Skeletal muscle metabolism during high-intensity sprint exercise is unaffected by dichloroacetate or acetate infusion

Howlett, Richard A., George J. F. Heigenhauser, and Lawrence L. Spriet. Skeletal muscle metabolism during high-intensity sprint exercise is unaffected by dichloroacetate or acetate infusion. J. Appl. Physiol. 87(5): 1747–1751, 1999.—This study investigated whether increased provision of oxidative substrate would reduce the reliance on nonoxidative ATP production and/or increase power output during maximal sprint exercise. The provision of oxidative substrate was increased at the onset of exercise by the infusion of acetate (AC); increased resting acetylcarnitine (PDH-a); and dichloroacetate (DCA; increased acetyl-CoA and greater activation of pyruvate dehydrogenase (PDH-a)). Subjects performed 10 s of maximal cycling on an isokinetic ergometer on three occasions after either DCA, AC, or saline (Con) infusion. Resting PDH-a with DCA was increased significantly over AC and Con trials (3.5 ± 0.4 vs. 0.5 ± 0.1 and 0.74 ± 0.1 mmol·kg dry muscle·min⁻¹). DCA and AC significantly increased resting acetyl-CoA (35.2 ± 4.4 and 22.7 ± 2.9 vs. 10.2 ± 1.3 mmol·kg dry muscle) and acetyl carnitine (12.9 ± 1.4 and 11.0 ± 1.0 vs. 3.3 ± 0.6 mmol·kg dry muscle) over Con. Resting contents of phosphocreatine, lactate, ATP, and glycolytic intermediates were not different among trials. Average power output and total work done were not different among the three 10-s sprint trials. Postexercise, PDH-a in AC and Con trials had increased significantly but was still significantly lower than in DCA trial. Acetyl-CoA did not increase in any trial, whereas acetyl carnitine increased significantly only in DCA. Exercise caused identical decreases in ATP and phosphocreatine and identical increases in lactate, pyruvate, and glycolytic intermediates in all trials. These data suggest that there is an inability to utilize extra oxidative substrate (from either stored acetyl carnitine or increased PDH-a) during exercise at this intensity, possibly because of O₂ and/or metabolic limitations.

DURING HIGH-INTENSITY sprint exercise [i.e., power outputs two- to threefold greater than those required to elicit maximal O₂ consumption (V₀₂max)], the reliance on nonoxidative or so-called “anaerobic” ATP production is extremely high (17, 20, 24). The majority of the ATP required to maintain muscular contractions at these power outputs comes from phosphocreatine (PCr) utilization and glycolytic ATP production, with glycosogen as the substrate. However, there is some aerobic ATP production at these power outputs, especially as exercise duration increases (i.e., ~20% of total ATP provision in 30-s sprint). As well, it has been previously demonstrated that the amount of oxidative ATP production in the working muscle can be increased during this type of exercise. In a study utilizing repeated bouts of sprint exercise, the amount of oxidative ATP production rose substantially during the third and final bout, when oxidative metabolism was fully activated (20). Because the ATP yield from oxidative metabolism is much greater than that of PCr or glycogen breakdown to lactate, a small increase in oxidative metabolism may reduce the reliance on glycyls and/or PCr use at the onset of sprinting.

Recent studies at submaximal power outputs have demonstrated that the amount of nonoxidative ATP production required during the rest-to-work transition can be attenuated (lower PCr degradation and lactate accumulation from glycolysis) by increasing oxidative ATP production (12, 25–27). In these studies, the extra substrate was provided via an increase in pyruvate dehydrogenase activity (PDH-a) mediated by dichloroacetate (DCA) infusion. In some of these studies, it was suggested that the extra substrate came from the large resting stores of acetyl carnitine, which provide acetyl-CoA for entry into the tricarboxylic acid (TCA) cycle (25–27). Another study showed similar effects of DCA infusion but suggested that the increased oxidative metabolism was simply due to increased flux through PDH after exercise began and not from acetyl carnitine, which actually accumulated early in exercise (12).

The infusion of sodium acetate (AC) has also been used previously to increase acetyl carnitine and acetyl-CoA contents in resting human skeletal muscle to levels that are similar to DCA infusion, but without an increase in PDH-a (21). Therefore, by using both AC and DCA infusions during separate trials, and assuming that oxidative metabolism would increase, it should be possible to discriminate the source of extra oxidative substrate.

The purpose of this investigation was therefore threefold. First, to determine whether an increased provision of oxidative substrate would increase oxidative energy provision and decrease the reliance on nonoxidative ATP provision during high-intensity exercise, as it does at lower power outputs (i.e., 65% V₀₂max). Second, to test, by using DCA and AC infusions, whether the increased substrate for oxidative metabolism was derived from high accumulations of acetyl carnitine before exercise or from increased PDH flux at the onset of exercise. Third, to test whether the maximal power output during cycling would increase if oxidative metabolism increased, without a decrease in the contribution from nonoxidative metabolism. Our hypothesis

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was that DCA would cause a decrease in nonoxidative metabolism during 10 s of high-intensity cycling, whereas AC would not, suggesting that PDH flux limits TCA cycle flux, and thus oxidative metabolism, during the rest-to-work transition in maximally exercising human muscle.

METHODS

Subjects. Ten (3 women, 7 men) healthy subjects volunteered to participate in this study. Their (mean ± SE) age, height, weight, and VO\textsubscript{2max} were 23.5 ± 0.7 yr, 177.4 ± 14.0 cm, 72.2 ± 4.4 kg, and 3.7 ± 0.4 l/min (51.3 ± 2.2 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}), respectively. The subjects were not well trained, but most were engaged in some regular physical activity. Subjects were informed of possible risks involved in the study, and informed consent was received from all subjects. The Ethics Committees of the University of Guelph and McMaster University approved the study.

Experimental infusions. AC (4 M) was obtained from the McMaster University Medical Center and delivered intravenously at a dose of 4 mmol/kg body mass. DCA was prepared at a concentration of 100 mg/ml (pH 7.0) by the pharmacy at McMaster University Medical Center and delivered at a dose of 100 mg/kg body mass. Both were delivered intravenously to subjects in 500 ml of normal saline solution over the course of 1 h immediately before exercise. For control trials, 500 ml of saline were infused over the same time course.

Preexperimental protocol. The custom-made cycle ergometer has been described previously (16). Briefly, it is an isokinetic ergometer that is braked by a 3-horsepower electric motor, enabling the maximal allowable cadence to be set at 100 rpm, regardless of the subject’s power output. Pedal forces are measured by strain gauges in the crankarms, allowing calculation of average and peak power and total work of each leg, or both combined, for each stroke and the whole exercise bout. All subjects underwent a practice session consisting of several all-out sprints on the isokinetic cycle to familiarize themselves with the intense nature of the exercise and were strongly advised of the importance of an all-out effort for each of three experimental trials. Subjects also underwent a test of VO\textsubscript{2max} on a cycle ergometer (Excalibur, Quinton Instruments, Seattle, WA) using a metabolic cart (SensorMedics model 2900, Yorba Linda, CA).

Experimental protocol. On 3 separate experimental days (each separated by 1 wk), subjects arrived at the laboratory at the same time of day. One hour before each exercise trial, a catheter was inserted into the antecubital vein of the subject, and an infusion of saline, AC, or DCA (in randomized order) was started while the subject rested on a bed. Subjects had one leg prepared for needle biopsies 30 min before the start of the exercise, with two incisions made through the skin superficial to the vastus lateralis muscle under local anesthesia (2% lidocaine without epinephrine) (2). A resting biopsy was taken before exercise. Subjects then moved to the electronically braked isokinetic cycle ergometer and pedaled with maximum effort for 10 s. Immediately after the 10-s exercise bout, a biopsy was taken, with the subject remaining on the cycle ergometer. Samples were immediately frozen in liquid N\textsubscript{2} (3–5 s from the insertion of the needle), removed from the needle, and stored in liquid N\textsubscript{2} until analysis.

Analyses. A small piece of frozen wet muscle (10–20 mg) was removed under liquid N\textsubscript{2} for the determination of the activity of PDH in its active form (PDH-a), as described by Constantin-Teodosiu et al. (6) and modified by Putman et al. (22). The remainder of the biopsy sample (70–100 mg) was freeze-dried; dissected of all visible blood, connective tissue, and fat; and powdered for subsequent analysis.

One aliquot of freeze-dried muscle was extracted with 0.5 M HClO\textsubscript{4} (containing 1 mM EDTA) and neutralized with 2.2 M KHCO\textsubscript{3}. This extract was used for determination of creatine, PCr, ATP, d-glucose 6-phosphate (G-6-P), lactate, glyceral 3-phosphate (G-3-P), and glucose by enzymatic spectrophotometric assays (1, 10). Pyruvate was determined on this extract fluorometrically (19). Acetyl-CoA and acetylcarbinol were determined by radiometric measures (4). Muscle glycogen content was determined on a second aliquot of freeze-dried muscle from resting samples (10). To account for possible diluting effects of blood or connective tissue in dried muscle samples, all metabolite contents and the activity of PDH-a were normalized to the highest total creatine content measured in the six biopsies from each subject.

Calculations. The rate of ATP provision [mmol ATP·kg dry muscle (dm)\textsuperscript{-1}·s\textsuperscript{-1}] from nonoxidative sources was calculated over the 10-s period of maximal cycling in each trial, as described by Spriet et al. (24)

\[
\text{ATP provision rate} = 1.5\Delta(\text{lactate + pyruvate}) + \Delta[\text{PCr}]
\]

where \(\Delta\) is difference and brackets indicate concentration.

Statistics. All data are presented as means ± SE. For all dependent variables, a two-way ANOVA (time × trial) with repeated measures was employed. Significance was set at \(\alpha = 0.05\), and, when obtained, a Tukey post hoc test was used to identify where significant differences occurred.

RESULTS

Power and work output. Peak average power was not significantly different among Con, AC, and DCA trials (1,166 ± 35, 1,096 ± 42, and 1,120 ± 38 W, respectively). The total work done in 10 s (11.6 ± 0.9, 10.8 ± 0.9, and 11.3 ± 0.9 kJ, respectively) was also not different among trials.

PDH-a. DCA infusion resulted in a significant increase in resting PDH-a level compared with Con and AC trials, which were not significantly different from each other (Fig. 1). PDH activation in Con and AC both increased to the same extent from rest but was significantly lower than DCA postexercise.

Acetylated compounds. At rest, the acetyl-CoA content in the AC trial was significantly greater than Con,
and DCA was significantly greater than both (Fig. 2A). Exercise increases in acetyl-CoA were not significant in any group, so AC was significantly greater than Con, and DCA was significantly greater than the other two trials postexercise. Resting acetylcarnitine contents in the AC and DCA trials were not different, but both were significantly greater than Con levels (Fig. 2B). Postexercise acetylcarnitine contents rose significantly only in the DCA trial, resulting in DCA being significantly greater than both Con and AC results.

Muscle metabolites. Resting muscle contents of ATP and PCr were not different among trials (Table 1). Both ATP and PCr decreased significantly after exercise in all trials, but the postexercise values were not different among trials.

Resting lactate, pyruvate, G-3-P, G-6-P, and free glucose contents were not different among trials (Table 1). The muscle contents of all these metabolites rose significantly with exercise, although the 10-s values were not different among trials.

Resting muscle glycogen content was similar among Con, AC, and DCA trials (442.7 ± 32.0, 445.1 ± 38.4, and 430.6 ± 39.7 mmol/kg dm, respectively) before exercise.

![Figure 2](https://example.com/figure2.png)

**Fig. 2.** Skeletal muscle acetyl-CoA (A) and acetylcarnitine (B) accumulation at rest and after 10 s of sprint exercise after saline (Con), AC, or DCA infusion. *Significantly different than Con at same time point. †Significantly different than AC at same time point. ‡Significantly different than Pre for same trial.

Nonoxidative ATP provision. The average rates of nonoxidative ATP provision during maximal cycling were not different among the Con, AC, and DCA trials (13.6 ± 0.8, 12.8 ± 0.9, and 14.4 ± 1.0 mmol·kg dm⁻¹·s⁻¹).

**DISCUSSION**

The high-intensity sprint exercise employed in the present study required extremely high ATP turnover rates to maintain the high power output produced by the contracting muscles. The nonoxidative ATP turnover rate, calculated as the ATP yield from PCR degradation and from glycolysis (lactate accumulation), was in the range of 13–14 mmol·kg dm⁻¹·s⁻¹ for all three trials. This ATP demand was two- to threefold in excess of that which can be maintained solely by oxidative phosphorylation, and, as a result, there was a very large reliance on nonoxidative ATP production or substrate-level phosphorylation. However, if oxidative metabolism could be activated more quickly at the onset of sprinting, it would lead to a large increase in energy provision. The complete oxidation of 1 mmol pyruvate yields 15 mmol ATP, sparing either 15 mmol of PCR utilization or 10 mmol of lactate accumulation or, perhaps, leads to an increase in power output if nonoxidative ATP provision was not spared. If substrate availability is limiting to oxidative metabolism at the onset of exercise, then the increased delivery of substrate by either DCA or AC infusion should lead to one of the above scenarios.

However, the present results conflict with those of previous DCA infusion studies performed at much lower and constant power outputs (12, 25–27). In these studies, DCA caused reduced reliance on nonoxidative ATP provision, reflected by decreased PCR degradation, and decreased glycolysis (reduced lactate accumulation). In one study, nonoxidative ATP production was decreased by ~35% in the first 2 min of exercise (12).

**Table 1. Muscle metabolite contents before and after 10 s of sprint exercise with saline, AC, or DCA infusion**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Time</th>
<th>Con</th>
<th>AC</th>
<th>DCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>Pre</td>
<td>22.4 ± 1.2</td>
<td>22.7 ± 1.1</td>
<td>24.9 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>17.0 ± 1.0*</td>
<td>18.2 ± 1.4*</td>
<td>17.0 ± 1.6*</td>
</tr>
<tr>
<td>PCr</td>
<td>Pre</td>
<td>93.7 ± 2.4</td>
<td>96.3 ± 3.2</td>
<td>94.9 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>40.2 ± 3.2*</td>
<td>39.3 ± 2.8*</td>
<td>39.6 ± 2.6*</td>
</tr>
<tr>
<td>Lactate</td>
<td>Pre</td>
<td>5.5 ± 1.5</td>
<td>5.6 ± 0.6</td>
<td>4.2 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>58.8 ± 3.7*</td>
<td>52.9 ± 3.2*</td>
<td>61.6 ± 4.5*</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>Pre</td>
<td>0.11 ± 0.03</td>
<td>0.13 ± 0.02</td>
<td>0.11 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>2.02 ± 0.25*</td>
<td>1.99 ± 0.28*</td>
<td>1.82 ± 0.17*</td>
</tr>
<tr>
<td>G-3-P</td>
<td>Pre</td>
<td>2.5 ± 0.8</td>
<td>2.8 ± 0.6</td>
<td>2.4 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>6.6 ± 0.5*</td>
<td>6.5 ± 0.6*</td>
<td>8.0 ± 1.1*</td>
</tr>
<tr>
<td>G-6-P</td>
<td>Pre</td>
<td>0.9 ± 0.2</td>
<td>1.2 ± 0.3</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>18.5 ± 1.3*</td>
<td>15.8 ± 0.9*</td>
<td>17.8 ± 1.6*</td>
</tr>
<tr>
<td>Glucose</td>
<td>Pre</td>
<td>2.2 ± 0.5</td>
<td>2.9 ± 0.6</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>4.0 ± 0.8*</td>
<td>4.4 ± 0.4*</td>
<td>4.2 ± 0.5*</td>
</tr>
</tbody>
</table>

Values are means ± SE in mmol/kg dry muscle. Con, control-saline; AC, sodium acetate; DCA, dichloroacetate; PCR, phosphocreatine; G-3-P, glycerol 3-phosphate; G-6-P, d-glucose 6-phosphate; Post, after infusion. *Significantly different than before infusion (Pre) for the same trial.
In the present investigation, the experimental perturbations were successful in setting up conditions to allow us to discriminate which source of oxidative substrate (increased resting acetyl carnitine and/or increased PDH-a) would contribute to the reduction in nonoxidative ATP production. It was clear that DCA infusion increased both PDH activation and acetyl carnitine content, whereas AC infusion increased acetyl carnitine both at rest and during exercise. However, for some reason(s) there was an inability of the muscle fibers at this power output to utilize this extra substrate. Possible reasons for this inability include a mitochondrial O₂ limitation and/or finite temporal activation of oxidative metabolism at sites other than PDH, such as the TCA cycle or the electron transport chain itself.

Whether O₂ can be limiting to oxidative phosphorylation is controversial. Some authors believe that the delivery of O₂ at the start of exercise is often inadequate, as increases in blood flow cannot meet the demands of exercise at the start of exercise (13, 15). However, others have shown that the increases in O₂ delivery are more than adequate to meet the increased demands of the cell (7). Experiments on isolated mitochondria have shown that the critical PO₂ to sustain cytochrome turnover is in the order of <1 Torr (5). This result has been used as evidence that mitochondria are not O₂ limited, as measured PO₂ during the transition from rest to moderate contractions was reported to be greater than this (7). However, the PO₂ at the mitochondria has been difficult to measure. Recent reports have suggested that it is relatively low, based on the saturation of myoglobin, but still theoretically above the suggested that it is relatively low, based on the saturation of myoglobin, but still theoretically above the suggested that it is relatively low, based on the saturation of myoglobin, but still theoretically above the critical PO₂ (23). Many authors have disputed whether the in vitro critical PO₂ is relevant to the in vivo regulation of oxidative phosphorylation and that the mitochondria are actually sensitive to O₂ concentrations well above this PO₂ (28). Studies have shown that even in moderate-intensity steady-state exercise, a change in cell PO₂ results in predictable changes in high-energy phosphate metabolism (3, 11). Whereas it is unknown whether an O₂ limitation existed in the present investigation in any trial, it is reasonable to assume that, if a deficit in O₂ delivery can occur at the start of exercise, it is likely that it would occur at this very high-intensity, short-duration exercise. MacDonald et al. (15) showed that increased blood flow and O₂ delivery at the start of exercise caused a faster O₂ uptake on-response during higher power output exercise (above ventilatory threshold) but not during lower intensity exercise (below ventilatory threshold).

The provision of NADH via the metabolic pathways is another determinant in the rate of oxidative phosphorylation. Some authors suggest that there is an inherent inertia to metabolic systems and that the activation of biochemical pathways determines the rate of oxidative metabolism. In studies utilizing perfused canine muscle where O₂ delivery was increased before the onset of exercise, either through increased flow or increased O₂ dissociation, there was no change in O₂ uptake on-kinetics in the experimental trial vs. control (8, 9).

These authors concluded that metabolic activation determined the rate at which oxidative metabolism and O₂ utilization were activated. In the present study, it is possible that full activation of the systems for oxidative metabolism is not achieved in 10 s in any of the trials. It has been shown that maximal PDH activation is not achieved until between 6 and 15 s of exercise of this type, although glycolysis only produces ATP via anaerobic glycolysis (18). A similar time period was also observed in the present study (12), where maximal PDH flux was not observed until between 6 and 15 s of exercise with DCA, but it is possible that all of this increased flux was accomplished later in that 30-s period if metabolic activation was limiting in both trials. DCA infusion spared PCr degradation compared with control, but there was still an obligatory fall in the energy state of the cell in both trials, demonstrating that oxidative metabolism required stimulatory signals and/or a finite time period to be activated, even if an excess of substrate was present. It is also possible that, despite full activation of PDH and/or presence of excess substrate, downstream pathways, such as the TCA cycle or electron transport, are not immediately active.

Another possibility for the lack of observed effect exists. Due to the extremely intense nature of this exercise, there is a very large change in the cellular milieu occurring in a short period (10 s). Degradation of PCr and ATP results in the accumulations of ADP and Pi, stimulating oxidative phosphorylation. However, the ATP requirement for this type of sprint exercise is so large that there is a need for maximal rates of energy provision from all ATP sources (high-energy phosphates, glycogen breakdown, and oxidative metabolism) in all trials, completely overwhelming all regulatory points. The rates of PCr degradation, glycolysis, lactate accumulation, and O₂ use in the present study and others demonstrate the extreme energy demand of this type of exercise in humans (14, 24). It may be necessary for this maximal exercise to be performed for a longer period, when oxidative metabolism represents a quantitatively larger percentage of the total ATP production than at the onset of exercise (20). It is interesting to note that only DCA infusion resulted in a significant postexercise increase in acetyl carnitine, suggesting that there was potentially greater PDH flux at the start of exercise, but, rather than being oxidized, it was shuttled to acetyl carnitine. This suggests a limitation in O₂ availability and/or oxidative metabolism distal to PDH may exist and that the lack of effect was not simply due to the relative contribution of oxidative ATP production.

In summary, the present study demonstrated that the availability of extra substrate for oxidative metabolism before maximal sprint exercise did not increase oxidative ATP provision (no change in power output) or decrease the reliance on nonoxidative ATP production. This was true despite significant increases in PDH-a before the DCA trial and increased acetyl carnitine in both the DCA and AC trials. The exercising muscles were not able to increase oxidative metabolism or power output, suggesting that a limitation in O₂ avail-
ability and/or oxidative metabolism distal to PDH may exist at this intensity.

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