Prior heavy exercise elevates pyruvate dehydrogenase activity and muscle oxygenation and speeds O$_2$ uptake kinetics during moderate exercise in older adults

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DURING THE TRANSITION TO moderate-intensity exercise there is a delay in the full activation of mitochondrial oxidative phosphorylation, as estimated by pulmonary O$_2$ uptake (V$_{O2p}$) kinetics (2, 21, 38), such that it can take several minutes before the transition to exercise (33), or the interaction between these two (22, 54). In older compared with young adults, V$_{O2p}$ kinetics, reflecting the adaptation of muscle O$_2$ utilization, are slowed during the transition to moderate- and high-intensity exercise (1, 5, 9, 11). Whether this slower response in older adults is a result of an increased sluggishness in activating muscle enzymes and providing oxidative substrate (occasionally referred to as metabolic inertia), an impaired O$_2$ delivery, or both has not been established.

Recently, we demonstrated that the activation of the mitochondrial enzyme pyruvate dehydrogenase (PDH) was attenuated during the transition to moderate-intensity exercise in older compared with young adults (23). To our knowledge this is the first direct demonstration, in older adults, that the activation of a rate-limiting oxidative enzyme is impaired during the transition to exercise. These data suggest a potential role for oxidative enzyme activation and provision of substrate to the TCA cycle and ETC in the observed slowing of V$_{O2p}$ kinetics in older adults.

There also is evidence that O$_2$ availability is impaired during the transition to exercise in older adults. Heart rate kinetics, an index of bulk blood flow, are slowed during the transition to exercise (12, 13, 51), while muscle (conduct artery) blood flow (39, 43, 44) is lower and its distribution within muscle is altered (away from more oxidative to more glycolytic fibers), thereby lowering microvascular PO$_2$ and diffusive O$_2$ delivery to active muscle (3, 11, 39, 43, 49). These results, from both human and rat work, suggest an impaired O$_2$ availability, which could delay the activation of oxidative phosphorylation and V$_{O2p}$ kinetics during the transition to exercise.

Prior heavy-intensity exercise speeds V$_{O2p}$ kinetics during subsequent moderate-intensity exercise in older adults (12, 51) and in younger adults exhibiting relatively slower V$_{O2p}$ kinetics (22, 24). In young adults, the faster V$_{O2p}$ kinetics accompanying prior heavy-intensity exercise have been attributed, in part, to either a greater activation of PDH activity (22), elevated bulk muscle blood flow (41), and/or improved local microvascular O$_2$ availability (22, 24) during baseline exercise immediately preceding the transition to moderate-intensity exercise. Whether or not the speeding of V$_{O2p}$ kinetics observed previously in older adults after prior heavy-intensity exercise (12, 51) is accompanied by a greater activation of PDH has not been examined.

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The purpose of this study was to examine the effect of a prior bout of heavy-intensity exercise on VO2p kinetics, PDH activation, muscle metabolite content, and muscle oxygenation in older adults during subsequent moderate-intensity exercise. We hypothesized that following a prior bout of heavy-intensity exercise, the transition to moderate-intensity exercise would be associated with (1) faster VO2p kinetics; (2) an elevated activity of PDH; (3) a decreased reliance on substrate-level phosphorylation, as indicated by a decreased breakdown of phosphocreatine (ΔPCR); and (4) elevated muscle oxygenation, as determined by near-infrared spectroscopy (NIRS).

METHODS

**Subjects.** Six older healthy male adults (age, 70 ± 5 yr; VO2peak, 31 ± 4 ml·kg⁻¹·min⁻¹) volunteered and gave written informed consent to participate in the study. All subjects were recreationally active but not involved in a specific training program at the time of the study. The study was approved by the University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects and conformed with the Declaration of Helsinki. Data from this group of older adults (4 out of 6) were used, in part, in a previous publication from our laboratory, which compared the responses during the transition to moderate-intensity exercise (without prior warm-up exercise) to the responses observed in a group of young adults (23).

**Exercise protocol.** Subjects reported to the laboratory on six separate occasions at approximately the same time of day and ~2 h after consuming a small meal high in carbohydrate and low in fat. On the first day of testing, subjects performed an incremental ramp exercise test (20 W/min) to the limit of tolerance on an electronically-braked cycle ergometer (model H-300-R; Lode) for determination of the estimated lactate threshold (θL) and VO2peak (for definitions see Ref. 22). From the results of this ramp test, work rates (WR) were identified that elicited a steady-state VO2p corresponding to ~90% θL (i.e., moderate-intensity exercise), and Δ50% (VO2p at θL plus ~50% of the difference between the VO2p at θL and VO2peak; i.e., heavy-intensity exercise).

During one of the subsequent five visits to the laboratory, subjects performed two step transitions in WR of moderate intensity (Mod1 and Mod2) separated by a step increase in WR of heavy intensity, as previously described (22). Briefly, three biopsy sites were prepared after which the subject was moved to the cycle ergometer and began 20-W cycling. Biopsy samples were taken after 5-min baseline cycling and at 30 s and 6 min of the transition to Mod1 while the subject remained seated on the cycle ergometer. After ~1-h resting recovery, during which time three additional biopsy sites were prepared on the opposite leg, the subject returned to the cycle ergometer and exercised for 6 min at the 20-W baseline, followed by 6 min of heavy-intensity exercise, 6-min baseline exercise (at 20 W) and 6-min moderate-intensity exercise (i.e., Mod2). Muscle biopsy samples were taken after 5-min recovery from heavy-intensity exercise (i.e., 1 min before the transition to Mod2) and at 30 s and 6 min of Mod2. Each biopsy procedure took <10 s, and additional time was added onto the protocol to account for this sampling time.

**Muscle analysis.** A small piece of frozen muscle (~10–15 mg) was chipped from each muscle sample under liquid N2 and used for determination of the active form of PDH as previously described (10, 45). The remaining muscle sample was freeze-dried, powdered, dissected free of all visible blood and connective tissue, and extracted as described previously (22). Creatine, PCR, ATP, lactate, and pyruvate were analyzed by spectrophotometric assays (6, 26), while acetyl-CoA was determined radioisotopically (8). All muscle measurements were normalized to the highest total creatine measured among the six biopsy samples from each subject.

**Calculations.** Muscle content of free ADP (ADPf) was calculated by assuming equilibrium of the creatine kinase and adenylyl kinase reactions (15). ADPf was calculated by using the measured ATP, creatine, PCR, estimated H⁺ concentration ([H⁺]), and the creatine kinase equilibrium constant of 1.66 ·10⁶; [H⁺]. It was estimated from the measured muscle pyruvate and lactate contents as described by Sahlin et al. (50). Free inorganic phosphate (Pi) was calculated by adding the estimated free phosphate content of 10.8 mmol·(kg dry wt)⁻¹ (15) to the difference in PCR content relative to the baseline value.

**Statistical analysis.** Parameter estimates for VO2p and for the two moderate-intensity exercise bouts were compared using a one-way ANOVA for repeated measures with τVO2p being compared using a one-tailed test. The active form of PDH and muscle metabolite contents were compared using a two-way ANOVA for repeated measures with main effects of exercise bout and time. Significant main effects and interactions were subsequently analyzed by using a Tukey’s post hoc test. The relationship between the Mod1 τVO2p and the Mod1–Mod2 τVO2p difference was determined by Pearson product moment correlation analysis. Statistical significance was accepted at P < 0.05. Data are presented as means ± SD.
RESULTS

V\textsubscript{O}2\textsubscript{p} kinetics. In the present study, subjects exercised at a moderate-intensity of 88 ± 7 %\textsubscript{VO}2\textsubscript{peak} [45% ± 6 V\textsubscript{O}2\textsubscript{peak}; power output, 66 ± 14 W] and at a heavy-intensity of Δ50% [~80% V\textsubscript{O}2\textsubscript{peak}; power output, 140 ± 28 W]. The V\textsubscript{O}2\textsubscript{p} response profile during the transition to each bout of moderate-intensity exercise for a representative subject (including the exponential model best-fit line) is shown in Fig. 1, while the summary of the parameter estimates for the on-transient V\textsubscript{O}2\textsubscript{p} response to Mod1 and Mod2 is presented in Table 1. The phase 2 V\textsubscript{O}2\textsubscript{p} time constant (τV\textsubscript{O}2\textsubscript{p}) was reduced (P < 0.05) in Mod2 (29 ± 5 s) compared with Mod1 (39 ± 14 s). A significant correlation (r = 0.904, P < 0.05) existed between the time course of adaptation of V\textsubscript{O}2\textsubscript{p} during Mod1 (τV\textsubscript{O}2\textsubscript{p} Mod1) and the reduction in τV\textsubscript{O}2\textsubscript{p} that occurred during Mod2 [ΔτV\textsubscript{O}2\textsubscript{p} (Mod1 − Mod2)].

Muscle PDH activity. During Mod1, PDH activity was increased above baseline (1.15 ± 0.29 mmol acetyl-CoA·min\textsuperscript{-1}·kg wet wt\textsuperscript{-1}) at 30-s exercise (1.69 ± 0.67 mmol acetyl-CoA·min\textsuperscript{-1}·kg wet wt\textsuperscript{-1}) with a further increase (P < 0.05) observed at 6-min exercise (2.31 ± 0.42 mmol acetyl-CoA·min\textsuperscript{-1}·kg wet wt\textsuperscript{-1}). After heavy-intensity exercise, PDH activity was elevated (P < 0.05) at baseline (2.13 ± 0.63 mmol acetyl-CoA·min\textsuperscript{-1}·kg wet wt\textsuperscript{-1}), with no changes observed at 30-s exercise (2.05 ± 0.58 mmol acetyl-CoA·min\textsuperscript{-1}·kg wet wt\textsuperscript{-1}) or 6-min exercise in Mod2 (2.55 ± 0.74 mmol acetyl-CoA·min\textsuperscript{-1}·kg wet wt\textsuperscript{-1}). PDH activity was not different between Mod1 and Mod2 at 30 s and 6 min of the exercise transition (Fig. 2). The apparent trend between the change in τV\textsubscript{O}2\textsubscript{p} and the change in PDH activity from Mod1 to Mod2 at baseline (Fig. 3A; P = 0.18) and 30 s of exercise (Fig. 3B; P = 0.20) did not achieve statistical significance.

Muscle metabolite content. Muscle pyruvate, lactate, and acetyl-CoA contents, and calculated [H\textsuperscript{+}] are presented in Table 2. Muscle pyruvate content did not change significantly during exercise or as a consequence of prior heavy-intensity exercise. Muscle lactate content and [H\textsuperscript{+}], were greater in Mod2 than Mod1 at baseline and after 30-s exercise. Acetyl-CoA content remained at baseline levels throughout Mod1. After heavy-intensity exercise, acetyl-CoA was greater (P < 0.05) at all times compared with Mod1 (Table 2).

Muscle contents of creatine, PCr, ATP, calculated ADP\textsubscript{r}, and Pi are presented in Table 3. Absolute PCr content and PCr breakdown (ΔPCr) are presented in Fig. 4, A and B, respectively. During Mod1, PCr content tended to be lower (P = 0.06) at 30-s exercise (64.8 ± 14.5 mmol·kg dry wt\textsuperscript{-1}) compared with baseline (83.7 ± 4.9 mmol·kg dry wt\textsuperscript{-1}), and was significantly lower (P < 0.05) after 6-min exercise (51.7 ± 17.9 mmol·kg dry wt\textsuperscript{-1}). Baseline PCr content was lower (P < 0.05) prior to Mod2 than Mod1. During the transition to Mod2 a significant decrease (P < 0.05) in PCr content was not observed until 6-min exercise (Table 3); PCr breakdown from baseline (ΔPCr) was reduced (P < 0.05) at both 30 s and 6 min in Mod2 compared with Mod1 (Fig. 4B). Changes in muscle Cr content were similar but opposite to those observed for PCr content.

Muscle ATP content was unchanged compared with baseline during both Mod1 and Mod2 (Table 3). Muscle ADP\textsubscript{r} content increased (P < 0.05) at both 30-s and 6-min exercise in Mod1. Although muscle ADP\textsubscript{r} content tended to be higher at baseline in Mod2 compared with Mod1, these apparent differences were not significant. In Mod2 a significant increase (P < 0.05) in muscle ADP\textsubscript{r} content was seen only after 6-min exercise (Table 3); the increase in muscle ADP\textsubscript{r} content after 6-min exercise was ~60% lower in Mod2 than Mod1. Increases (P < 0.05) in muscle Pi content were seen after 6-min exercise in Mod1 and Mod2, with the increase in Pi content being ~45–60% lower in Mod2 than in Mod1 (Table 3).

NIRS-derived muscle oxygenation. Δ[O\textsubscript{2}H\textsubscript{b}] decreased (P < 0.05) from baseline to end-exercise in both Mod1 and Mod2 and was elevated at both baseline and end-exercise in Mod2 compared with Mod1. Δ[H\textsubscript{bTOT}] increased (P < 0.05) from baseline to end-exercise in Mod1 and remained unchanged throughout Mod2; Δ[H\textsubscript{bTOT}] was greater (P < 0.05) at baseline
and end-exercise in Mod2 compared with Mod1 (Table 4). There were no significant differences in the time course of the \([\text{HHb}]\) response (i.e., \([\text{HHb}]\)-TD, \([\text{HHb}]\), \([\text{HHb}]\)-MRT) between Mod1 and Mod2 (Table 4), despite a significant reduction in the \(\dot{V}\text{O}_2\) in Mod2.

**DISCUSSION**

This is the first study to examine the adaptation of \(\dot{V}\text{O}_2\) kinetics reflecting the kinetics of muscle \(O_2\) utilization (2, 21, 38), activation of the mitochondrial PDH complex, changes in muscle metabolite concentrations, and changes in muscle oxygenation during the transition to moderate-intensity exercise before (Mod1) and after (Mod2) a priming bout of heavy-intensity exercise in older adults. The major new findings of the present study were that in older adults, the faster \(\dot{V}\text{O}_2\) kinetics seen during the transition to Mod2 was coincident with a significantly higher preexercise PDH activity, which was not different from that seen in the steady-state of either Mod2 or Mod1, elevated NIRS-derived markers of local muscle \(O_2\) availability (i.e., \(\Delta[O_2\text{Hb}]\) and \(\Delta[Hb\text{TOT}]\)), higher muscle contents of ADP, and lower preexercise muscle PCR content. Thus after heavy-intensity priming exercise in older adults there was a greater availability of oxidative substrates prior to the onset of Mod2, which likely attenuated the delay required for increased mitochondrial delivery of oxidative substrates (reducing equivalents, ADP, \(P_i\), and \(O_2\) delivery) during the transition to exercise and resulted in faster activation of oxidative phosphorylation as implied by the faster \(\dot{V}\text{O}_2\) kinetics during the onset of Mod2 compared with Mod1.

**Table 1. Summary of parameter estimates for \(\dot{V}\text{O}_2\) on-transient to moderate-intensity exercise in Mod1 and Mod2 in older adults**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mod1</th>
<th>Mod2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.74±0.08</td>
<td>0.91±0.07*</td>
</tr>
<tr>
<td>Amplitude</td>
<td>0.40±0.10</td>
<td>0.33±0.10*</td>
</tr>
<tr>
<td>End exercise</td>
<td>1.14±0.13</td>
<td>1.24±0.10*</td>
</tr>
<tr>
<td>(\tau\text{VO}_2), s</td>
<td>38.5±13.6</td>
<td>29.3±5.2*</td>
</tr>
</tbody>
</table>

Values are means ± SD (n = 6) in liters per minute, unless noted. Mod1, moderate-intensity exercise; Mod2, moderate-intensity exercise preceded by heavy-intensity warm-up exercise.*Significantly (\(P < 0.05\)) different from Mod1.

**Table 2. Muscle contents of pyruvate, lactate, calculated \(H^+\) concentration ([\(H^+\]_c]), and acetyl CoA during Mod1 and Mod2 in older adults**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Baseline</th>
<th>30 s</th>
<th>360 s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mod1</td>
<td>0.29±0.16</td>
<td>0.35±0.18</td>
<td>0.35±0.25</td>
</tr>
<tr>
<td>Mod2</td>
<td>0.53±0.25</td>
<td>0.39±0.22</td>
<td>0.37±0.21</td>
</tr>
</tbody>
</table>

| Lactate   |          |      |       |
| Mod1      | 10.5±3.6 | 9.7±1.2 | 18.6±12.6 |
| Mod2      | 28.5±11.8| 25.6±15.5*| 23.1±9.3 |

\([H^+]_c,10^{-6}\) mol/l

| Acetyl-CoA |          |      |       |
| Mod1      | 96.6±3.2 | 95.8±1.0| 104.9±12.7 |
| Mod2      | 115.4±12.9*| 112.4±16.3* | 109.2±9.4 |

Values are means ± SD (n = 6 for pyruvate, lactate, and \([H^+]_c\); n = 5 for acetyl-CoA) in mmol/kg dry wt.*Significantly (\(P < 0.05\)) different from Mod1.
relationship exists between the previous findings (12, 51). Previously, we reported that a linear relationship was seen also in the present study (measureable reduction in supports the contention that the likelihood of observing a phase 2 \( \dot{V}_{O2} \) kinetics (bout of heavy-intensity exercise (i.e., /H9270 Mod1 (Fig. 4), consistent with the faster \( \dot{V}_{O2} \) kinetics observed during that transition. Also, while muscle lactate content decreased during Mod2 (Table 2), re-
creased as a consequence of the heavy-intensity exercise, during the exercise transition (i.e., without prior heavy-intensity exercise) that normally found in younger adults (51). In addition, in the present study, the \( \dot{V}_{O2} \) kinetics for \( \dot{V}_{O2} \) Mod2, consistent with \( \dot{V}_{O2} \) in older adults being constrained by any one or the combined availability of these substrates in the unprimed Mod1 condition.

**PDH activity, oxygenation, and muscle \( \dot{V}_{O2} \) ADP concentration.** The mitochondrial PDH complex catalyzes the rate-limiting step for provision of carbohydrate-derived substrate to the TCA cycle (in the form of acetyl-CoA and ETC (reducing equivalents in the form of NADH) at the onset of exercise. However, the role of PDH activation in constraining the increase in oxidative metabolism, and therefore muscle \( \dot{V}_{O2} \) utilization and \( \dot{V}_{O2} \) kinetics, is controversial. Several studies have demonstrated a decrease in substrate-level phosphoryla-

\( \dot{V}_{O2} \) kinetics. In the present study, the time constant for the phase 2 \( \dot{V}_{O2} \) kinetics (\( \tau \dot{V}_{O2} \)) estimated during the transition to Mod2 in older adults (~39 s) was similar to that reported previously in older adults (~40–50 s), and was greater than that normally found in younger adults (~20–25 s) (1, 5, 9, 11, 12, 22–24, 51). In addition, in the present study, the \( \tau \dot{V}_{O2} \) for older adults was reduced when moderate-intensity exercise was performed subsequent to a bout of heavy-intensity exercise (\( \tau \dot{V}_{O2} \) Mod2, ~29 s; \( \tau \dot{V}_{O2} \) Mod1, ~39 s), consistent with previous findings (12, 51). Previously, we reported that a linear relationship exists between the \( \tau \dot{V}_{O2} \) estimated during the transition to Mod1 (i.e., without prior heavy-intensity exercise) and the reduction in the \( \tau \dot{V}_{O2} \) that occurs subsequent to a prior bout of heavy-intensity exercise (i.e., \( \tau \dot{V}_{O2} \) Mod1 – \( \tau \dot{V}_{O2} \) Mod2) in both young (22, 24) and older adults (51). This relationship was seen also in the present study (\( r = 0.904 \)) and supports the contention that the likelihood of observing a measureable reduction in \( \tau \dot{V}_{O2} \) is greater in subjects presenting with slower (i.e., \( \tau \dot{V}_{O2} \) ~40s) rather than faster (i.e., \( \tau \dot{V}_{O2} \) ~20 s) \( \dot{V}_{O2} \) kinetics.

Faster \( \dot{V}_{O2} \) kinetics, reflecting faster activation of mitochondrial oxidative phosphorylation and muscle \( \dot{V}_{O2} \) utilization (2, 21, 38) is expected to reduce the requirement for substrate-level phosphorylation (and nonoxidative ATP synthesis), thereby lowering PCR breakdown and lactate accumulation during the exercise transition (7, 31, 40, 46, 53). In the present study, PCR breakdown was reduced in Mod2 compared with Mod1 (Fig. 4), consistent with the faster \( \dot{V}_{O2} \) kinetics observed during that transition. Also, while muscle lactate content increased as a consequence of the heavy-intensity exercise, muscle lactate content decreased during Mod2 (Table 2), reflecting decreased lactate production through glycolysis, enhanced conversion to pyruvate (catalyzed by LDH), and subsequent oxidation through the mitochondrial PDH reaction, enhanced lactate efflux from muscle, or a combination of these factors during the transition to Mod2 (18).

### Table 3. Muscle contents of creatine, ATP, calculated free ADP (ADPf), and Pi during Mod1 and Mod2 in older adults

<table>
<thead>
<tr>
<th>Condition</th>
<th>Baseline</th>
<th>30 s</th>
<th>360 s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mod1</td>
<td>12.0±6.8</td>
<td>30.6±7.4†</td>
<td>44.9±9.9†</td>
</tr>
<tr>
<td>Mod2</td>
<td>21.1±7.5*</td>
<td>27.2±6.7</td>
<td>35.3±8.2†</td>
</tr>
<tr>
<td>PCr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mod1</td>
<td>83.7±4.9</td>
<td>64.8±14.5</td>
<td>51.7±17.9†</td>
</tr>
<tr>
<td>Mod2</td>
<td>75.8±7.1*</td>
<td>68.2±6.0</td>
<td>61.1±9.2†</td>
</tr>
<tr>
<td>ATP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mod1</td>
<td>32.8±10.5</td>
<td>30.7±10.6</td>
<td>29.9±9.2</td>
</tr>
<tr>
<td>Mod2</td>
<td>27.2±4.8</td>
<td>32.1±6.7</td>
<td>25.1±2.9</td>
</tr>
<tr>
<td>ADPf</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mod1</td>
<td>48.6±12.6</td>
<td>99.5±46.5†</td>
<td>149.8±34.2‡†</td>
</tr>
<tr>
<td>Mod2</td>
<td>66.5±34.4</td>
<td>90.7±35.0</td>
<td>106.4±53.2‡†</td>
</tr>
<tr>
<td>Pi</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mod1</td>
<td>10.8</td>
<td>29.4±17.2†</td>
<td>41.7±24.4†</td>
</tr>
<tr>
<td>Mod2</td>
<td>19.9±9.5</td>
<td>27.1±9.4</td>
<td>34.1±13.1†</td>
</tr>
</tbody>
</table>

*Values are means ± SD (n = 6) in mmol/kg dry wt except ADPf, which is in μmol/kg dry wt. PCR, phosphocreatine. *P < 0.05 vs. Mod1; †P < 0.05 vs. baseline. ‡P < 0.05 vs. 30 s.

**Fig. 4.** Muscle phosphocreatine (PCR) at baseline and at 30 s and 6 min of moderate-intensity exercise during Mod1 (black bars) and Mod2 (white bars) (A) with net breakdown of PCR (∆PCR) in Mod1 (black bar) and Mod2 (white bar) between baseline and 30 s and baseline and 360 s (B). Values are means ± SD in mmol/kg dry wt. *P < 0.05 vs. Mod1; †P < 0.05 vs. baseline.
The limited number of older subjects (despite a reasonably strong positive relationship being seen in PDH activation kinetics and slow VO₂p kinetics, a prior bout of exercise cannot dismiss the findings that in older adults having slowed VO₂p kinetics is consistent with PDH activation playing a significant role in oxidative phosphorylation.

Table 4. Summary of parameter estimates for near-infrared spectroscopy response to cycle ergometer exercise in Mod1 and Mod2 in older adults

<table>
<thead>
<tr>
<th></th>
<th>Mod1</th>
<th>Mod2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ[O₂Hb]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline, μM</td>
<td>1.8±4.7</td>
<td>22.8±5.7*</td>
</tr>
<tr>
<td>End exercise, μM</td>
<td>-1.5±3.7†</td>
<td>6.0±3.2*‡</td>
</tr>
<tr>
<td>Δ[Hb]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline, μM</td>
<td>-5.0±9.1†</td>
<td>15.8±9.3*</td>
</tr>
<tr>
<td>End exercise, μM</td>
<td>4.4±8.8†</td>
<td>13.6±8.7*</td>
</tr>
</tbody>
</table>

Values are means ± SD (n = 6) and represent the change [Hb] from a 0 set baseline before the start of cycling exercise. Δ[Hb]-TD, time delay; Δ[Hb]-MRT, HHb mean response time (τ + TD). *P < 0.05 vs. Mod1; †P < 0.05 vs. baseline.

In contrast to young adults in this and in our previous study (23), there was a demonstrated delay in achieving the full, steady-state activation of PDH in older adults. These results are possibly consistent with an attenuated activation of PDH contributing, in part, to the relatively slow VO₂p kinetics observed in older adults in Mod1, while the elimination of this delay during the transition to Mod2, a consequence of prior exercise, would be expected to deliver greater substrate to the TCA cycle and ETC and thus possibly contribute to the faster VO₂p kinetics in Mod2. In this study, a significant relationship between the changes in τVO₂p and the changes in PDH activity was not observed between Mod1 and Mod2 at either baseline (P = 0.18; Fig. 3A) or 30 s of exercise (P = 0.20; Fig. 3B), despite a reasonably strong positive relationship being seen in this limited number of older subjects (r = 0.6). However, we cannot dismiss the findings that in older adults having slowed PDH activation kinetics and slow VO₂p kinetics, a prior bout of heavy-intensity exercise resulted in faster VO₂p kinetics and that this speeding occurred in association with an elevated PDH activity and removal of the delay in activating PDH to the required steady-state level.

PDH activation can also provide information about the activation status of other rate-determining enzymes, specifically within the TCA cycle, as these enzymes are activated by many of the same metabolites responsible for activating PDH (52). Thus the elevated PDH activity prior to Mod2 suggests that other dehydrogenases within the TCA cycle also were activated to increase TCA cycle flux and provide reducing equivalents for the ETC during the transition to Mod2. In support of this is the observation that contraction-induced activation of oxidative phosphorylation in Xenopus single muscle fibers resulted in a faster fall in intracellular PO₂ [estimated MRT ~26 s (29)] than was observed following a DCA-mediated activation of PDH [estimated MRT ~33 s (32)], suggesting that full activation of oxidative phosphorylation requires more than just priming the mitochondrial PDH complex.

In addition to elevated PDH activity, the NIRS-derived nitric oxide concentration changes in muscle oxygenation (Δ[O₂Hb]) and total hemoglobin-myoglobin were both elevated following the heavy-intensity exercise and throughout Mod2. These findings are consistent with previous studies examining prior exercise in both young and older adults and suggest a greater muscle perfusion prior to and throughout Mod2 (12, 22, 24). A greater muscle oxygenation is consistent with previous demonstrations of increased HR in older adults (12, 51) and greater limb conduit artery blood flow in young adults (16, 17, 41) during baseline exercise following heavy-intensity exercise and very early in the transition to a subsequent bout of exercise. There is evidence that O₂ availability is impaired during the transition to exercise in muscle from older animals and human subjects (3, 11, 12, 14, 39, 43, 44, 49), and thus prior exercise in older adults likely improves O₂ availability in Mod2 compared with Mod1. A greater muscle perfusion would improve the ratio of local muscle perfusion to O₂ utilization during the exercise transition, and thereby reduce the reliance on fractional O₂ extraction. Given an increase in perfusion (relative to O₂ utilization), the fall in microvascular PO₂ should be attenuated, thereby maintaining a higher O₂ driving pressure for diffusive O₂ delivery from the microvascular red blood cell to the muscle mitochondria. Although Behnke et al. (4) reported a more rapid fall in microvascular PO₂ during the second of two contraction bouts in rat spinotrapezius muscle preparations, likely a consequence of faster muscle O₂ utilization kinetics, a transient undershoot in microvascular PO₂, which is a feature of older animal preparations (3) was not observed, suggesting the limitations may be different in young and old animals. Also, the greater Δ[HbTOT] (and microvascular hematocrit) would increase the functional capillary surface area for O₂ diffusion, thereby contributing to a higher diffusional O₂ conductance between the microvasculature and muscle.

Changes in the NIRS-derived deoxyhemoglobin concentration (Δ[HHb]) reflect the balance between O₂ availability and utilization in the microvasculature surrounding the muscle and therefore provide an index of fractional O₂ extraction. In the present study, there was no significant difference in the time course of Δ[HHb], although the group mean τΔ[HHb] and τΔ[HHb]-MRT were both ~13 s greater in Mod2 despite faster VO₂p kinetics (τVO₂p ~10 s lower in Mod2). The similar (or trend towards slower) deoxygenation kinetics agrees with previous reports (12, 22) and is consistent with a greater muscle blood flow and O₂ delivery to muscle O₂ demand during the transition to Mod2.

A higher mitochondrial ADPf and Pi at baseline also may have contributed to the faster VO₂p kinetics observed in this study during Mod2. Baseline muscle PCr content was lower in Mod2 compared with Mod1 likely due to inadequate recovery time. This is consistent with findings of lower baseline PCr following heavy-intensity exercise from both biopsy studies (18) and magnetic resonance spectroscopy (48). It was dem-
shown recently that an intact PCR system serves as an energy buffer to limit the increase in cytosolic and mitochondrial ADP. In studies utilizing iodoacetamide-induced creatine kinase (CK) inhibition (27, 36) and CK knockout mice (25), muscle oxidative phosphorylation was activated more quickly than under control conditions when an active PCR system would normally buffer the increase in ADP. Glancy et al. (19) demonstrated that the time constant of O2 utilization by isolated mitochondrial preparations was related linearly to the total creatine (and PCR content) of the preparation. Also, Jones et al. (35) observed slower PCR kinetics when muscle PCR content was increased following a 5-day period of creatine loading, although pulmonary O2 uptake kinetics were not changed by a similar creatine loading regime (34a). Thus the lower baseline PCR content before Mod2 in the current study may contribute to faster muscle O2 utilization kinetics by shifting the CK reaction toward a higher ADP-to-ATP ratio during the exercise transition providing a greater drive for oxidative phosphorylation and resulting in faster VO2p kinetics.

In the present study, the baseline muscle ADPf content, while tending to be higher, was not significant, which may reflect the fact that the ADPf represents a calculated, whole muscle value, which may not directly reflect a mitochondrial ADPf concentration.

Muscle ADPf content was lower at end-exercise in Mod2 compared with Mod1, in spite of a higher end-exercise VO2p. The muscle ADPf content required to support a given steady-state rate of oxidative ATP synthesis is expected to be lower if provision of the other oxidative substrates is greater (28, 30, 56). As discussed above, provision of O2 and NADH may have been higher in Mod2, which may have reduced the requirement for a higher ADPf content to support oxidative phosphorylation. Thus the findings of elevated muscle PDH activity, NIRS-derived Δ[HbT] and Δ[HbT], and higher muscle contents of ADPf and P, prior to the onset of Mod2 compared with Mod1 suggest that the speeding of VO2p kinetics observed after heavy-intensity priming exercise in the older adults is a result of attenuating the delay in activating rate-limiting mitochondrial enzymes and providing substrate (reducing equivalents, ADP, P) including O2 for mitochondrial oxidative phosphorylation.

**Perspectives and Significance**

This study has provided evidence that prior heavy-intensity exercise improves control of oxidative phosphorylation in older adults. This improvement would be expected to increase exercise tolerance, and thus, if applied appropriately, these findings may have practical implications for exercise prescription in older populations. In addition, we have demonstrated that this improvement in the control of oxidative phosphorylation in older adults appears to be the result of an integrative upregulation in the provision of all oxidative substrate (O2, elevated HbT, and O2Hb; NADH, elevated PDH activity; ADP, lowered baseline PCR). Further work examining VO2p kinetics and exercise tolerance in older adults should therefore attempt to apply an integrative approach that examines the roles of all oxidative substrate.

In conclusion, this study demonstrated faster VO2p kinetics in older adults during the transition to moderate-intensity exercise when the transition was preceded by a bout of heavy-intensity exercise. Consistent with a greater contribution of oxidative phosphorylation during the transition to Mod2, there was a reduced net breakdown of PCR and a fall in muscle lactate content. An elevated PDH activity at baseline, which attenuated any delay in reaching the required steady-state PDH activity, and muscle content of ADPf and P, and greater NIRS-derived Δ[Hb] and Δ[HbT] combined to attenuate any delay in delivering substrate for oxidative phosphorylation, and likely contributed to the faster adjustment of muscle O2 utilization and VO2p kinetics during Mod2. In addition, these results are consistent with the slowed VO2p kinetics typically seen in older adults as being the result of an impaired metabolic activation, in part related to the mitochondrial PDH complex (and/or possibly other oxidative rate-determining enzymes), in combination with impaired O2 availability.

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