The Effect of Aerobic Training Years on Systemic Oxygen Utilisation, and Peripheral $O_2$ Extraction in the Vastus Lateralis, Gastrocnemius and Pre-Frontal Cortex in Young Men

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ABSTRACT

Introduction: This study was designed to determine if systemic $O_2$ utilisation ($\dot{V}O_2$), and tissue $O_2$ extraction (deoxyhemoglobin [HHb]) in the vastus lateralis (VL), gastrocnemius (GAST) and pre-frontal cortex (PFC) differed between aerobically short term trained (STT 6 - 24 months) and long term trained (LTT > 5 yr) men aged 18 - 30 years, who were matched for current training load.

Methods: Fourteen STT and 13 LTT participants completed ramp incremental (RI) and square-wave constant load (SWCL) (3 min at 25%, 80% and 25% and 20 min at 90% of ventilatory threshold [VT]) tests on a cycle ergometer. Results: ANCOVA revealed that $\dot{V}O_2$ was higher in LTT compared to STT in the RI ($p < 0.001$) and SWCL ($p = 0.004$) tests. There was no difference in $\Delta$HHb in the VL, GAST or PFC between LTT and STT during both tests. However, there was a group x intensity interaction in the GAST during SWCL. Conclusion: These results suggest that in men aged 18 - 30 years, with up to 24 months of aerobic training, additional training years can increase systemic $O_2$ utilisation without increased current training load and that peripheral adaptations have little influence on the observed increases.

KEYWORDS: Aerobic training, oxygenation, $\dot{V}O_{2\text{peak}}$. 

Introduction

Aerobic training consistently increases peak aerobic capacity ($\dot{V}O_{2\text{peak}}$) in healthy adults¹-³, with a dose response relationship existing (to a limit) between increased $\dot{V}O_{2\text{peak}}$ and the training load (intensity, duration and frequency) of the intervention⁴-⁶. In previously untrained young men, training studies of between 9 - 52 weeks report a plateau in $\dot{V}O_{2\text{peak}}$ gains⁴,⁵,⁷, however, this could be due to
the training stimulus not changing as adaptations occur. It is yet to be investigated if (independent of current training load) further gains in VO2peak can result from additional training years in young men. If as suggested, the primary mediator of increases in VO2peak is training load not years of training, the VO2peak of young men that are matched for age and training load but differ in years of training should be similar. However, we expect that training years would affect VO2peak, as reported in similar studies of older8 and younger9 women from our laboratory.

The gains in VO2peak following aerobic training result from increased central O2 delivery (cardiac output) to, and O2 extraction (arterio-venous O2 difference) by peripheral muscles. In young men, the relative contribution that central and peripheral adaptations make to increasing VO2peak is not known and varies depending on the training load and duration of the intervention6,10-12. Further, a meta-analysis of short term (5 - 13 wk) training studies reported that central adaptations are linearly associated with and likely responsible for increases in VO2peak, with little to no changes in peripheral O2 extraction (arterio-venous O2 difference)10. However, except for one study that assessed single limb O2 uptake6, the other studies focused on changes in whole body O2 extraction (arterio-venous O2 difference) which reflects O2 extraction of all tissues in the body, not isolated muscles.

Near-Infrared Spectroscopy (NIRS) provides a method of non-invasively monitoring changes in local tissue O2 extraction, through changes in deoxyhemoglobin (HHb) and oxyhemoglobin (O2Hb)13-15. The HHb pattern reflects the dynamic balance between O2 delivery and O2 utilisation at the site of the measurement, and thus tissue O2 extraction16,17. Changes in HHb response (ΔHHb) has been used to report higher tissue O2 extraction at the vastus lateralis (VL) of trained compared to untrained young and older men18,19 and young20 and older21 women during transitions from low to moderate exercise, and to report a correlation between VO2peak and peak HHb in the VL and rectus femoris muscles22. However, the effect of aerobic training years on peak muscle tissue O2 extraction, or tissue O2 extraction at a constant sub-ventilatory threshold (VT) load for an extended period remains to be investigated in a cohort of young men. Furthermore, the reported large decrease in O2Hb and maintained or continued increases in HHb in the pre-frontal cortex (PFC) during high intensity exercise in highly trained (VO2peak of 62 ± 7.9 mL · kg⁻¹ · min⁻¹) but not untrained young men13 have led to theories of a potential relationship between PFC oxygenation and exercise performance14,23,24. Therefore, a better understanding of tissue oxygenation responses at various sites during different intensity exercise, and following different durations (yr) of training may help clarify the influence of aerobic training on these determinants of aerobic capacity.

The aim of this current study was to simultaneously investigate the difference in VO2 and ΔHHb in the VL and gastrocnemius (GAST) muscles and the PFC between STT (6 - 24 months) and LTT (> 5 yr) aerobically trained young men aged 18 - 30 years who were matched for current training load. Participants completed ramp incremental (RI) and sub-VT square-wave constant load (SWCL) cycling
tests. It was hypothesised that: 1) $\dot{V}O_2$ and $\Delta HHb$ would be higher in the VL and GAST of LTT compared to the STT during the RI and each measured relative intensity (25%, 80% and 90% VT) during SWCL; and 2), there would be no difference in $\Delta HHb$ in the PFC between LTT and STT at any exercise intensity during both tests.

Methods
Ethical approval
This study was approved by the Human Research Ethics Committee at the University of the Sunshine Coast, Australia (S/14/676) and participants provided written informed consent.

Participants
The sample of young (18 - 30 yr) Caucasian men consisted of one group of 14 STT (6 - 24 months) men, and one group of 13 LTT (> 5 yr) men. Each participant reported having exceeded 200 min of moderate to vigorous aerobic training per week (including cycling), not missing more than two weeks of training over any six-month period, as determined from self-reported physical activity training logs (refer Table 1). Current training load (volume and exercise intensity) is a primary factor influencing $\dot{V}O_2$ and $\Delta HHb$ during exercise, with both being higher in higher trained individuals$^{18,22}$. All participants reported their current training load as a typical training week across the previous six months. Participants’ physical characteristics and training history are presented in Table 1. Following medical screening (Physical Activity Readiness Questionnaire$^{25}$ and Medical Health Questionnaire), exclusion included any health related issues or medications that would affect participant safety and or exercise capacity or $O_2$ utilisation/extraction.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>STT</th>
<th>LTT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>23.5 (3.6)</td>
<td>24.0 (3.6)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>84.3 (14.4)</td>
<td>79.7 (12.1)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>180.4 (8.8)</td>
<td>184.1 (8.7)</td>
</tr>
<tr>
<td>VL adipose (thickness mm)</td>
<td>7.4 (2.6)</td>
<td>3.9 (1.2) *</td>
</tr>
<tr>
<td>GAST adipose (thickness mm)</td>
<td>4.9 (3.0)</td>
<td>3.0 (0.6) *</td>
</tr>
<tr>
<td>Current training load (AU)</td>
<td>1189 (331)</td>
<td>1302 (324)</td>
</tr>
<tr>
<td>Current training (yr)</td>
<td>1.5 (0.5)</td>
<td>8.3 (2.7) *</td>
</tr>
<tr>
<td>VT $\dot{V}O_2$ (mL · kg$^{-1}$ · min$^{-1}$)</td>
<td>28.5 (4.7)</td>
<td>36.9 (5.2) *</td>
</tr>
<tr>
<td>VT % of Peak (mL · kg$^{-1}$ · min$^{-1}$)</td>
<td>62.8 (6.9)</td>
<td>65.5 (4.8)</td>
</tr>
</tbody>
</table>

Values are mean (SD). (STT n = 14, LTT n = 13)

* Significant ($p < 0.05$) difference between LTT and STT.

Current training (yr) = current number of continuous years of training; Current training load (AU) = volume x intensity (light = 1, moderate = 2 and high = 3).

Study design
The study used a cross-sectional, two group (STT and LTT), within subject repeated measures (exercise intensity within RI and
SWCL) design. The independent variable used was current training duration (yr). The dependent variables were systemic O₂ utilisation (VO₂), heart rate (HR), rating of perceived exertion (RPE) (Borg’s 1 - 10 category-ratio [CR-10])\(^{26}\), and ΔHHb in the VL, GAST and PFC. The current training load was not significantly different between STT and LTT (p = 0.909).

Each participant completed two testing sessions in a temperature controlled (20 - 23º C) Exercise Physiology Laboratory. Prior to testing, participants were required to abstain from caffeine and food for four hours, and alcohol and intense exercise for 24 hours.

**Procedures**

**Session One**

The aim of session one was to determine participant characteristics, and VO₂, HR, RPE (Table 2) and ΔHHb in the VL, GAST and PFC during a RI (increments = 1 W per 2-s) cycling test to volitional cessation on a Velotron cycle ergometer (Racermate, Seattle, USA). Anthropometric, pulmonary function (Spiro II spirometer, Medical International Research, Rome, Italy), HR and RPE data were recorded using standard measures as previously described\(^{6}\). The VO₂, HR, and ΔHHb in the VL, GAST and PFC were recorded continuously during exercise, while RPE was recorded within the last 10 s of each minute (except for the warm-up) and at the point of volitional cessation. To encourage peak results, participants received verbal feedback and encouragement from the tester. Expired gas was analysed to determine VO₂peak and VT, with VO₂peak determined as the highest 15-s average VO₂ value within the last minute of this test, and VT determined using the V-slope method described by Beaver, Wasserman, Whipp\(^{27}\). Briefly, this method defines the point where the rate of rise in CO₂ output (VCO₂) relative to minute ventilation exceeds the relative rise in VO₂. This VT was time aligned with the power (watts) of the cycle ergometer to calculate the power outputs for the subsequent SWCL test. As direct comparisons were not made between the RI and SWCL tests or for systemic VO₂ and muscle O₂ extraction (i.e. VO₂ to ΔHHb ratio), VO₂peak was not left shifted to accommodate for any potential phase I- phase II VO₂ lag time.

**Session Two**

Session two was conducted three to 14 days after session one. The aim of session two was to determine the participants’ VO₂, HR, RPE (Table 3), and ΔHHb in the VL, GAST and PFC while cycling at a relative intensity of 25%, 80% and 90% of the VT power output obtained from the RI test. The timing and intensities for the SWCL test were; 3 minutes at 25%, 80%, 25%; 20 minutes at 90%; and a further 3 minutes at 25% of calculated VT.

The VO₂, HR, and ΔHHb 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 using methods as previously described\(^{6}\). Participants were not encouraged or provided feedback during the test, to minimise cognitive stimulus.

**Tissue Deoxyhemoglobin**

Tissue oxygenation (HHb and O₂Hb) data were measured simultaneously and continuously in
the left VL, GAST and PFC using a single-channel NIRS system (PortaMon and Portalite, Artinis Medical Systems BV, Zetten, Netherlands). The muscle optodes were fixed to the skin at the mid-belly of the muscle using adhesive tape and wrapped with low compression black elastic bandage, and the PFC optode was fixed to the skin at the left PFC using adhesive tape, then covered with a black headband.

All NIRS primary data ($\Delta$HHb and $\Delta$O$_2$Hb) were recorded at 10 Hz. The last 20-s of resting values were averaged to obtain baseline values. Any 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 $\Delta$O$_2$Hb, $\Delta$HHb (the primary variable for the present study) is less affected by changes in hemodynamics and is therefore a better indicator of tissue O$_2$ extraction. Therefore, only $\Delta$HHb data have been presented in this present study.

Statistical Analyses
All statistical analyses were performed using SPSS (version 24, SPSS Inc., Chicago, IL). Prior to statistical analysis, data were checked for normality (Shapiro-Wilk test of normality and Mauchly test for Sphericity), and that the relevant assumptions for each test were met.

To identify the presence of any significant group (STT vs LTT) differences in current training load, independent t-tests were conducted. To identify the presence of any significant group (STT vs LTT) and exercise intensity main effects or interactions, two-way Analysis of Covariance (ANCOVAs) were conducted on each dependent variable within each test. 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 physiological responses to exercise, training load was included as a covariate in all analyses. The level for statistical significance was set to $p < 0.05$ for all analyses. Partial-eta squared was used to indicate the effect size as small ($\eta^2 > 0.01$), medium ($\eta^2 > 0.06$) or large ($\eta^2 > 0.14$) as per Cohen.

Results
The $\dot{V}$O$_2$peak, HR and RPE exhibited significant intensity main effects with large effect sizes in both the RI and SWCL tests, while the $\Delta$HHb in the VL, GAST and PFC exhibited significant intensity main effects with large effect sizes in the SWCL but not the RI test. These variables increased with exercise intensity as physiologically expected (descriptive statistics are shown in Tables 2 and 3). Significant group main effects with large effect sizes were observed for $\dot{V}$O$_2$ in both the RI and SWCL tests, with $\dot{V}$O$_2$ higher in LTT. There were significant group by intensity interactions with large effect sizes for $\dot{V}$O$_2$, and $\Delta$HHb in GAST in the SWCL test, with the difference increasing with increased intensity. Results for group main effects and group by intensity interactions are shown in Table 4.
### Table 2. Systemic oxygen utilisation, ventilation, heart rate and rating of perceived exertion of short term trained and long term trained young men at 90% ventilatory threshold and peak exercise during Ramp Incremental cycling.

<table>
<thead>
<tr>
<th>Variable</th>
<th>90% VT</th>
<th>Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>STT</td>
<td>LTT</td>
</tr>
<tr>
<td>VO₂ (mL · kg⁻¹ · min⁻¹)</td>
<td>26.0 (3.6)</td>
<td>33.8 (4.4)</td>
</tr>
<tr>
<td>HR (Beats · min⁻¹)</td>
<td>141.1 (13.7)</td>
<td>140.4 (11.4)</td>
</tr>
<tr>
<td>RPE b</td>
<td>4.2 (0.8)</td>
<td>4.5 (0.9)</td>
</tr>
</tbody>
</table>

Values are mean (SD).

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

VT: Ventilatory threshold; STT: Short term trained; LTT: Long term trained; VO₂: Oxygen utilisation; HR: Heart rate; RPE: Rating of perceived exertion.

### Table 3. Systemic oxygen utilisation, heart rate and rating of perceived exertion of short term trained and long term trained young men at 25%, 80% and 90% ventilatory threshold during Square-Wave Constant Load cycling.

<table>
<thead>
<tr>
<th>Variable</th>
<th>25% VT</th>
<th>80% VT</th>
<th>90% VT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>STT</td>
<td>LTT</td>
<td>STT</td>
</tr>
<tr>
<td>VO₂ (mL · kg⁻¹ · min⁻¹)</td>
<td>10.9 (1.6)</td>
<td>12.8 (2.2) *</td>
<td>23.0 (3.5)</td>
</tr>
<tr>
<td>HR (Beats · min⁻¹)</td>
<td>89.1 (8.5)</td>
<td>90 (7.3)</td>
<td>120.7 (10.8)</td>
</tr>
<tr>
<td>RPE b</td>
<td>1.1 (0.3)</td>
<td>1.2 (0.4)</td>
<td>3.5 (1.5)</td>
</tr>
</tbody>
</table>

Values are mean (SD).

a significant group main effect; b significant intensity main effect; ab significant group by intensity interaction; * significant difference between STT and LTT p < 0.05; #HR at 25%, 80% and 90% VT; STT n = 14, LTT n = 11.

VT: Ventilatory threshold; STT: Short term trained; LTT: Long term trained; VO₂: Oxygen utilisation; HR: Heart rate; RPE: Rating of perceived exertion.
Table 4. Results of two-way Analysis of Covariance (ANCOVAs) for systemic oxygen utilisation, heart rate, rating of perceived exertion and HHb of young men during ramp incremental and square-wave constant load cycling.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Test</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Df</td>
<td>F</td>
<td>p</td>
<td>η²</td>
<td>β</td>
<td>Df</td>
<td>F</td>
<td>p</td>
<td>η²</td>
<td>β</td>
<td>η²</td>
</tr>
<tr>
<td>VO₂ (mL · kg⁻¹ · min⁻¹)</td>
<td>RI</td>
<td>1, 24</td>
<td>24.412</td>
<td>&lt; 0.001*</td>
<td>0.504</td>
<td>0.997</td>
<td>1, 24</td>
<td>2.952</td>
<td>0.099</td>
<td>0.110</td>
<td>0.378</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SWCL</td>
<td>1, 24</td>
<td>9.981</td>
<td>0.004*</td>
<td>0.294</td>
<td>0.858</td>
<td>2, 48</td>
<td>7.803</td>
<td>0.001*</td>
<td>0.245</td>
<td>0.939</td>
<td></td>
</tr>
<tr>
<td>HR (Beats · min⁻¹)</td>
<td>RI</td>
<td>1, 22</td>
<td>0.005</td>
<td>0.994</td>
<td>&lt; 0.001</td>
<td>0.051</td>
<td>1, 22</td>
<td>0.410</td>
<td>0.529</td>
<td>0.018</td>
<td>0.094</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SWCL</td>
<td>1, 22</td>
<td>0.435</td>
<td>0.516</td>
<td>0.019</td>
<td>0.097</td>
<td>2, 44</td>
<td>0.528</td>
<td>0.536</td>
<td>0.026</td>
<td>0.141</td>
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<tr>
<td>RPE</td>
<td>RI</td>
<td>1, 24</td>
<td>0.400</td>
<td>0.533</td>
<td>0.016</td>
<td>0.093</td>
<td>1, 24</td>
<td>1.702</td>
<td>0.204</td>
<td>0.066</td>
<td>0.240</td>
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<tr>
<td></td>
<td>SWCL</td>
<td>1, 24</td>
<td>0.014</td>
<td>0.907</td>
<td>0.001</td>
<td>0.051</td>
<td>2, 48</td>
<td>0.298</td>
<td>0.744</td>
<td>0.012</td>
<td>0.095</td>
<td></td>
</tr>
<tr>
<td>VL HHb</td>
<td>RI</td>
<td>1, 23</td>
<td>0.450</td>
<td>0.509</td>
<td>0.019</td>
<td>0.099</td>
<td>1, 23</td>
<td>1.200</td>
<td>0.285</td>
<td>0.050</td>
<td>0.183</td>
<td></td>
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<tr>
<td></td>
<td>SWCL</td>
<td>1, 24</td>
<td>0.230</td>
<td>0.636</td>
<td>0.009</td>
<td>0.075</td>
<td>2, 48</td>
<td>0.117</td>
<td>0.890</td>
<td>0.005</td>
<td>0.067</td>
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<td>GAST HHb</td>
<td>RI</td>
<td>1, 22</td>
<td>3.899</td>
<td>0.061</td>
<td>0.151</td>
<td>0.471</td>
<td>1, 22</td>
<td>0.013</td>
<td>0.910</td>
<td>0.001</td>
<td>0.051</td>
<td></td>
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<tr>
<td></td>
<td>SWCL</td>
<td>1, 22</td>
<td>2.284</td>
<td>0.145</td>
<td>0.094</td>
<td>0.304</td>
<td>2, 44</td>
<td>7.938</td>
<td>0.001*</td>
<td>0.265</td>
<td>0.941</td>
<td></td>
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<tr>
<td>PFC HHb</td>
<td>RI</td>
<td>1, 23</td>
<td>0.237</td>
<td>0.631</td>
<td>0.010</td>
<td>0.075</td>
<td>1, 23</td>
<td>0.145</td>
<td>0.707</td>
<td>0.006</td>
<td>0.065</td>
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<td></td>
<td>SWCL</td>
<td>1, 22</td>
<td>0.093</td>
<td>0.763</td>
<td>0.004</td>
<td>0.060</td>
<td>2, 44</td>
<td>0.066</td>
<td>0.936</td>
<td>0.003</td>
<td>0.059</td>
<td></td>
</tr>
</tbody>
</table>

*Significant p < 0.05

RI: Ramp incremental; SWCL: Square-wave constant load; VO₂: Oxygen utilisation; HR: Heart rate; RPE: Rating of perceived exertion, VL: Vastus lateralis; GAST: Gastrocnemius; PFC: Pre-frontal cortex; HHb: deoxyhaemoglobin.
Figure 1. Mean (SD); ΔHHb in the VL, GAST and PFC during Ramp Incremental cycling.  Panel (A) ΔHHb in the VL. Panel (B) ΔHHb in the GAST. Panel (C) ΔHHb 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.
Figure 2. Mean (SD) ΔHHb in the VL, GAST and PFC during Square-Wave Constant Load cycling. Panel (A) ΔHHb in the VL. Panel (B) ΔHHb in the GAST. Panel (C) ΔHHb in the PFC. Diamond shape STT, square shape LTT.
Figure 3. Mean (SD); ΔHHb in the VL, GAST and PFC during Ramp Incremental cycling. Panel (A) ΔHHb in the VL. Panel (B) ΔHHb in the GAST. Panel (C) ΔHHb in the PFC at 90% of VT and peak exercise. Pattern fill STT, solid fill LTT.
Figure 4. Mean (SD); ΔHHb in the VL, GAST and PFC during Square-Wave Constant Load cycling. Panel (A) ΔHHb in the LV. Panel (B) ΔHHb in the GAST. Panel (C) ΔHHb in the PFC at 90% of VT and peak exercise. Pattern fill STT, solid fill LTT.
* Significant (p < 0.05) differences between STT and LTT.
Discussion

This study examined the effect of aerobic training years (STT vs LTT) on \( \dot{V}O_2 \) and \( O_2 \) extraction (ΔHHb) in the VL, GAST and PFC at peak, and sub-VT exercise intensities. The primary findings were that: (i) \( \dot{V}O_2 \) was higher in LTT compared to STT in both the RI and SWCL tests; and (ii) although a significant group x intensity interaction was observed for ΔHHb in the GAST in the SWCL test, ΔHHb in the VL, GAST and PFC was not significantly different between LTT and STT in either test. This suggests that the higher \( \dot{V}O_2 \) in LTT was due to higher central \( O_2 \) delivery rather than peripheral \( O_2 \) extraction.

Systemic Oxygen Utilisation

As hypothesised, \( \dot{V}O_{2\text{peak}} \) was significantly higher in LTT compared to STT. The \( \dot{V}O_{2\text{peak}} \) values of 56.3 ± 6.9 mL·kg\(^{-1}\)·min\(^{-1}\) for LTT and 45.2 ± 4.9 mL·kg\(^{-1}\)·min\(^{-1}\) for STT are also consistent with the training load and experience reported by the participants\(^{30}\). However, these present results do not support suggestions that in young men, \( \dot{V}O_{2\text{peak}} \) plateaus within 12 months of commencing aerobic training\(^{4,5}\). While differences in current training years between previous studies and the present study provides a possible explanation for the difference in results, past training volumes of participants in the present study (which was not assessed) may have influenced the results. Additionally, compared to the STT, the genetic determinants of trainability of the LTT participants may have been stronger, and a reason why the LTT in the present study had continued aerobic training\(^{31,32}\).

Concomitant to \( \dot{V}O_{2\text{peak}} \), as expected, \( \dot{V}O_2 \) at VT was higher in the LTT compared to the STT. However, there was no difference when the \( \dot{V}O_2 \) was expressed as a percentage of \( \dot{V}O_{2\text{peak}} \), indicating increases in VT paralleled that of \( \dot{V}O_{2\text{peak}} \). As some training methods such as high intensity interval training increase VT as a percentage of \( \dot{V}O_{2\text{peak}} \)\(^{33,34}\), this finding may suggest that the training stimulus of both LTT and STT were similar.

Despite exercising at the same calculated relative exercise intensity during the study, \( \dot{V}O_2 \) was higher in LTT compared to STT during the SWCL test. This difference increased with increased exercise intensity from 1.9 mL·kg\(^{-1}\)·min\(^{-1}\) at 25% to 5.2 mL·kg\(^{-1}\)·min\(^{-1}\) at 80% and 11.1 mL·kg\(^{-1}\)·min\(^{-1}\) at 90% of VT. This combined with other findings in older men\(^{18}\) and older\(^{8,21}\) and younger women\(^{9}\) suggests that regardless of age and sex, aerobic training increases systemic \( O_2 \) utilisation while exercising at constant loads below VT.

In the present study, heart rates were not different between LTT and STT at any intensity in either test, supporting that LTT and STT were matched for age and were cycling at similar relative intensities during the SWCL test. Although peak heart rates are a function of age and not training, training induced faster heart rate dynamics during transitions from lower to higher exercise intensities could impact the delivery of substrates or \( O_2 \) required for oxidative phosphorylation\(^{3,18}\). Additionally, RPE was not different at any intensity during the SWCL test, further supporting that exercise intensities were matched between STT and LTT.
For ΔHHb in the VL and GAST, the present data were contrary to the hypothesis with ΔHHb not being different between LTT and STT during either test (Figures 2 and 3). This suggests that in young men, beyond approximately two years of aerobic training, additional training years does not independently further increase $O_2$ extraction ($\Delta$HHb) in the VL or GAST. It has been suggested that muscle tissue oxygenation ($\Delta$HHb and $\Delta O_2$Hb) correlates with $\dot{V}O_{2peak}$ in young men\textsuperscript{22,35}. However, the results of the present study do not support this, with ΔHHb in the VL and GAST being not different between LTT and STT at peak exercise despite the higher $V_{O_2}$ in the LLT.

The effect of exercise training on the peak HHb in the VL of young men has previously only been reported in one study in untrained young men. Following a short term (6 wk) high intensity running program, peak HHb was significantly higher during cycling exercise\textsuperscript{33}. In addition, changes in sub-maximal HHb have been limited to studies comparing changes in the ratio of tau (response time to a step increment in work load) HHb in the VL to tau pulmonary $V_{O_2}$ (tau ΔHHb:tau $\Delta V_{O_2}$) during transitions from low to moderate intensity exercise\textsuperscript{18-19,36-37}. The reduced $\Delta$HHb:$\Delta V_{O_2}$ ratio was reported to reflect an improved matching of $O_2$ distribution to extraction. However, tau HHb was unchanged in these previous studies\textsuperscript{18-19,36-37}.

In the present study, the difference in ΔHHb in the GAST between STT and LTT did not reach statistical significance. However, the ΔHHb was substantially higher in LTT at 90% VT and peak exercise during RI, and at 80% and 90% during SWCL, and therefore, likely to be physiologically meaningful. Due to the high between-participant variability in ΔHHb during testing, it is possible that the results may not be completely indicative of potential differences. Three studies have previously published oxygenation (HHb or $O_2$Hb) changes in the GAST during exercise\textsuperscript{6,38-39}. However, only one study from our laboratory investigated the effect of aerobic training\textsuperscript{6}. That study showed no significant difference in ΔHHb in the GAST between STT and LTT older women at peak or sub-VT exercise. This, in addition to studies in our laboratory of older men and young women showing differences in ΔHHb in the GAST between LTT compared to STT suggests that training induced changes in $O_2$ extraction in the GAST may differ as a function of age, sex and exercise intensity. Possible explanations for these varied results include sex differences in muscle fibre distribution\textsuperscript{40}, and age-related reduction in leg blood flow and perfusion pressure in older women\textsuperscript{41}.

In the present study, the ΔHHb pattern in the GAST at the onset of exercise opposed that of the VL. That is, ΔHHb in the VL gradually increasing from the onset of exercise to a plateau, whereas ΔHHb in the GAST decreased substantially at the onset of exercise then progressively increased with increased exercise intensity (Figures 1 and 2). This response has previously been reported in older women\textsuperscript{8} and young women\textsuperscript{9}. As metabolic and muscle fibre recruitment processes are influenced by fibre type characteristics (slow-twitch, low $O_2$ extraction to fast-twitch, higher $O_2$ extraction), variations
in muscle fibre characteristics between the VL and GAST may provide a possible explanation for the differences in HHb patterns\textsuperscript{13,40,42-43}.

For HHb in the PFC, the present results support the hypothesis in that ΔHHb was not different between STT and LTT in either test. This supports recent consensus that cerebral oxygenation does not limit the exercise performance of healthy young adults under normoxic conditions\textsuperscript{13,44}. A link between cerebral oxygenation, central motor drive and exercise performance emerged following reports of reduced O\textsubscript{2}Hb and increased HHb near maximal exercise\textsuperscript{14,23-24,45-48}, and increased performance following administration of O\textsubscript{2} near maximal exercise in hypoxic\textsuperscript{23,49} and normoxic conditions\textsuperscript{50}. The present results oppose suggestions that training status (aerobic capacity) impacts cerebral oxygenation\textsuperscript{48,51}. Indeed, our data showing VO\textsubscript{2} values differed between groups and ΔHHb in the PFC did not differ, suggests that aerobic capacity does not affect PFC oxygenation. However, it is possible that the difference in aerobic capacity between LTT and STT was not great enough to impact PFC oxygenation. Given that aerobic capacity only appears to impact cerebral oxygenation in high level athletes\textsuperscript{13}, it is also possible that true maximum effort was not achieved, and/or aerobic capacity was not high enough to impact PFC oxygenation.

LIMITATIONS
A potential limitation of this present study is that while regular cycling was a requirement for recruitment, the contribution cycling had on individual training volumes was not determined. Furthermore, the adipose tissue thickness of the STT was higher than the LTT. However, while high levels of adipose tissue may affect NIRS measures, all participants were within the recommended ranges (< 34 mm) for ΔHHb not to be affected\textsuperscript{52}.

PRACTICAL IMPLICATIONS AND FUTURE RESEARCH
The results of this study has implications for aerobic training in young men. Specifically, the present data suggest additional years of regular training in young men, without increasing weekly training load, may provide significant further increases in VO\textsubscript{2} with the increase resulting predominately from central not peripheral adaptations. Moreover, ΔPFC O\textsubscript{2} extraction does not change with additional years of training in young men aged 18 – 30 yr. Future research should include training studies that exceed 12 months duration and monitor central delivery to and extraction of O\textsubscript{2} in multiple peripheral tissues during various absolute and relative exercise intensities.

Conclusions
It is concluded that independent of current training load, regular aerobic training beyond approximately two years duration significantly increases the VO\textsubscript{2} of young men during ramp incremental and square-wave constant load exercise. This increase occurs without increases in tissue O\textsubscript{2} extraction in the vastus lateralis and gastrocnemius muscles.

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