Enhanced pyruvate dehydrogenase activity does not affect muscle O$_2$ uptake at onset of intense exercise in humans

JENS BANGSBO, MARTIN J. GIBALA, PETER KRUSTRUP, JOSÉ GONZÁLEZ-ALONSO, AND BENGT SALTIN

Copenhagen Muscle Research Centre, Institute of Exercise and Sport Sciences, University of Copenhagen, Copenhagen, Denmark

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Enhanced pyruvate dehydrogenase activity does not affect muscle O$_2$ uptake at onset of intense exercise in humans. Am J Physiol Regulatory Integrative Comp Physiol 282: R273–R280, 2002.—It has been proposed that the activation state of pyruvate dehydrogenase (PDH) may influence the rate of skeletal muscle O$_2$ uptake during the initial phase of exercise; however, this has not been directly tested in humans. To remedy this, we used dichloroacetate (DCA) infusion to increase the active form of PDH (PDH$_a$), and subsequently, measured leg O$_2$ uptake and markers of anaerobic ATP provision during conditions of intense dynamic exercise, when the rate of muscle O$_2$ uptake would be very high. Six subjects performed brief bouts of one-legged knee-extensor exercise at $\sim$110% of thigh peak O$_2$ uptake (65.3 $\pm$ 3.7 W) on several occasions: under noninfused control (Con) and DCA-supplemented conditions. Needle biopsy samples from the vastus lateralis muscle were obtained at rest and after 5 s, 15 s, and 3 min of exercise during both experimental conditions. In addition, thigh blood flow and femoral arteriovenous differences for O$_2$ and lactate were measured repeatedly during the 3-min work bouts (Con and DCA) to calculate thigh O$_2$ uptake and lactate release. After DCA administration, PDH$_a$ was four- to eightfold higher ($P < 0.05$) than Con at rest, and PDH$_a$ remained $\sim$130% and 100% higher ($P < 0.05$) after 5 and 15 s of exercise, respectively. There was no difference between trials after 3 min. Despite the marked difference in PDH$_a$ between trials at rest and during the initial phase of exercise, thigh O$_2$ uptake was the same. In addition, muscle phosphocreatine utilization and lactate production were similar after 5 s, 15 s, and 3 min of exercise in DCA and Con. The present findings demonstrate that increasing PDH$_a$ does not alter muscle O$_2$ uptake and anaerobic ATP provision during the initial phase of intense dynamic knee-extensor exercise in humans.

Although the mechanisms responsible for the delay in muscle O$_2$ uptake at the onset of exercise remain controversial (for review see Ref. 25), recent studies have suggested that this phenomenon is not due to limited O$_2$ availability but, rather, the ability of the muscle cells to utilize O$_2$ (3, 9–11). One mechanism potentially involved in a local limitation to O$_2$ uptake is “delayed” activation of the enzyme pyruvate dehydrogenase (PDH). PDH is considered rate limiting for pyruvate oxidation in skeletal muscle, and its activity is subject to strict regulatory control. The active form of PDH (PDH$_a$) and PDH flux must be kept low in resting muscle to prevent unnecessary carbohydrate utilization and flux through the enzyme must be rapidly accelerated during strenuous exercise to meet the increased demand for carbohydrate-derived acetyl-CoA (14). A possible role for PDH in regulating muscle O$_2$ uptake has been suggested by several recent studies that employed the pharmacological agent dichloroacetate (DCA). DCA administration was shown to markedly increase PDH$_a$ in skeletal muscle and attenuate markers of anaerobic ATP provision during electrically evoked contractions in animals and during submaximal exercise in humans (12, 22–24). For example, Timmons et al. (22) reported that pretreatment with DCA resulted in a $\sim$50% reduction in muscle phosphocreatine utilization during 8 min of knee-extensor exercise at an intensity of $\sim$45% of leg peak O$_2$ uptake ($V_{O2peak}$). This observation was confirmed by Howlett and colleagues (12), who showed that DCA infusion attenuated muscle degradation after 30 s of exercise and lactate accumulation after 2 min of submaximal cycle exercise at $\sim$65% of maximal O$_2$ uptake ($V_{O2max}$). It was hypothesized that the availability of acetyl groups to the tricarboxylic acid (TCA) cycle, which is increased after PDH activation by DCA, is a key determinant of muscle O$_2$ uptake at the start of exercise (22, 23). To our knowledge, however, there have been no direct measurements of muscle O$_2$ uptake during exercise after DCA administration in humans.

Thus the aim of the present study was to determine whether manipulation of PDH$_a$ would alter muscle O$_2$ uptake or markers of anaerobic ATP provision during the initial phase of intense exercise in humans (i.e., when the rate of muscle O$_2$ uptake would be high and pyruvate is the primary source for the citric acid cycle). To examine this issue, subjects performed intense dynamic knee-extensor exercise on several occasions: un-
nder noninfused control (Con) and DCA-supplemented conditions.

METHODS

Subjects. Six healthy men, ranging in age from 21 to 24 yr, with an average height of 178 (range 172–183) cm and a mean body mass of 72.5 (range 67.9–78.3) kg, participated in the experiment. The subjects were fully informed in advance of the risks and discomfort associated with the experimental procedures, and all provided written consent. The study was approved by the Ethics Committee of Frederiksberg and Copenhagen.

Experimental protocol. Subjects were instructed to consume a light breakfast ~3 h before an experimental trial. After the subjects reported to the laboratory and rested in the supine position for ~30 min, a catheter was inserted into a femoral artery under local anaesthesia. The tip was positioned 1–2 cm proximal to the inguinal ligament. A catheter was also inserted into the femoral vein of the leg to be exercised, ~1–2 cm distal to the inguinal ligament. A thermistor for measurement of venous blood temperature was inserted through the catheter and advanced 8–10 cm proximal to the tip.

At ~1 h after insertion of the catheters, subjects performed a 3-min bout of dynamic knee-extensor exercise in the supine position on an ergometer that permitted the exercise to be confined to the quadriceps muscle (1). The external power output was 65.3 ± 3.7 W, corresponding to ~110% of thigh VO2peak, and the kicking frequency was 60 rpm. At this work intensity, subjects would typically have been exhausted after ~4 min. Just before exercise, the leg was passively moved for 5 s to accelerate the flywheel. Blood was drawn from the femoral artery and vein ~10 and 5 s before the exercise and ~2, 6, 10, 14, 29, 45, 60, 90, 120, 145, and 165 s during exercise. In addition, femoral venous blood flow was measured by the thermodilution technique (2) approximately every 30 s after ~1 min of the first exercise bout as well as ~3 s before and after 5, 35, 60, 90 and 160 s of exercise when the exercise bout was repeated after 1 h of rest. Values of blood flow obtained at the same time during the first exercise bouts agreed with the values after 60 min of rest for Con (43.6 ± 0.30 vs. 4.45 ± 0.23 l/min after 67 s, 4.83 ± 0.46 vs. 5.13 ± 0.44 l/min after 99 s, and 5.63 ± 0.70 vs. 5.39 ± 0.58 l/min after 152 s) and DCA (4.32 ± 0.48 vs. 4.58 ± 0.23 l/min after 67 s and 5.77 ± 0.50 vs. 5.48 ± 0.54 l/min after 166 s) with a coefficient of variation (CV) of 5.4%. Thus, in Con and DCA, the blood flow values after the rest period were used in the calculations. An occlusion cuff placed just below the knee was inflated (220 mmHg) during the exercise to avoid blood con-

Calculations. The uptake/release of O2 and lactate across the thigh was calculated by multiplying the blood flow by the femoral arteriovenous difference in O2 content and lactate concentration, respectively. Pyruvate exchange was calculated in a similar manner, except leg plasma flow was used. A continuous blood flow curve was constructed for each subject using a linear connection of consecutive data points to obtain time-matched values for blood flow and blood measurements. Lactate production during exercise was calculated as the sum of muscle lactate accumulation and total lactate release, determined as the area under the lactate release curve, expressed per unit active muscle.

To determine the mean transit time (MTT) of the blood from the femoral arterial to the collecting site in the femoral vein, four of the subjects performed the main experimental protocol a third time on a separate occasion (without DCA infusion), as described previously (3). Briefly, the experimental conditions and position of the catheters were the same as in the main experiments. Before and frequently during the
exercise, indocyanine green dye (Cardiogreen, Becton Dickinson) was injected rapidly into the femoral artery, and immediately thereafter the artery was flushed with isotonic saline. Blood was withdrawn from the femoral vein for measurements of dye concentration using a linear densitometer, and MTT was detected as the time from injection to the time when the curve peaked, corrected by transit time of catheter. The MTT values for Con were used to make calculations during the DCA experiment. This assumption appears valid, since no differences in thigh blood flow during exercise were observed between Con and DCA (see above). On the basis of MTT, the average time to which the collected artery and venous blood represented capillary blood was estimated as described previously (3). All blood variables are presented in relation to mean time at the capillary. For the two subjects where no MTT values were obtained, average values were used.

Statistics. Data were analyzed using two-way analysis of variance with repeated measures. A significance level of 0.05 was chosen. If a significant interaction was detected, data were subsequently analyzed using a Newman-Keuls post hoc test. Unless otherwise noted, values are means ± SE.

RESULTS

Muscle PDH. In Con muscle, PDH$_a$ at rest was ~10% of PDH$_a$ at the end of the 3-min exercise bout, and it increased to 28 and 38% after 5 and 15 s, respectively (Table 1). Compared with Con, DCA infusion elevated (P < 0.05) PDH$_a$ by four- to eightfold at rest (Table 1), by 130 and 100% after 5 and 15 s of exercise, respectively, but no difference was observed at the end of exercise (Fig. 1).

Thigh O$_2$ uptake. In Con and DCA, thigh blood flow increased from ~1.7 l/min just before exercise to 5.4 l/min at the end of exercise (Fig. 2).

Arterial O$_2$ content increased (P < 0.05) from ~189 to 200 ml/l during Con and DCA. Femoral venous O$_2$ content was the same before Con and DCA (~170 ml/l) and decreased in a similar manner during Con and DCA, reaching 55 ml/l at the end of exercise. Correspondingly, arteriovenous O$_2$ and leg O$_2$ uptake before and during exercise were the same in Con and DCA (Fig. 3).

Muscle creatine phosphate. Muscle creatine phosphate decreased by ~15 and 25% during the first 5 and 15 s of exercise, respectively, reaching a level corresponding to 30% of rest (24 mmol/kg dry wt) at the end of exercise in Con and DCA (Table 1). Consequently, the average rates of creatine phosphate degradation were the same in Con and DCA (Fig. 4).

Thigh pyruvate exchange. In DCA, arterial and venous pyruvate were lower (P < 0.05) than in Con (Fig. 5A). After 90 s of exercise, pyruvate release was higher (P < 0.05) in DCA than in Con (Fig. 5B).

Lactate production. No differences between Con and DCA were observed in the increase of muscle lactate in the first 5 and 15 s of exercise or in the last phase of exercise, with the total increase during exercise being ~80 mmol/kg dry wt in Con and DCA (Table 1). Arterial lactate was lower (P < 0.05) in DCA than in Con.

Table 1. Muscle PDH$_a$, creatine phosphate, lactate, and water content before and after intense knee-extensor exercise without and with DCA infusion

<table>
<thead>
<tr>
<th></th>
<th>3-Min Bout</th>
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<th>0- to 15-s Bout</th>
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<td></td>
<td>0 s</td>
<td>180 s</td>
<td>0 s</td>
<td>5 s</td>
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<tr>
<td>PDH$_a$, mmol acetyl-CoA·min$^{-1}$·kg wet wt$^{-1}$</td>
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<tr>
<td>Con</td>
<td>0.40 ± 0.11</td>
<td>3.44 ± 0.15†</td>
<td>0.26 ± 0.05</td>
<td>1.01 ± 0.28†</td>
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<tr>
<td>DCA</td>
<td>1.71 ± 0.29*</td>
<td>3.23 ± 0.33†</td>
<td>2.01 ± 0.52*</td>
<td>2.30 ± 0.36*</td>
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<tr>
<td>Creatine phosphate, mmol/kg dry wt</td>
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<tr>
<td>Con</td>
<td>83.5 ± 5.1</td>
<td>27.3 ± 11.3†</td>
<td>79.8 ± 3.8</td>
<td>72.1 ± 2.1†</td>
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<tr>
<td>DCA</td>
<td>84.2 ± 3.1</td>
<td>24.3 ± 4.3†</td>
<td>83.8 ± 0.8</td>
<td>72.2 ± 2.5†</td>
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<tr>
<td>Lactate, mmol/kg dry wt</td>
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<tr>
<td>Con</td>
<td>5.5 ± 0.9</td>
<td>84.1 ± 12.8†</td>
<td>4.7 ± 0.7</td>
<td>9.2 ± 2.5†</td>
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<tr>
<td>DCA</td>
<td>2.1 ± 0.3*</td>
<td>78.0 ± 12.5†</td>
<td>2.6 ± 0.8</td>
<td>5.8 ± 1.6†</td>
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<tr>
<td>%H$_2$O</td>
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<tr>
<td>Con</td>
<td>76.6 ± 0.2</td>
<td>78.3 ± 0.3†</td>
<td>76.8 ± 0.4</td>
<td>78.0 ± 0.8</td>
</tr>
<tr>
<td>DCA</td>
<td>77.5 ± 0.6</td>
<td>78.6 ± 0.6</td>
<td>77.6 ± 0.3*</td>
<td>77.9 ± 0.5</td>
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Values are means ± SE. PDH$_a$, active fraction of pyruvate dehydrogenase; Con, control; DCA, dichloroacetate. *Significantly different from Con. †Significantly different from preexercise.

Fig. 1. Active fraction of muscle pyruvate dehydrogenase (PDH$_a$, mmol acetyl-CoA·min$^{-1}$·kg wet wt$^{-1}$) before (before 5-s bout) and after 5, 15, and 180 s of an intense knee-extensor exercise bout without (Con, open bars) and with prior dichloroacetate (DCA) infusion (solid bars). Values are means ± SE. *Significantly different from preexercise value. †Significantly different from Con.
before and throughout exercise, and femoral venous lactate was lower \((P < 0.05)\) before and during the first 14 s of exercise (Fig. 6A). In DCA, net lactate release was higher \((P < 0.05)\) after 30 s of exercise and toward the end of exercise than in Con (Fig. 6B). The average rates of lactate production were the same in Con and DCA (Fig. 7).

**DISCUSSION**

The major findings from the present study were that although DCA infusion increased PDH\(_a\) at rest and during the first 15 s of intense exercise compared with the control condition, muscle \(\dot{V}O_2\) uptake, creatine phosphate degradation, and lactate production were not affected. These observations suggest that the delayed muscle respiratory response during the initial phase of intense dynamic exercise in humans \((\sim 110\%\) of thigh \(\dot{V}O_2\) peak) is not due to a lag in PDH activation.

Under control conditions, PDH\(_a\) after 15 s of exercise was only about one-third of that at the end of 3 min of work, at which time the enzyme was likely fully converted to the active form (19). Few data are available regarding the time course for PDH activation during the initial seconds of exercise. However, Parolin et al. (16) reported that PDH increased from 14% active at rest to 48% after 6 s of maximal isokinetic cycling and was almost totally activated by 15 s. In addition, Howlett et al. (13) recently reported a 65% transformation of PDH to the active form after 10 s of maximal sprint cycling. The slower activation in the present study than in these previous two studies may be related to exercise intensity, e.g., maximal cycling (>300% of the intensity eliciting \(\dot{V}O_2\) max) vs. the power output (\(\sim 110\%\) of thigh \(\dot{V}O_2\) peak) in the present study, which was sustained for 3 min. Although a number of mechanisms could be involved, one potential factor is difference in mitochondrial \(Ca^{2+}\) released from the sarcoplasmic reticulum, which is believed to be important for the initial activation of PDH during contraction (26).
The observations that PDH was only partly activated and that there was a significant accumulation of muscle lactate after 5 and 15 s of exercise indicate that the rate of pyruvate production from glycolysis was in excess of mitochondrial pyruvate oxidation and could be interpreted to suggest that flux through PDH was limiting for O₂ uptake at the start of exercise. However, we did not observe any elevation in the rate of muscle O₂ uptake after DCA administration, despite the fact that this manipulation markedly increased PDHₐ at rest as well as after 5 and 15 s of exercise compared with the control trial. It should be considered whether the measurements of leg O₂ uptake are sensitive enough to detect a possible difference in O₂ uptake. Moreover, the DCA treatment did not change the rate of creatine phosphate degradation or lactate production in the exercising muscles, indicating that the anaerobic ATP provision was not changed with DCA infusion. Thus, the data suggest that the activation state of PDH does not limit mitochondrial respiration during the initial phase of exercise. Some variation in arteriovenous O₂ difference was also observed in the 1st min of exercise (CV = 14%) and less during the last 2 min (CV = 6%). However, there was no evidence for a tendency of the O₂ extraction to be higher in DCA, since both the subjects had higher arteriovenous O₂ difference values in the first phase of exercise in Con than in DCA. In another knee-extensor study, a measurably higher O₂ extraction (<20 ml/l) was observed after a few seconds of repeated exercise (4). Thus, the lack of difference in arteriovenous O₂ difference and thigh O₂ uptake between Con and DCA appears not to be caused by the measurement of O₂ extraction and blood flow not being sensitive enough. Moreover, the DCA treatment did not change the rate of creatine phosphate degradation or lactate production in the exercising muscles, indicating that the anaerobic ATP provision was not changed with DCA infusion. Thus, these data suggest that the activation state of PDH does not limit mitochondrial respiration during the initial phase of exercise.

Fig. 5. Plasma pyruvate and pyruvate exchange. Arterial (triangles) and femoral venous (squares) plasma (A) and thigh pyruvate release (circles, B) related to time at capillary level before and during an intense knee-extensor exercise bout without (Con, open symbols) and with prior DCA infusion (filled symbols). Values are means ± SE. *DCA significantly different from Con.

Fig. 6. Blood lactate and thigh lactate release. Arterial (triangles) and femoral venous (squares) lactate (A) and thigh lactate release (circles, B) related to time at capillary level before and during an intense knee-extensor exercise bout without (Con, open symbols) and with prior DCA infusion (filled symbols). Values are means ± SE. *DCA significantly different from Con.
Fig. 7. Rate of muscle lactate production, determined as the sum of rate of lactate accumulation (below horizontal line) and rate of lactate release (above horizontal line), during the first 15 s (0–15 s) and remaining 165 s (15–180 s) of an intense knee-extensor exercise bout without (Con, open bars) and with prior DCA infusion (solid bars). Values are means ± SE. *Significantly different from 0–15 s.

Fig. 8. Anaerobic energy turnover. Individual anaerobic ATP turnover (mmol/kg dry wt), determined as the sum of creatine phosphate and lactate (Table 1) might indicate that the anaerobic energy production was (nonsignificantly) lower during the first 15 s in the DCA than in the Con trial, but this difference was largely due to one subject who had a markedly higher anaerobic energy production in Con (Fig. 8). The difference in aerobic energy production calculated from average values would have corresponded to a difference in $O_2$ uptake and $O_2$ extraction of ∼125 ml/min and 50 ml/l, respectively, between Con and DCA. These differences are far above the detection limit of the method, since a significant difference in $O_2$ extraction of 20 ml/l has been observed when intense exercise was repeated (4).

The extent of PDH activation after DCA administration in the present study was greater than that reported by Timmons and co-workers (22, 23) and smaller than that found by Howlett et al. (12), so this appears not to be the reason for the different findings. A more likely explanation is the difference in the intensity of the muscle contractions between the present study and several other studies in which submaximal

been hypothesized that, by activating PDH and elevating the level of muscle acetyl-CoA and acetylcarnitine, DCA increases the availability of substrate for the TCA cycle and, thereby, increases the rate of mitochondrial respiration after onset of exercise (23). It has also been suggested that DCA might function to expand the pool of TCA cycle intermediates (TCAI) in resting skeletal muscle and, therefore, augment TCA cycle flux on contraction. However, it was recently shown that DCA actually lowers the total concentration of muscle TCAI at rest and does not alter the steady-state concentration of TCAI during exercise (8).

In contrast to some of the studies cited above, we did not observe any differences between trials in creatine phosphate degradation or lactate accumulation during exercise. At first glance, the mean values for muscle creatine phosphate and lactate (Table 1) might indicate that the anaerobic energy production was (nonsignificantly) lower during the first 15 s in the DCA than in the Con trial, but this difference was largely due to one subject who had a markedly higher anaerobic energy production in Con (Fig. 8). The difference in aerobic energy production calculated from average values would have corresponded to a difference in $O_2$ uptake and $O_2$ extraction of ∼125 ml/min and 50 ml/l, respectively, between Con and DCA. These differences are far above the detection limit of the method, since a significant difference in $O_2$ extraction of 20 ml/l has been observed when intense exercise was repeated (4).

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Although the effect of DCA infusion on muscle $O_2$ uptake has not been studied previously, a number of recent investigations have examined the effect of DCA administration on skeletal muscle anaerobic metabolism in animals and humans. When PDH in resting canine gracilis muscle was activated by pretreatment with DCA, the estimated anaerobic energy contribution during electrically induced contraction was reduced, suggesting an increased aerobic contribution (24). In a human study conducted by the same group of investigators (22), a threefold increase in resting muscle PDH activity and a fivefold increase in muscle acetylcarnitine were observed after DCA infusion. During a subsequent bout of one-legged knee-extensor exercise, muscle creatine phosphate was significantly higher (30%) after 8 min of contraction than in a control situation; however, there were no differences between conditions in muscle lactate. In another human study using partial ischemia, Timmons et al. (23) observed that DCA infusion reduced creatine phosphate degradation and muscle lactate accumulation by ∼50% after 3 min of submaximal, single-leg knee-extension exercise, but there were no differences after 8 min of exercise. In agreement with these findings, Howlett et al. (12) reported that muscle creatine phosphate utilization and lactate accumulation after 2 min of submaximal exercise were reduced after pretreatment with DCA. On the other hand, Parolin et al. (17) reported that, under hypoxic conditions, DCA infusion did not affect muscle creatine phosphate utilization during the 1st min of exercise, whereas muscle creatine phosphate was higher and muscle lactate was lower after 15 min of exercise than in a control situation. The precise explanation for these observations is not clear, since DCA exerts a complex myriad of effects in numerous tissues (for review see Ref. 21). It has...
workloads were employed (12, 22). In support of this suggestion, Howlett et al. (13) did not observe any effect of DCA infusion on muscle phosphocreatine degradation and lactate accumulation during 10 s of maximal cycling. It should also be considered that the lower muscle lactate concentration observed in mixed muscle biopsies during submaximal exercise after DCA infusion (12, 22, 23) may not be solely due to an enhanced rate of pyruvate oxidation. Lactate release was not measured in these previous studies; however, in the present study, we observed a higher rate of lactate release after 30 s of exercise after DCA administration. Moreover, during submaximal exercise, not all the muscle fibers may be activated, and the elevated activity of PDH in the inactive and partly active muscle fibers in the contracting muscles may have led to a greater conversion of lactate to pyruvate subsequent to an elevated rate of pyruvate oxidation. This hypothesis is supported by the observation that muscle lactate was not lowered after 30 s, but after 2 min of exercise in the study by Howlett et al. (12), indicating that removal of lactate, rather than production of lactate, was influenced. Although the present findings do not rule out the importance of PDH activation and/or acetyl group availability in determining muscle O_2 utilization at the start of submaximal exercise, these factors do not appear to be important in regulating muscle respiration and anaerobic metabolism during the initial phase of intense contraction. As recognized by Howlett and colleagues (13), there could be a limit to oxidative metabolism within the muscle at sites other than PDH, such as the TCA cycle or the electron transport chain.

In summary, the present findings demonstrate that DCA infusion increased PDH_a at rest and during the initial phase of intense exercise but did not alter muscle O_2 uptake, creatine phosphate utilization, or muscle lactate production. These data suggest that the activation state of PDH is not limiting for muscle O_2 utilization during the first phase of intense dynamic exercise (~110% of VO_2 peak) in humans.

**Perspectives**

The workload for this study was selected to ensure that muscle activation was complete, that muscle O_2 demand was high, and also that pyruvate-derived acetyl-CoA was the primary fuel source for mitochondrial respiration. Our findings indicate that the rate of PDH activation does not limit muscle O_2 uptake during the initial phase of intense dynamic exercise in humans. Evidence has also been presented to suggest that, under these conditions, muscle O_2 uptake is not limited by O_2 delivery. Thus, if it is assumed that the delay resides within the muscle, future studies should examine the citric acid cycle and electron transport chain for potential sites of limitation to muscle O_2 uptake during the initial phase of contraction.

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