we found that ventilation during RI and SWCL exercise followed the expected response of healthy adults during exercise (47). Combined, these ventilatory responses suggest that that higher $\dot{V}O_{2peak}$ and/or the additional years of aerobic training alters ventilation mechanics in older but not young women during exercise.

Maximal HR was not different between groups which further supports maximal HR being a function of age rather than training status (4, 38). The rating of perceived exertion at peak exercise was the same (9.8 ± 0.4) and close to maximum (10) for both groups indicating that participants produced a maximum effort in the RI test. The intensities for the SWCL were calculated as percentages of VT (which has a subjective measurement component), however, there being no difference in HR and RPE between groups during the SWCL test supports that the relative exercise intensity was not different between the groups.

Tissue Deoxyhemoglobin

Unique to our study was the simultaneous measurement of HHb in the tissue of two skeletal muscles and PFC in young women. For HHb in the VL and GAST, the results supported the hypothesis in that HHb was significantly higher in LTT compared to STT in the RI test. Although the difference in HHb in the VL between groups did not reach statistical significance during the SWCL test, HHb was substantially higher in LTT compared to STT and therefore, likely to be meaningful. Due to the relatively high between- participant variability it is possible that there was a type-II error and a greater number of participants should confirm the expected difference. Significant improvements in peripheral O_2 extraction (faster tau HHb) during transitions from a low to moderate intensity have been reported in young women following 12 weeks aerobic training (39). Furthermore, Murias et al. (39) reported rapid initial improvements in tau HHb in the first nine weeks of training with minimal improvements in both tau HHb and $\dot{V}O_2$ between weeks 9 - 12. The results of the current study provide evidence that improvements in peripheral O_2 extraction may continue well beyond the early rapid adaptations previously reported by Murias et al. (39).

Prior to the current study, HHb in the GAST during exercise had only been investigated in three studies (11, 24, 33), none of these targeted at young women. The first study (24) compared the O_2Hb pattern of the GAST and VL in young men during treadmill running. While the authors reported that the deoxygenation pattern differed between muscles, no supporting data were presented.

The second study (33) of young untrained men reported increased HHb in the VL but not the GAST with consecutive high intensity interval bouts. The third study (11) of STT and LTT older (40 - 60 yr) women reported no difference in HHb in the GAST during RI and SWCL exercise. The findings of the study of older women contrast with the current study indicating that peak O_2 extraction is reduced in older (40 - 60 yr) compared to young (18 - 30 yr) women. Age related differences in muscle mass and changes in peripheral blood flow are possible explanations for the varied peak HHb results between older women (11) and the young women in the current study, as age related reductions in muscle mass in older women is associated with concomitant reductions in leg blood flow and perfusion pressure (44). Furthermore, the opposing HHb patterns observed in the GAST and VL in the current study were also reported in the study of older women (11). In these cases, at the onset of exercise HHb in the GAST decreased considerably then progressively increased with intensity, whereas HHb in the VL gradually increased from the onset of exercise.

Variations in muscle fiber characteristics and the recruitment pattern of these fibers between the GAST and VL during RI exercise provide a possible reason for differences in HHb patterns as metabolic and recruitment processes are influenced by fiber type characteristics (6, 27). Compared to the VL, the GAST has a high percent of Type I fibers which have a high perfusion pressure and rate of O_2 extraction and a low percentage of Type IIa and IIx fibers which have a low rate of O_2 extraction (30). The sequential recruitment of muscle fibers during progressive intensity exercise changes from slow-twitch to fast-twitch fibers (6).

In support of the hypothesis, HHb in the PFC was not different between groups during the RI and SWCL tests. These results concur with the one other study which reported HHb data in the PFC of young women during exercise (41) and suggests that HHb in the PFC does not appear to change following aerobic training and is unlikely to influence or limit peak performance in young women.

Furthermore, with no difference between young and older STT and LTT women (11), age and training status does not appear to affect O_2 extraction in the PFC in adult women during exercise. However, as some literature on young men indicate a possible link exists between imbalances in O_2 extraction in the PFC and maximum exercise capacity (45, 46), there could be a sex difference in PFC oxygenation of young adults during high intensity exercise. The regulation of blood flow in the PFC requires complex integration of various mechanisms including but not limited to cardiac output, cerebral metabolism, PaO_2 and $PaCO_2$ and the autonomic nervous system, therefore, the contribution of the potential controlling mechanisms on O_2 extraction in the PFC required further investigation.

Limitations

A first potential limitation of this current study was the cycling experience of participants. While regular cycling was a requirement for recruitment, the contribution of cycling (current and past) on the total training load of participants was unknown. A second limitation is the self-reported training logs might not be a complete or accurate representation of training history.

Practical applications

This current study has implications for aerobic training in young women in that the results indicate that, in addition to the typical initial adaptive improvements in systemic oxygen utilization and peripheral O_2 extraction following commencement of regular aerobic training, continued regular aerobic training > 24 months provides significant further improvements for the same current training load. Future research should include training studies extending beyond 12 months duration. The difference in tissue oxygen extraction between the young women in the current study and that reported in studies of younger men highlights the importance of using sex specific evidence when developing training programs and therefore, more studies should focus on women.

Conclusion

For young recreationally-active women, regular aerobic training beyond 24 months duration significantly improves $\dot{V}O_2$ during RI and SWCL exercise for the same current training load. Concomitant with these adaptations is increased peripheral O_2 extraction (HHb) in the GAST and VL during RI exercise. In the PFC however, O_2 extraction (HHb) is unaffected by additional training years.

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Author Contributions

Conceived and designed the experiment: GB, CS.

Performed the experiment: GB.

Analyzed the data: GB, GPL, CDA, CS.

Wrote the paper: GB, GPL, CDA, CS.

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Figure Legends

Fig. 1. Mean HHb in the VL, GAST and PFC during ramp incremental cycling. Panel (A) HHb in the VL. Panel (B) HHb in the GAST. Panel (C) HHb in the PFC. STT 0 - 8.5 min (n = 13); 8.5 - 10.5 min (n = 7 - 11); 10.5 - 12.5 min (n = 3 - 6). LTT 0 - 9.25 min (n = 13); 9.25 - 11.75 (n = 9 - 12); 11.75 - 14 (n = 2 - 7). Square trace LTT, diamond trace STT.

Fig. 2. Mean HHb in the VL, GAST and PFC during square-wave constant load cycling. Panel (A) HHb in the VL. Panel (B) HHb in the GAST. Panel (C) HHb in the PFC. Square trace LTT, diamond trace STT.

Fig 3. Group mean HHb in the VL, GAST and PFC during ramp incremental cycling. Panel (A) HHb in the VL. Panel (B) HHb in the GAST. Panel (C) HHb in the PFC at 90% of VT and peak exercise. Pattern fill STT, solid fill LTT.

* Significant ($p < 0.05$) differences between groups.

Fig 4. Group mean HHb in the VL, GAST and PFC during square-wave constant load cycling. Panel (A) HHb in the VL. Panel (B) HHb in the GAST. Panel (C) HHb in the PFC at 25%, 80% and 90% of VT. Pattern fill STT, solid fill LTT.

* Significant ($p < 0.05$) differences between groups.

Table 1. Participant characteristics of the short-term trained and long-term trained young women.

Characteristic	STT	LTT
Age (yr)	25.1 (3.6)	23.4 (3.2)
Mass (kg)	61.0 (7.3)	60.9 (13.4)
Height (cm)	166.0 (5.2)	169.4 (5.6)
LV adipose (thickness)	10.5 (2.6)	8.8 (3.3)
GAST adipose (thickness)	6.2 (1.6)	4.9 (1.6)
Current training load (AU)	940.2 (470.5)	957.1 (507.2)
Current training (yr)	1.5 (0.5)	7.31 (2.0) *
VT $\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	25.9 (4.0)	30.6 (6.1) *
VT % of Peak ($mL \cdot kg^{-1} \cdot min^{-1}$)	69.1 (5.6)	72.5 (7.5)

Values are mean (SD).

* Significant difference between groups $p < 0.05$

Current training load = min x exercise intensity (light = 1, moderate = 2 and high = 3)

STT: Short-term trained; LTT: Long-term trained; LV: Left *vastus lateralis*; GAST: *Gastrocnemius*; AU: Arbitrary units; VT: Ventilatory threshold.

Table 2. Systemic oxygen utilization, ventilation, heart rate and rating of perceived exertion of short-term trained and long-term trained young women at 90% VT and peak exercise during ramp incremental cycling.

	90% VT		Peak	
Variable	STT	LTT	STT	LTT
$\dot{V}O_2$ (mL · kg^{-1} · min^{-1})$^{a, b}$	23.4 (4.6)	28.0 (5.7) *	37.5 (5.34)	42.0 (6.0) *
V_E (L · min^{-1})$^{a, b, ab}$	36.3 (6.6)	44.6 (7.4) *	84.9 (14.0)	102.3 (15.6) *
V_T (L)^{b,}	1.6 (0.4)	1.9 (0.3)	1.9 (0.3)	2.0 (0.3)
BF (Breaths · min^{-1})^{b}	23.2 (5.4)	24.6 (4.7)	47.6 (9.8)	53.2 (10.1)
HR (Beats · min^{-1})$^{\# a, b}$	143.6 (10.8)	144.8 (11.5)	178.9 (9.3)	179.8 (9.0)
RPE^{b}	4.8 (0.8)	4.2 (0.6)	9.8 (0.4)	9.8 (0.4)

Values are mean (SD).

^{a} significant group main effect; ^{b} significant intensity main effect; ^{ab} significant group by intensity interaction; * Significant difference between groups $p < 0.05$; $^{\#}$ HR at 90% VT, STT n = 13, LTT n = 12. HR at Peak, STT n = 13, LTT n = 13.

VT: Ventilatory threshold; STT: Short-term trained; LTT: Long-term trained; $\dot{V}O_2$: Oxygen extraction; V_E: Minute ventilation; V_T: Tidal volume; BF: Breathing frequency; HR: Heart rate; RPE: Rating of perceived exertion.

Table 3. Systemic oxygen utilization, ventilation, heart rate and rating of perceived exertion of short-term trained and long-term trained young women at 25%, 80% and 90% VT during square-wave constant load cycling.

	25% VT		80% VT		90% VT	
Variable	STT	LTT	STT	LTT	STT	LTT
$\dot{V}O_2$ ($\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) [a, b, ab]	10.0 (1.9)	11.9 (2.1) *	19.6 (3.3)	24.0 (3.8) *	26.2 (3.1)	32.4 (4.9) *
V_E ($\text{L} \cdot \text{min}^{-1}$) [a, b, ab]	16.3 (2.7)	19.1 (3.1) *	30.5 (4.8)	37.8 (6.1) *	49.3 (8.3)	60.2 (9.4) *
V_T (L) [b, ab]	0.9 (0.2)	1.0 (0.3)	1.5 (0.3)	1.6 (0.3)	1.6 (0.3)	1.8 (0.3) *
BF ($\text{Breaths} \cdot \text{min}^{-1}$) [b]	18.9 (3.2)	19.3 (4.6)	21.8 (3.8)	23.8 (5.0)	32.2 (6.5)	33.8 (6.4)
HR ($\text{Beats} \cdot \text{min}^{-1}$)[#] [b]	98.7 (5.9)	95.2 (9.4)	127.3 (8.0)	127.9 (9.3)	156.9 (14.3)	157.8 (13.1)
RPE [b]	1.2 (0.4)	1.1 (0.3)	3.2 (0.9)	3.1 (0.9)	5.9 (1.2)	6.0 (1.0)

Values are mean (SD).

[a] significant group main effect; [b] significant intensity main effect; [ab] significant group by intensity interaction; * Significant difference between groups p = < 0.05; [#] HR at 25% VT; STT n = 12, LTT n = 12. HR at 80% and 90% VT; STT n = 11, LTT n = 12.

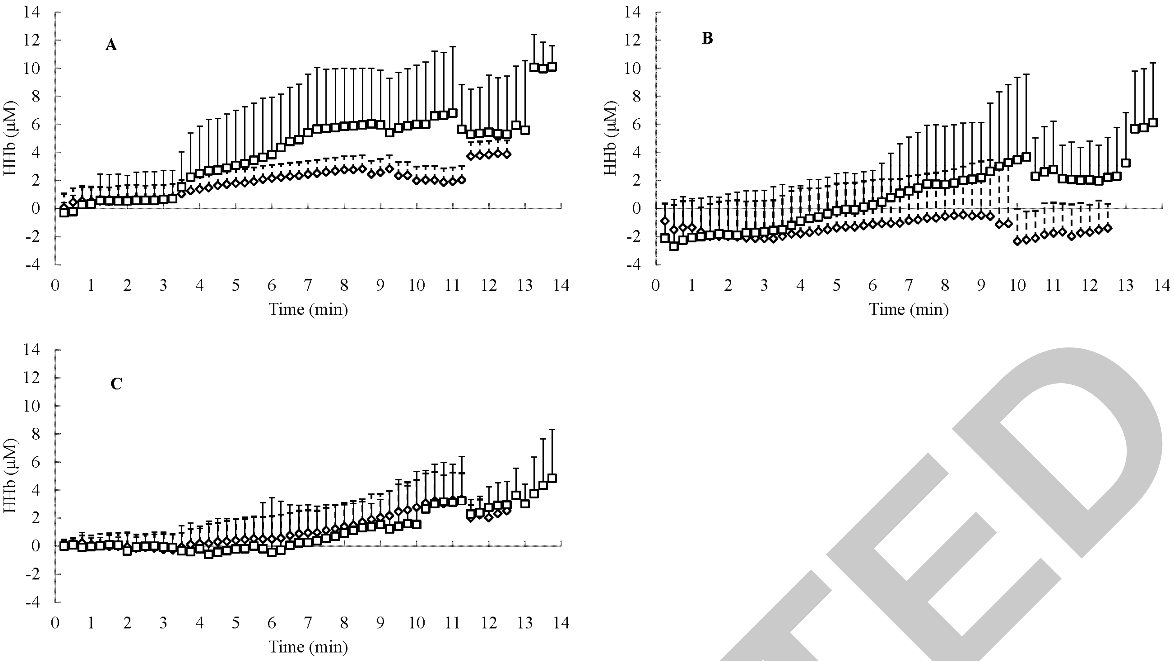
VT: Ventilatory threshold; STT: Short-term trained; LTT: Long-term trained; $\dot{V}O_2$: Oxygen utilization; V_E: Minute ventilation; V_T: Tidal volume; BF: Breathing frequency; HR: Heart rate; RPE: Rating of perceived exertion.

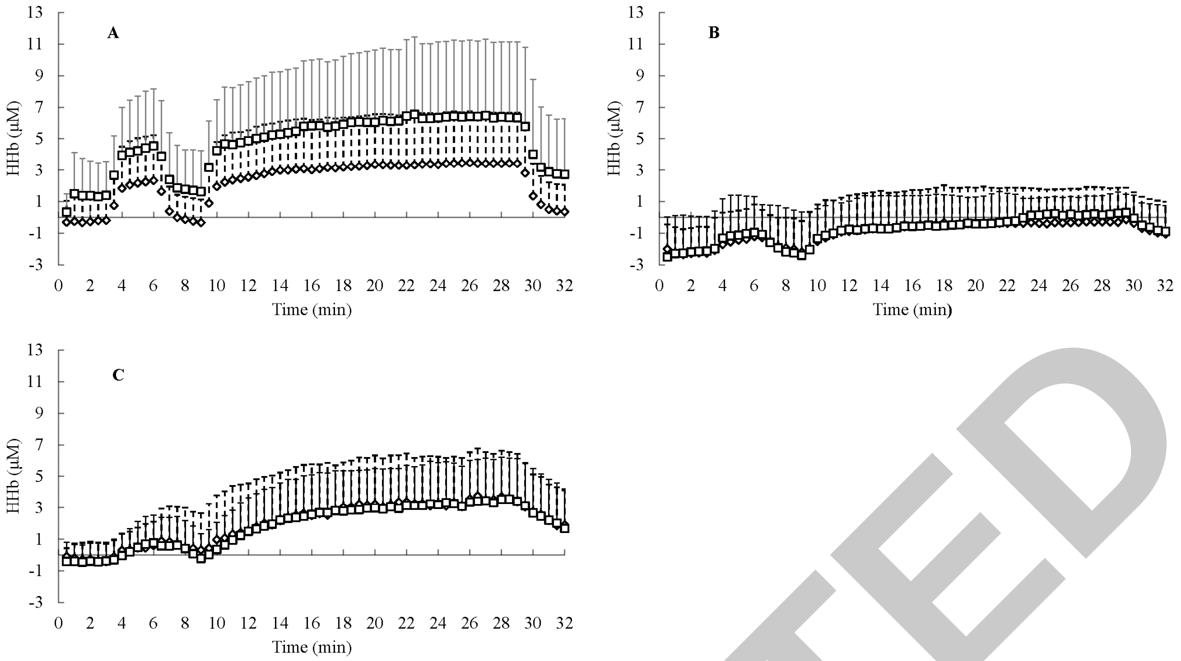
Table 4. Results of 2-way analysis of covariance (ANCOVA) for ventilatory parameters, and HHb during ramp incremental and square-wave constant load cycling.

		Main effect for group					Group x intensity interaction				
Variable	Test	Df	F	p	η_P^2	ß	Df	F	p	η_P^2	ß
$\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	RI	1, 23	5.884	0.024*	0.204	0.642	1, 23	0.007	0.932	< 0.001	0.051
	SWCL	1, 23	13.535	0.001*	0.370	0.941	2, 46	9.805	< 0.001*	0.299	0.977
V_E ($L \cdot min^{-1}$)	RI	1, 23	9.835	0.005*	0.300	0.851	1, 23	5.405	0.029*	0.190	0.605
	SWCL	1, 23	12.394	0.002*	0.350	0.921	2, 46	5.165	0.009*	0.183	0.802
V_T (L)	RI	1, 23	1.665	0.210	0.068	0.236	1, 23	1.513	0.231	0.062	0.218
	SWCL	1, 23	2.797	0.108	0.108	0.361	2, 46	1.901	0.161	0.076	0.357
BF (Breaths $\cdot$ min^{-1})	RI	1, 23	1.512	0.230	0.062	0.218	1, 23	2.304	0.143	0.091	0.307
	SWCL	1, 23	0.537	0.471	0.023	0.108	2, 46	0.483	0.620	0.021	0.124
HR (Beats $\cdot$ min^{-1})	RI	1, 22	0.108	0.746	0.005	0.061	1, 22	0.002	0.964	< 0.001	0.050
	SWCL	1, 19	0.035	0.854	0.002	0.054	2, 38	5.868	0.911	0.005	0.063
RPE	RI	1, 23	2.273	0.145	0.090	0.304	1, 23	3.039	0.095	0.177	0.386
	SWCL	1, 23	0.019	0.891	0.001	0.052	2, 46	0.091	0.913	0.004	0.063
VL HHb	RI	1, 23	4.558	0.044*	0.165	0.534	1, 23	0.426	0.520	0.018	0.096
	SWCL	1, 23	3.706	0.067	0.139	0.454	2, 46	1.355	0.268	0.056	0.277
GAST HHb	RI	1, 23	5.542	0.027*	0.194	0.616	1, 23	1.597	0.219	0.065	0.228
	SWCL	1, 22	0.082	0.777	0.004	0.059	2, 44	0.276	0.760	0.012	0.091
PFC HHb	RI	1, 22	0.033	0.858	0.001	0.053	1, 22	0.081	0.779	0.004	0.059
	SWCL	1, 22	0.044	0.836	0.002	0.055	2, 44	0.021	0.980	0.001	0.053

*Significant $p = < 0.05$

RI: Ramp incremental; SWCL: Square-wave constant load; $\dot{V}O_2$: Oxygen utilization; V_E: Minute ventilation; V_T: Tidal volume; BF: Breathing frequency; HR: Heart rate; RPE: Rating of perceived exertion, VL; *Vastus lateralis*: GAST; *Gastrocnemius*: PFC; Pre-frontal cortex: HHb; deoxyhemoglobin.


A
B
C
HHb (μM)
14
12
10
8
6
4
2
0
-2
-4
Time (min)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14


A
B
C
HHb (µM)
13
11
9
7
5
3
1
-1
-3
0
2
4
6
8
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12
14
16
18
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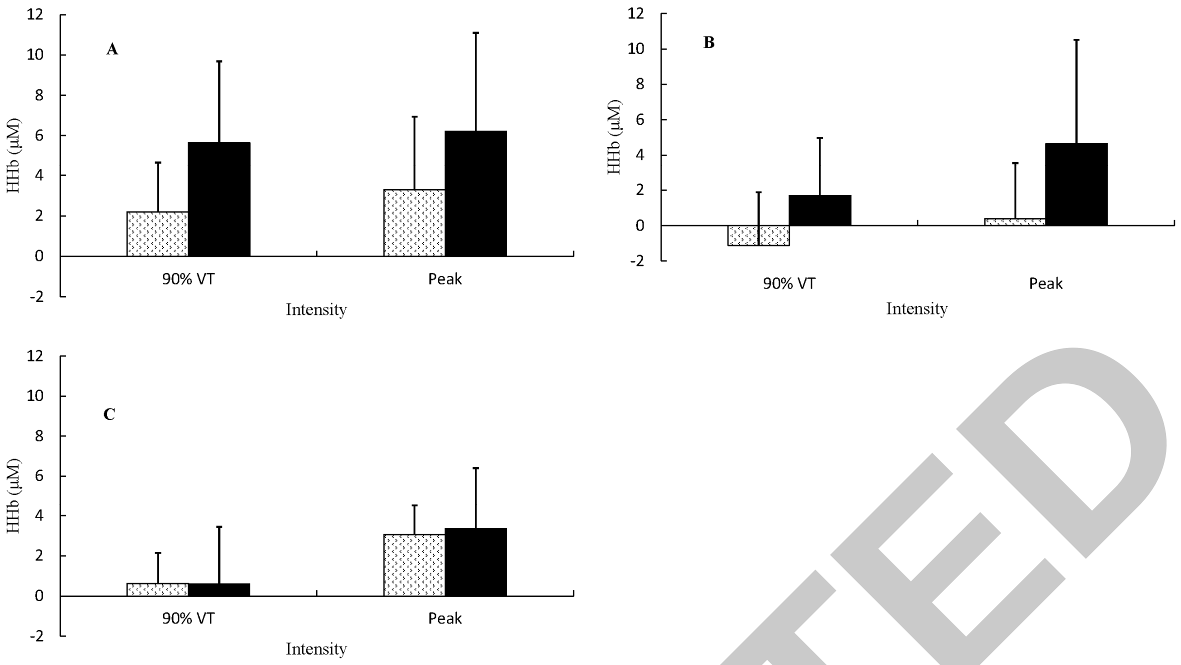
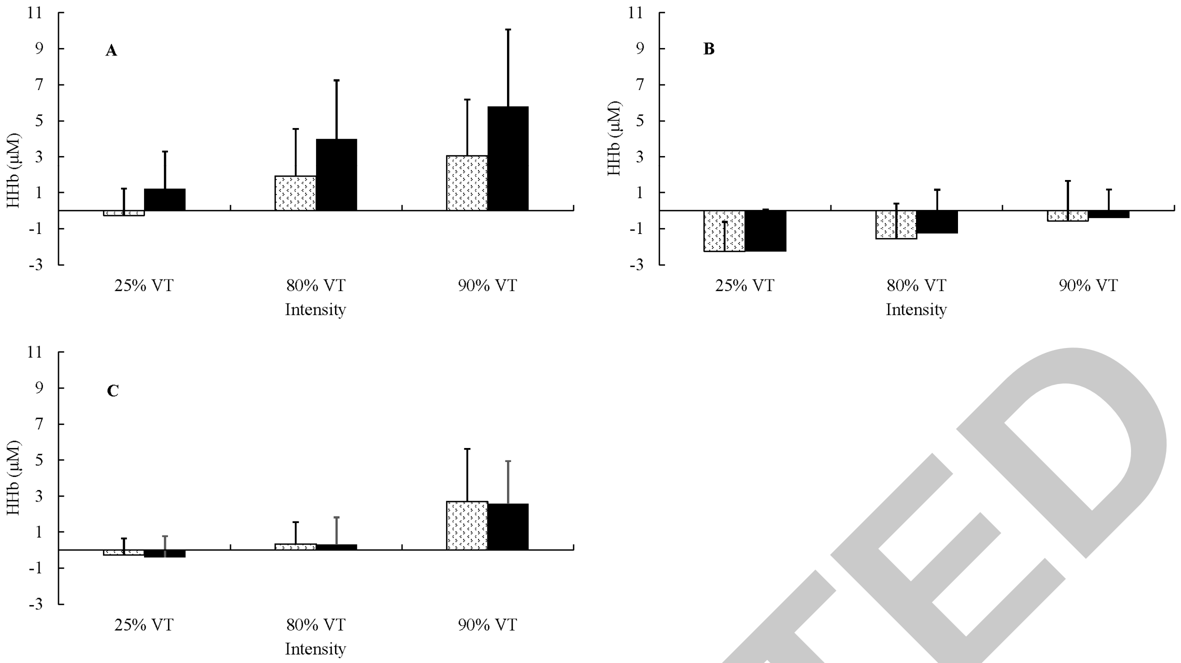
32
Time (min)


A
B
C
HHb (µM)
12
10
8
6
4
2
0
-2
90% VT
Peak
Intensity


A
B
C
HHb (µM)
11
9
7
5
3
1
-1
-3
25% VT
80% VT
90% VT
Intensity