O₂ uptake kinetics, pyruvate dehydrogenase activity, and muscle deoxygenation in young and older adults during the transition to moderate-intensity exercise


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The adaptation of pulmonary O₂ uptake (V₂O₂p) kinetics is slowed in older compared with young adults during the transition to moderate-intensity exercise. In this study, we examined the relationship between V₂O₂p kinetics and mitochondrial pyruvate dehydrogenase (PDH) activity in young (n = 7) and older (n = 6) adults. Subjects performed cycle exercise to a work rate corresponding to 90% of estimated lactate threshold. Phase 2 V₂O₂p kinetics were slower (P < 0.05) in older (τ = 40 ± 17 s) compared with young (τ = 21 ± 6 s) adults. Relative phosphocreatine (PCr) breakdown was greater (P < 0.05) at 30 s in older compared with young adults. Absolute PCr breakdown at 6 min was greater (P < 0.05) in older compared with young adults. In young adults, PDH activity increased (P < 0.05) from baseline to 30 s, with no further change observed at 6 min. In older adults, PDH activity during baseline exercise was similar to that seen in young adults. During the exercise transition, PDH activity did not increase (P > 0.05) at 30 s of exercise but was elevated (P < 0.05) after 6 min. The change in deoxyhemoglobin (HHb) was greater for a given V₂O₂p in older adults, and there was a similar time course of HHb accompanying the slower V₂O₂p kinetics in the older adults, suggesting a slower adaptation of bulk O₂ delivery in older adults. In conclusion, the slower adaptation of V₂O₂p in older adults is likely a result of both an increased metabolic inertia and lower O₂ availability.

 adaptation of muscle O₂ consumption during the transition to exercise.

In healthy older adults, the adaptation of V₂O₂p (and presumably muscle O₂ consumption) is slowed during the transition to exercise (1, 6, 12, 16). However, what has not been established is whether this slower response is a result of control imposed by impaired O₂ delivery or metabolic inertia. There is evidence that muscle O₂ delivery is attenuated in the older adult and may contribute to slower V₂O₂p kinetics (i.e., lower heart rate and/or slower heart rate kinetics (12, 16, 17, 42), lower steady-state limb blood flow (18, 34, 35), and indications of impaired local microvascular flow in both animals (5, 16, 30, 34, 40) and humans (16, 34)). However, there is a paucity of information on muscle metabolic adaptations during the transition to exercise in older adults. It was shown that the stimulation of respiration by the addition of oxidative substrate (ADP and ADP + Cr) was blunted in skinned muscle fibers and isolated mitochondria taken from older compared with young adults (45). Also, an age-associated decline in electron transport chain (ETC) activity was observed in human (9), monkey (50), and rat (2) muscle. Thus it also is possible that a sluggish activation of muscle metabolism (i.e., metabolic inertia) may contribute to the slower V₂O₂p kinetics during the transition to exercise in older adults.

In young adults, the mitochondrial enzyme pyruvate dehydrogenase (PDH) has been proposed as a possible site of metabolic inertia, since it controls the entry of carbohydrate-derived substrate into the TCA cycle and thus the provision of reducing equivalents (in the form of NADH) to the ETC. Prior activation of PDH (using dichloroacetate or prior exercise) was shown to lower lactate accumulation and PCr breakdown (25, 44), speed the fall in intracellular Po₂ (26), and speed the activation of V₂O₂p (20). Although there is evidence that resting PDH activity is lower in heart muscle of older monkeys (50) and rats (31), we are not aware of any studies that have examined the effects of ageing on the activation of mitochondrial PDH and its relationship to the adaptation of V₂O₂p during the transition to exercise in humans.

Therefore, the purpose of the present study was to examine the relationship between pulmonary V₂O₂ kinetics, activation of mitochondrial PDH (i.e., PDH activity), and the rate of muscle...
deoxygenation (an index of the balance between O2 delivery and O2 utilization) during the transition to moderate-intensity exercise in healthy young and older adults. We hypothesized that V\textsubscript{O2p} kinetics would be slowed in older compared with younger adults and that this response would be accompanied by a slower activation of the mitochondrial PDH complex and an increased reliance on substrate-level phosphorylation as demonstrated by a greater breakdown of muscle PCr. Also, the time course of muscle deoxyhemoglobin would be similar despite the slower V\textsubscript{O2p} kinetics suggestive of a slowed adaptation of bulk O2 delivery.

METHODS

Subjects. Seven young [age, 24 ± 3 yr; \textit{V}\textsubscript{O2peak}, 51 ± 3 (SD) ml·kg\textsuperscript{-1}·min\textsuperscript{-1}] and six older (age, 70 ± 5 yr; \textit{V}\textsubscript{O2peak}, 31 ± 4 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}) healthy adults volunteered and gave written informed consent to participate in the study. Subjects were recreationally active but not involved in a training program during participation in the study. The study was approved by The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects and conformed to the Declaration of Helsinki.

Exercise protocol. On six separate occasions, subjects reported to the laboratory at approximately the same time of day for each subject and ~2 h after consuming a small meal high in carbohydrate and low in fat. An incremental ramp exercise test (young, 25 W/min; old, 20 W/min) to the limit of tolerance on an electronically braked cycle ergometer (model H-300-R; Lode) was performed on the first day of testing for determination of the estimated lactate threshold (\textit{\theta}L) and \textit{V}\textsubscript{O2peak}, as described previously (23). These results were used for determination of a moderate-intensity work rate (WR) that elicited a steady-state \textit{V}\textsubscript{O2} corresponding to ~90% \textit{\theta}L.

During four visits to the laboratory, subjects performed step transitions to the moderate-intensity WR; the duration of each step transition was 6 min, and each transition was preceded by 6 min of baseline (20 W) cycling. The time delay between gas exchange onset and the time where \textit{DH}Hb showed a consistent increase in response (HHb-TD) was described previously (23). The subsequent increase in \textit{DH}Hb was modeled using the exponential function described in Eq. 1 as described previously (20). The mean response time (MRT = HHb - TD + \textit{HH}b) was calculated to provide a description of the overall time course for muscle \textit{DH}Hb. Analysis of the \textit{DO}2Hb and \textit{DH}Hb\textsubscript{TOT} signals was limited to determining the steady-state baseline and end-exercise values.

Muscle sampling. During one visit to the laboratory, muscle biopsies were obtained from the vastus lateralis muscle using the needle biopsy technique (8). Before exercise, three biopsy sites were prepared by making incisions through the skin to the deep fascia under local anesthesia (2% lidocaine without epinephrine). The subject then moved to the cycle ergometer and began baseline cycling at 20 W. A muscle biopsy sample was taken after 5 min baseline cycling and again at 30 and 360 s of the transition to moderate-intensity exercise. Muscle biopsy samples were frozen immediately in liquid N\textsubscript{2} and stored at -80°C until analyzed.

Muscle analysis. Near-infrared spectroscopy. Near-infrared spectroscopy (NIRS; Hamamatsu NIRO 300, Hamamatsu Photonics KK, Japan) was used to measure changes in concentration of local muscle oxy (\textit{DO}2Hb\textsubscript{oxy}), deoxy (\textit{DH}Hb\textsubscript{deoxy}), and total (\textit{DH}Hb\textsubscript{TOT}) hemoglobin + myoglobin of the vastus lateralis muscle as described previously (16). Briefly, optodes were housed in an optically dense holder (intertrode spacing, 5 cm) and secured to the skin surface over the muscle belly midway between the lateral epicondyle and greater trochanter of the femur. The intensity of incident and transmitted light was recorded continuously at 1 Hz and, along with relevant specific extinction coefficients and optical path-length (differential path-length factor assumed = 3.83), used for online estimation of concentration changes (relative to a “zero” set at resting baseline before the start of the protocol) in O2Hb, HHb, and Hb\textsubscript{TOT}.

The NIRS-derived \textit{DH}Hb signal is a reliable estimator of changes in intramuscular deoxygenation and represents the balance between local muscle O2 delivery and O2 utilization, which reflects microvascular O2 and muscle O2 extraction within the NIRS field of interrogation (15, 20).

Modeling of the \textit{V}\textsubscript{O2} and NIRS responses. Breath-by-breath \textit{V}\textsubscript{O2} data from each transition were filtered and linearly interpolated to 1-s intervals, time aligned, and ensemble averaged to yield a single profile, and then time averaged into 10-s bins to give a single response for each subject. The on-transient phase 2 \textit{V}\textsubscript{O2} responses to moderate exercise were modeled using a monoeXponential of the form:

\[
Y(t) = Y_{\text{BLSL}} + \text{Amp} \times \{1 - e^{-t/\tau}\}
\]

where \textit{Y}(\textit{t}) represents the variable at any time (\textit{t}), \textit{Y}_{\text{BLSL}} is the baseline value of \textit{Y} before the step increase in \textit{WR}, \textit{Amp} is the amplitude (i.e., steady-state increase in \textit{Y} above baseline), \textit{\tau} is the time constant (i.e., the time taken to reach 63% of the steady-state response), and TD is the time delay.

Estimation of the time delay between exercise onset and the time where \textit{DH}Hb showed a consistent increase in response (HHb-TD) was described previously (23). The subsequent increase in DHHb was modeled using the exponential function described in Eq. 1 as described previously (20). The mean response time (MRT = HHb - TD + \textit{HH}b) was calculated to provide a description of the overall time course for muscle \textit{DH}Hb. Analysis of the \textit{DO}2Hb and \textit{DH}Hb\textsubscript{TOT} signals was limited to determining the steady-state baseline and end-exercise values.

Calculations. Muscle contents of free ADP (ADP\textsubscript{f}) and AMP were calculated by assuming equilibrium of the creatine kinase and adenylyl kinase reactions, respectively (19). ADP\textsubscript{f} was calculated by using the measured ATP, Cr, PCr, estimated H\textsuperscript{+} concentration, and the creatine kinase equilibrium constant of 1.66 × 10\textsuperscript{6}. H\textsuperscript{+} concentration was calculated from the measured pyruvate and lactate contents as described by Sahlin et al. (41). Free inorganic phosphate in exercise was calculated by assuming an estimated free phosphate content at rest of 10.8 mmol/kg dry wt (19) and adding this value to the difference in PCr content relative to the baseline value (24).

Statistical analysis. Parameter estimates for \textit{V}\textsubscript{O2} were compared using a one-way ANOVA. PDH activity and muscle metabolite contents were compared using a two-way ANOVA for repeated measures with main effects of age and time. Significant main effects and interactions were subsequently analyzed by using a Tukey’s post...
RESULTS

**VO2 kinetics and respiratory exchange ratio.** In the present study, moderate-intensity exercise represented 89 ± 4% \( \theta_h \) (52 ± 3% \( \dot{V}O_{2peak} \) at a power output of 100 ± 15 W) for young adults and 86 ± 6% \( \theta_h \) (45 ± 3% \( \dot{V}O_{2peak} \) at a power output of 65 ± 12 W) for older individuals; intensity expressed as percent \( \dot{V}O_{2peak} \) was lower \((P < 0.05)\) in the older adults. A summary of the parameter estimates for the on-transient \( \dot{V}O_{2p} \) response for young and older adults is presented in Table 1, and the \( \dot{V}O_{2p} \) responses for a representative young and older adult (with the exponential model line-of-best-fit) are shown in Fig. 1. The phase 2 \( \dot{V}O_2 \) time constant (\( r\dot{V}O_2 \)) was lower \((P < 0.05)\) in the young (21 ± 6 s) compared with older adults (40 ± 17 s).

The respiratory exchange ratio (RER; \( \dot{V}CO_2/\dot{V}O_2 \)) during steady-state baseline exercise was not different between the young (0.92 ± 0.03) and older (0.93 ± 0.03) adults. The RER increased \((P < 0.05)\) in both groups during the transition to moderate-intensity exercise, but the steady-state RER at end exercise was not different between groups (young, 0.97 ± 0.03; old, 0.99 ± 0.07).

**Muscle PDH activity.** The group mean response for PDH activity during the transition to moderate-intensity exercise in young and older adults is presented in Fig. 2A. In young adults, PDH activity increased \((P < 0.05)\) above baseline levels by 30 s exercise, with no further change observed at 6 min exercise. In older adults, however, an increase \((P < 0.05)\) in PDH activity was not observed until later in exercise (i.e., at 6 min) (Fig. 2A). Also, the increase in PDH activity relative to the increase in WR \( \DeltaPDH activity/\DeltaWR \) was not different between age groups at 30 s exercise (young, 0.012 ± 0.008 mmol acetyl CoA·min\(^{-1}\)·kg wet wt\(^{-1}\)·W\(^{-1}\); old, 0.006 ± 0.011 mmol acetyl CoA·min\(^{-1}\)·kg wet wt\(^{-1}\)·W\(^{-1}\)), but, at end-exercise, the \( \DeltaPDH activity/\DeltaWR \) was greater \((P < 0.05)\) in older (0.023 ± 0.009 mmol acetyl CoA·min\(^{-1}\)·kg wet wt\(^{-1}\)·W\(^{-1}\)) than young (0.013 ± 0.008 mmol acetyl CoA·min\(^{-1}\)·kg wet wt\(^{-1}\)·W\(^{-1}\)) (Fig. 2B).

**Muscle metabolite content.** Muscle pyruvate content did not change during the transition to moderate-intensity exercise in either the young or older adults and was not different between age groups at any time point (Table 2). The muscle lactate content and the calculated [H\(^+\)] did not change during moderate-intensity exercise in either age group (Table 2).

In young adults, muscle acetyl-CoA content increased throughout moderate-intensity exercise, reaching significance \((P < 0.05)\) at 6 min relative to both baseline and 30 s (Table 2). Acetyl-CoA content did not change during moderate-intensity exercise in older adults and was lower \((P < 0.05)\) compared with young adults at end exercise (Table 2).

Changes in muscle PCr content for young and older adults are presented in Table 3 and Fig. 3A, and PCr breakdown expressed as the change in PCr content from baseline relative to the increase in WR \( \DeltaPCr/\DeltaWR \) exercise is shown in Fig. 3B. PCr breakdown (expressed as \( \DeltaPCr/\DeltaWR \)) was greater \((P < 0.05)\) in older adults at both 30 s and 6 min of exercise (Fig. 3B). Muscle Cr content showed a reciprocal change during exercise in both young and older adults (Table 3). Muscle ATP content was similar in young and older adults and did not change from baseline at any point during exercise. In young adults, muscle ADP\(_f\) content remained at baseline levels throughout exercise. However, in older adults, ADP\(_f\) content was greater \((P < 0.05)\) at end exercise compared with baseline and 30 s exercise and was greater \((P < 0.05)\) than the ADP\(_f\) content seen in the young adults (Table 3).

NIRS-derived muscle oxygenation. A summary of the parameter estimates for the change in concentration of muscle deoxyhemoglobin-myglobin (i.e., \( \DeltaHHb \)) is presented in Table 4, and representative profiles of the \( \DeltaHHb \) response in young and older adults is presented in Fig. 1. No differences were observed in the time course of muscle deoxygenation (i.e., \( \DeltaHHb-TD \), \( \tau\HHb \), \( \DeltaHHb-MRT \)) during the transition to moderate-intensity exercise in young and older adults (Table 4). The change in \( \DeltaHHb \) relative to the change in \( \dot{V}O_2 (\Delta\dot{V}O_2/\Delta\dot{V}O_2) \) was greater \((P < 0.05)\) in older compared with young adults.

There were no differences in the change in concentration of muscle oxyhemoglobin-myglobin (i.e., \( \DeltaO2Hb \)) at baseline (young, −0.5 ± 5.6 μM; old, 4.8 ± 6.6 μM) or end exercise (young, −1.1 ± 5.8 μM; old, 3.6 ± 7.6 μM) between age groups or across exercise time. Also, the change in concentration in total hemoglobin-myglobin (i.e., \( \DeltaHbTOT \)) was not different between groups at baseline (young, −10.9 ± 8.8 μM; old, −0.8 ± 6.4 μM) and end exercise (young −3.1 ± 8.7 μM; old, 6.7 ± 12.5 μM) but was elevated \((P < 0.05)\) compared with baseline in both groups.

**DISCUSSION**

This is the first study to examine the adaptation of \( \dot{V}O_{2p} \), the activation of mitochondrial PDH, changes in muscle metabolite concentration, and muscle deoxygenation status and their control in young and older adults during the transition to constant-load, moderate-intensity exercise. The major new finding of this study was that the slower \( \dot{V}O_{2p} \) kinetics observed in older compared with younger adults at the onset of moderate-intensity exercise was accompanied by a slower activation of PDH in the first 30 s of exercise. This suggests that a slowed activation of PDH and provision of substrates to the mitochondrial TCA cycle and ETC may, in part, contribute to the slowed \( \dot{V}O_{2p} \) kinetics (reflecting a slower rate of increase in muscle \( O_2 \) utilization) in older adults. Also, the slower \( \dot{V}O_{2p} \) and PDH kinetics observed in older adults were accompanied...
by a greater substrate-level phosphorylation as demonstrated by a greater absolute PCR breakdown (at 6 min exercise) and a greater relative PCR breakdown (ΔPCr/ΔWR) (at 30 s and 6 min exercise). There was also a greater muscle deoxygenation (ΔHHb) amplitude relative to V˙O₂p but similar time course for muscle deoxygenation despite slower V˙O₂p kinetics in the older adults, reflecting a greater local muscle O₂ extraction (relative to O₂ availability) and lower blood flow within muscle in older compared with young adults.

The adaptation of oxidative phosphorylation during the transition to exercise is determined by the relative concentrations of NADH, O₂, and ADP (48). Provision of NADH to the ETC is determined by PDH activation and TCA cycle flux; O₂ availability is determined by convective and diffusive delivery of O₂ to and within muscle; and ADP concentration is determined by an imbalance between ATP breakdown and ATP resynthesis by means of substrate-level and oxidative phosphorylations. Therefore, an inability to provide one or more of these oxidative substrates in adequate amounts may limit the rate of increase of muscle O₂ utilization during the transition to exercise in older adults.

**Relationship between V˙O₂ kinetics and PDH activation.** In the present study, V˙O₂p kinetics was measured to provide a reliable estimate (i.e., within 10%) of the adaptation of muscle O₂ consumption (3, 22). We observed slower V˙O₂p kinetics in older adults, consistent with the findings of previous studies (1, 6, 12, 16, 42), and further demonstrated herein that the slower V˙O₂p (and muscle O₂ consumption) kinetics were associated with a slower activation of PDH during the transition to moderate-intensity exercise. In young adults, PDH activation

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Fig. 1. Absolute V˙O₂ (A) and change in deoxyhemoglobin (ΔHHb; B) response of a representative young (C, black line-of-best-fit) and older (● and gray line-of-best-fit) adult during the transition to moderate-intensity exercise. Inset shows the normalized V˙O₂ and ΔHHb response (ΔV˙O₂ and ΔHHb above baseline, 0–100%) (with line-of-best-fit) for these two representative subjects.
has been implicated as a possible site of metabolic inertia (44) either by limiting entry of carbohydrate-derived substrate into the TCA cycle (25, 32) or by limiting acetyl group availability during the transition to exercise (37). In older adults, PDH activity remained at baseline levels during the initial 30 s of exercise, with a significant increase not occurring until after 30 s of the exercise transition (Fig. 2). This suggests that the required flux through PDH needed to meet substrate demand by the TCA cycle and ETC (i.e., provision of acetyl-CoA and reducing equivalents, respectively) had not yet been achieved by 30 s exercise. Also, a decrease in acetyl-CoA was not observed in either age group (Table 2), suggesting that acetyl-CoA availability per se may not contribute to metabolic inertia in healthy older or younger adults. Therefore, in older adults, a slower transition of the PDH complex to a more active form has been implicated as a possible site of metabolic inertia (44)

Fig. 2. Pyruvate dehydrogenase activity (PDHa) at rest and at 30 s and 6 min of moderate-intensity exercise for young (open bars) and older (filled bars) adults (A) and the change in PDH activity relative to work rate (WR; ∆PDH activity/∆WR; B). Values are means ± SD. P < 0.05, significant difference vs. young (*), vs. baseline exercise (†), and vs. 30 s exercise (‡).

Table 2. Muscle contents of pyruvate, lactate, c[H⁺], and acetyl-CoA for young and older adults during baseline and moderate-intensity exercise

<table>
<thead>
<tr>
<th>Condition</th>
<th>Baseline</th>
<th>Moderate Exercise</th>
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<tr>
<td></td>
<td></td>
<td>30 s</td>
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<tr>
<td>Pyruvate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>0.23 ± 0.14</td>
<td>0.25 ± 0.16</td>
</tr>
<tr>
<td>Old</td>
<td>0.28 ± 0.14</td>
<td>0.35 ± 0.16</td>
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<tr>
<td>Lactate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>12.3 ± 4.0</td>
<td>14.8 ± 6.8</td>
</tr>
<tr>
<td>Old</td>
<td>10.9 ± 3.3</td>
<td>10.9 ± 3.3</td>
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<tr>
<td>C[H⁺]⁺</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>98.2 ± 3.7</td>
<td>100.7 ± 6.5</td>
</tr>
<tr>
<td>Old</td>
<td>96.9 ± 3.0</td>
<td>97.0 ± 3.1</td>
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<tr>
<td>Acetyl-CoA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>4.3 ± 1.0</td>
<td>5.2 ± 2.1</td>
</tr>
<tr>
<td>Old</td>
<td>4.2 ± 1.1</td>
<td>4.1 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SD. Muscle content values are expressed as mmol/kg dry wt except for calculated H⁺ concentration (c[H⁺⁺]), which is expresses as 10⁻³ mol/l. * P < 0.05, significant difference vs. young adults (+), vs. baseline exercise (†), and vs. 30 s exercise (‡).

Fig. 3. A: muscle phosphocreatine (PCr) content at baseline and 30 s and 6 min of moderate-intensity exercise in young (open bars) and older (filled bars) adults. B: changes in PCr content from rest relative to the change in WR. Values are means ± SD. P < 0.05, significant difference vs. young at same time point (*), vs. baseline exercise (†), and vs. 30 s exercise (‡).
within the first 30 s of exercise results in a slower rate of provision of oxidative substrate to the TCA cycle and ETC, which potentially limits the rate of activation of oxidative phosphorylation.

Although a slower PDH activation was observed in older adults early in the exercise transition, the PDH activity at 6 min exercise was not different between age groups despite the older adults exercising at a lower WR, and thus a lower ATP requirement and lower steady-state VO₂p. This greater activation of PDH relative to the ATP or O₂ requirements of exercise in the older adults suggests that a greater metabolic activation is required to maintain a similar relative intensity in older adults.

Mechanism of PDH activation. The mechanism responsible for the slower increase in PDH activity within the first 30 s of exercise in older adults is not known. However, it does not appear that the initial activation state of PDH nor substrate utilization was responsible because baseline PDH activity (Fig. 2) and steady-state baseline and end-exercise RER (a measure of substrate oxidation) were not different between older and young adults. The possible mechanisms that could explain the age-related attenuation of PDH activation during the transition to exercise include: 1) a difference in the protein content or relative activities of either or both of PDH kinase (PDK) and PDH phosphatase (PDP) (29, 31), resulting in a slower rate of dephosphorylation during the transition to exercise; and/or 2) reduced release of Ca²⁺ from the sarcoplasmic reticulum (28, 33), thereby reducing mitochondrial Ca²⁺ uptake, and/or a reduced sensitivity of PDP to Ca²⁺, thereby slowing PDP activation and PDH dephosphorylation and activation.

The elevated relative PDH activity observed at 6 min in older adults reflects a possible greater enzyme activation needed to achieve a required rate of TCA cycle and ETC flux, and thus oxidative ATP synthesis, to match the rate of ATP hydrolysis. This may be because of a loss of TCA cycle or ETC enzyme activity because of an age-related reduction in mitochondrial volume density (13), a decrease in oxidative capacity and ADP sensitivity of remaining mitochondria (13, 45), and/or a lower O₂ availability at the terminal oxidase of the ETC (5, 16, 30). Alternatively, the fivefold increase in muscle ADPp by the end of exercise in older adults may inhibit PDK, lower PDH phosphorylation, and relieve inhibition of the PDH complex (43).

O₂ availability, ADP provision, and VO₂p kinetics. Both convective and diffusive O₂ delivery are lower during the transition to and in the exercise steady state in older compared with young adults (5, 16, 40, 42). In the present study, the ΔHHb/ΔVO₂p was greater in older adults (~22 μM·L⁻¹·min⁻¹ compared with ~11 μM·L⁻¹·min⁻¹ in young adults), reflecting a greater extraction. This will result in a lower microvascular PO₂ at a given VO₂p in older adults and thus a lower diffusive O₂ flux to the mitochondria (5). Additionally, the similar time course of ΔHHb accompanying the slower VO₂ kinetics suggests a slower adaptation of bulk O₂ delivery in older adults. Thus the observed attenuation of PDH activation and accompanying delay in provision of reducing equivalents may occur in association with a reduced mitochondrial VO₂, and both likely contribute to the slower VO₂p kinetics in older adults.

Additionally, the greater muscle ADPf and Pi concentrations in older adults may reflect a greater requirement of substrate (i.e., NADH, ADP, and Pi) to achieve a lower mitochondrial oxidative phosphorylation (as reflected by the lower VO₂p). The greater PCR breakdown (relative to WR) in the older relative to younger adults represents a greater substrate-level phosphorylation and is consistent with the slower VO₂p and muscle O₂ utilization kinetics and delayed PDH activation. During the transition to exercise, the greater PCR breakdown seen in older adults would be associated with a greater muscle Cr concentration, and thus, in turn, would be expected to increase delivery of ADP to the mitochondria (via the PCR shuttle system and mitochondrial creatine kinase reaction). The close matching of PCR kinetics and VO₂p kinetics that is seen in younger adults (38, 39) is supportive of the control of oxidative phosphorylation residing, in part, with PCR and the creatine kinase system, presumably through regulation of ADP concentration (38). Thus the greater PCR breakdown observed in older adults is consistent with the ADP requirement being greater in older adults to achieve a given muscle O₂ consumption (and VO₂p) to support oxidative ATP synthesis. This is likely the consequence of lower provision of both NADH and O₂ during the transition to exercise and reflects an interaction that occurs between the various oxidative substrates to achieve a required rate of oxidative phosphorylation (49).

Limitations

A correlation between VO₂p kinetics and PDH activation at 30 s of exercise was not observed in the present study (P > 0.05), as might be expected if a slow activation of PDH was responsible for the slower VO₂p kinetics in older adults. This, in part, may be due to the limited number of muscle biopsy samples taken during the exercise transition and to the “timing” of our first muscle biopsy sample which, in young adults, was too late to observe any “kinetic” response.

Additionally, the contents of ADPf and Pi were not measured directly but were calculated based on the content of metabolites measured in muscle samples along with assumptions related to enzyme equilibria and resting metabolite contents (see METHODS). That ADPf did not increase significantly early during the exercise transient, in part, may reflect variability in measurement of the metabolites contributing to these calculations. Also, measured and calculated values reflect “whole muscle” and not “mitochondrial” values, which is of greater consequence to respiratory control.
Conclusions. In the present study, we demonstrated that slower $\dot{V}_O_2p$ kinetics in older compared to young adults were associated with an attenuated activation of the mitochondrial PDH complex and greater PCR breakdown early during the transition to moderate-intensity exercise. Also, slower $\dot{V}_O_2p$ kinetics, coupled with the similar NIRS-derived $\Delta Hb$ kinetics between groups and greater $\Delta Hb/\Delta V_O_2$ in older adults, suggests that muscle $O_2$ delivery (convective and diffusive) may be attenuated in older adults. Together, these findings suggest that the slower $\dot{V}_O_2p$ kinetics (and muscle $O_2$ consumption) in older adults is a consequence of an inherently sluggish activation of the muscle metabolic machinery (i.e., metabolic inertia) and lower $O_2$ availability. The greater PDH activity and ATP concentration at end exercise in older adults relative to steady-state $\dot{V}_O_2p$ suggest that a greater metabolic disturbance may be required to achieve a given rate of oxidative phosphorylation in older adults.

Perspectives and Significance

This study provides evidence that control of oxidative phosphorylation is dependent on the delivery and provision of adequate amounts of oxidizable substrate to the mitochondria. A limitation in providing one or more of these substrates necessitates a compensatory increase in the other substrates to achieve a given rate of mitochondrial oxidative phosphorylation. Thus, during the transition to exercise, there is an interaction between muscle metabolism and provision of ADP and reducing equivalents, and the maintenance of an adequate O2 flux through adjustments in delivery and distribution of blood flow and O2 to active muscle fibers. In the case of older adults, a delay in the activation of the mitochondrial PDH and possibly distribution of blood flow within muscle appears to slow the adaptation of muscle $O_2$ utilization (as seen by $\dot{V}_O_2p$), resulting in a compensatory increase in muscle ADP concentration. Future work examining $\dot{V}_O_2p$ kinetics or attempting to improve exercise tolerance in older adults should incorporate an integrated approach where both metabolic inertia and $O_2$ availability are given equal attention.

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