The total concentration of tricarboxylic acid (TCA) cycle intermediates (TCAI) increases severalfold during moderate-to-intense contraction in human skeletal muscle (11, 12). The vast majority of the increase in TCAI occurs within the first minute of exercise, and the overall magnitude of TCAI pool expansion, usually referred to as “anaplerosis,” appears exponentially related to work intensity (for review, see Ref. 13). Several investigators have suggested that the increase in muscle TCAI is necessary to optimize flux through the TCA cycle under conditions of increased energy demand (22, 29). However, an alternative explanation is that the increase in TCAI primarily represents a sink for pyruvate when its rate of formation from glycolysis exceeds its rate of oxidation in the TCA cycle (i.e., a mass action effect). Many of the reactions that can lead to the net formation of TCAI are directly or indirectly dependent on the concentration of pyruvate, including the near-equilibrium alanine aminotransferase reaction (pyruvate + glutamate → 2-oxoglutarate + alanine), which appears quantitatively most important for the increase in TCAI at the start of exercise in humans (10, 22). Viewed in this context, the increase in TCAI during exercise might simply be a consequence of the imbalance between glycolytic flux and mitochondrial pyruvate oxidation, the latter being regulated by the pyruvate dehydrogenase enzyme complex (PDH).

In spite of the potential merits of these conflicting hypotheses, however, few experimental data are available to support or refute either of them. One of the ways in which to begin to resolve this debate is to manipulate the concentrations of TCAI and determine what effect, if any, this has on skeletal muscle metabolism and exercise performance. In this regard, dichloroacetate (DCA) administration has been shown to markedly increase the active fraction of PDH (PDHα) at rest (26, 27) and during the initial phase of exercise in human skeletal muscle (9). Timmons and co-workers (26, 27) recently reported that DCA infusion caused an approximate threefold increase in resting PDHα, while Gibala and Krustrup (9) confirmed that the stimulatory effect of DCA on PDHα was maintained after 5 and 15 s of intense dynamic exercise. Timmons et al. (28) have also shown that PDHα was higher after 1 min of stimulation in ischemic canine muscle after DCA administration. Finally, Constantin-Teodosiu et al. (8) recently demonstrated that DCA infusion reduced the concentrations of citrate and malate in resting human muscle; however, the effect of DCA on TCAI during exercise has not been reported.

In view of these collective observations, the primary purpose of the present investigation was to examine
the effect of DCA infusion on the concentrations of muscle TCAI at rest and during dynamic knee extensor exercise in humans. We hypothesized that DCA would markedly accelerate the flux of pyruvate through PDH at rest and during the initial period of contraction; as a consequence, less pyruvate would be diverted through anaplerotic pathways, and thus the magnitude of TCAI pool expansion during exercise would be attenuated. In this manner, we hoped to examine the effect of reduced concentrations of TCAI on skeletal muscle metabolism during exercise in humans. We also measured PDH\textsubscript{a} in the muscle biopsy samples, because there is only one previous report of PDH activity during exercise after the muscle biopsy samples, because there is only one previous report of PDH activity during exercise after DCA administration in humans (9). Moreover, the focus of that study (9) was intense, maximal exercise; to our knowledge, the effect of DCA on PDH\textsubscript{a} during submaximal exercise in humans has not been previously reported.

METHODS

Subjects. Six healthy subjects (4 males, 2 females) with a mean age, height, and body mass of 22 ± 1 yr, 177 ± 4 cm, and 73 ± 6 kg, respectively, volunteered for the investigation. All subjects were recreationally active, but none were engaged in any form of regular physical training. Subjects were fully informed of the purposes and potential risks of the study, which was approved by the Ethical Committee for Copenhagen and Frederiksberg communities, and provided written consent.

Preexperimental procedures. Subjects were familiarized with the Krogh ergometer modified for one-legged knee extensor exercise as previously described (2). With this exercise model, electromyographic activity is absent in the hamstrings and glutei muscles and the majority of work done for knee extension is performed by the quadriceps femoris muscle. At least 3 days before the experiment, subjects performed an incremental exercise test with their dominant leg (kicking frequency: 60/min) to determine the maximal power output of the knee extensors. This was defined as the highest workload that could be sustained while the desired kicking frequency was maintained. The mean maximal peak workload for the group was 56 ± 6 W. Subjects were instructed to consume their habitual diet and refrain from exercise or strenuous physical activity for 48 h before the experiment.

Experimental protocol. On the subjects’ arrival at the laboratory on the day of the experiment, a Teflon catheter was inserted into the femoral vein of each leg for saline or DCA infusion. The area over the lateral aspect of each thigh was anesthetized and prepared for the extraction of needle biopsy samples from the vastus lateralis muscle (5). Subjects were moved to the exercise apparatus, where they rested supine for ~15 min. Subjects then received a 300-ml infusion of normal saline into a femoral vein over a 30-min period. The choice of venous catheter (i.e., right or left leg) was randomized and counterbalanced between subjects for dominance. A needle biopsy sample was obtained immediately after the saline infusion. Subjects rested for an additional 5 min and then performed the leg kicking exercise at ~70% of the one-legged maximal knee extension capacity for 15 min, followed immediately by intense exercise at 100% of maximum until exhaustion (Exh). Muscle biopsy samples were obtained from the exercising leg after 1 and 15 min of submaximal exercise and at Exh after the intense work bout. Heart rate and expired air measurements were made at rest, during the 5- to 10-min period of the submaximal exercise period, and continually during the intense work bout (MedGraphics CPX System, Klampenborg, Denmark).

After the first exercise bout, subjects remained seated or lying on the exercise apparatus and rested for 120 min. Subjects then received a 100 mg/kg body mass dose of DCA, which was diluted in 300 ml of normal saline and infused into the contralateral femoral vein over 30 min. Five minutes after the cessation of the infusion, subjects performed the second exercise bout at the same absolute work intensities as for the first bout, with the opposite leg. Muscle biopsy samples and cardiopulmonary measurements were obtained at the same time points as during the first trial.

Muscle analyses. Biopsy samples were immediately frozen (<5 s) in liquid nitrogen, removed from the needle while still frozen, and stored at −80°C. A 10- to 20-mg piece of frozen muscle was sectioned from each biopsy sample and used for the determination of the PDH\textsubscript{a} with the method of Constantin-Teodosiu et al. (7), as modified by Putman et al. (20). The remaining portion of each biopsy sample was freeze-dried, powdered, disected free of nonmuscle elements, and stored at −80°C. An ~10-mg portion of freeze-dried muscle was extracted with 0.5 M perchloric acid (containing 1 mM EDTA), neutralized with 2.2 M KHCO\textsubscript{3}, and assayed for citrate, isocitrate, succinate, fumarate, malate, pyruvate, lactate, phosphocreatine, and creatine with enzymatic methods (4, 14, 19) adapted for fluorometry (Hitachi F-2000 fluorescence spectrophotometer, Hitachi Instruments). To correct for differences in blood or connective tissue between samples, muscle metabolites were corrected to the highest total creatine value obtained in all biopsy samples for a given subject. Similarly, PDH\textsubscript{a} values were similarly adjusted to the highest peak total creatine concentration determined in neutralized PCA extracts of the wet muscle homogenates used for the PDH\textsubscript{a} analyses.

Statistics. Cardiorespiratory and muscle metabolite data were analyzed with a two-factor (condition × time) repeated-measures ANOVA. The net increase in TCAI from rest to 1 min of exercise was analyzed with a repeated-measures t-test. Statistical significance for all analyses was accepted as P < 0.05, and significant interactions and main effects were further analyzed with a Tukey’s honestly significant difference post hoc test. All data are expressed as means ± SE.

RESULTS

Cardiorespiratory and performance data. Heart rate, pulmonary O\textsubscript{2} uptake, and expired ventilation showed main effects for time (P < 0.05); however, there were no significant interactions between trials. The mean time to fatigue at the higher work intensity, after the 15-min period of submaximal work, was not different between the control and DCA conditions (4.7 ± 0.2 and 4.3 ± 0.3 min, respectively).

Muscle PDH\textsubscript{a}, TCAI, and other metabolites. PDH\textsubscript{a} was markedly elevated (P < 0.05) at rest after DCA infusion compared with control; however, there were no significant differences between trials after 1 and 15 min of submaximal exercise or at Exh (Fig. 1). The total concentration of the five measured TCAI was ~50% lower (P < 0.05) at rest after DCA infusion compared with control (Fig. 2). With respect to individual TCAI, however, only citrate showed a significant interaction and was lower (P < 0.05) at rest after DCA infusion compared with control (Table 1). The concentrations of the remaining four measured TCAI (isocitrate, succi-
DISCUSSION

The main finding from the present investigation was that DCA administration markedly reduced the concentrations of TCAI in resting human skeletal muscle but did not alter TCAI pool size after either moderate or intense leg extensor exercise. Although all of the intermediates were not measured in the present study, it has previously been demonstrated that the sum of citrate, isocitrate, succinate, fumarate, and malate comprises $\approx$90% of total TCAI, both at rest and during exercise in humans (10, 12), and thus provides a quantitative index of total pool size. The present data also confirm the recent observation that DCA reduced the concentrations of citrate and malate in resting human muscle (8) and extend this finding to indicate that DCA does not simply redistribute TCA cycle carbon among the various intermediates but, in fact, causes an overall reduction in total pool size at rest. The lower pool size was likely due to the marked activation of PDH that occurs after DCA administration, as recently demonstrated in humans (26, 27) and confirmed in the present study (Fig. 1). The higher PDH activity at rest apparently diverts pyruvate away from the TCAI pool, most likely through the near-equilibrium alanine aminotransferase reaction (23), and toward the production of acetyl-CoA. This subsequently results in the accumulation of acetylcarnitine (26, 27), because the excess acetyl groups formed are buffered via the carnitine acyltransferase reaction to prevent the depletion of the mitochondrial CoASH pool.

In addition to the stimulatory effect on PDH, it has also been suggested that DCA may inhibit glycolysis in resting skeletal muscle (6). Clark et al. (6), with an incubated rat muscle preparation, reported a higher insulin-stimulated glycogen synthase activity, higher glycogen accumulation, and a reduction in net glycolysis after DCA administration compared with control. DCA also reduced the muscle concentration of lactate (an effect that could also be attributed to increased PDH flux), but there was no change in muscle citrate. Notably, these latter observations are opposite to the findings of the present study and those of Constantin-Teodosiu et al. (8), in which a decrease in muscle citrate and no change in lactate were observed after DCA infusion in resting humans. The conflicting results highlight the difficulty in comparing studies with such dramatic methodological differences, i.e., incubated muscle preparations vs. intact humans. Moreover, although our data cannot rule out the possibility that a reduced rate of pyruvate formation contributed to the lower pool size at rest after DCA infusion, we feel this is unlikely given that we failed to observe any differences between trials in the resting muscle concentration of pyruvate, lactate, or the lactate-to-pyruvate ratio.

In spite of the marked changes observed after DCA administration at rest, PDH$_a$ and the total concentration of TCAI were not different between trials after 1 min or at any subsequent time during exercise. It has previously been shown that the stimulatory effect of DCA on PDH$_a$ is maintained after 15 s of intense exercise in humans (9) and after 1 min of electrical stimulation in ischemic canine muscle (28). Consequently, we hypothesized that less pyruvate would be diverted through alanine aminotransferase, the key anaplerotic reaction at the onset of exercise in humans (10), and thus the magnitude of TCAI pool expansion would be attenuated during exercise. To this end, we used a dose of DCA that was double that given in
previous human studies (9, 17, 18, 26, 27) to try and maximize the potential flux through PDH during the rest-to-work transition. Although this treatment was indeed successful at markedly elevating PDH₃ at rest (Fig. 1), the active enzyme fraction and the total concentration of TCAI were not different between conditions during exercise (Fig. 2). The lower pool size at rest after DCA infusion was compensated for by a higher rate of increase in muscle TCAI during the first minute of exercise (3.1 ± 0.5 vs. 2.5 ± 0.5 mmol·min⁻¹·kg dry wt⁻¹), which is in fact the highest ever reported for human muscle. These data also support recent work from this laboratory (10, 12) indicating that the rate of increase in muscle TCAI during moderate to intense exercise is severalfold higher than previously suggested based on earlier human (22, 24) and rodent investigations (3).

Although the present data cannot precisely resolve the reason for the faster rate of increase in muscle TCAI during exercise in the DCA trial, there are several interpretations that need to be considered. One possible explanation is that, despite the higher initial PDH₃ fraction after DCA infusion, the rate of pyruvate production from glycolysis rapidly exceeded PDH flux in both trials and provided sufficient substrate to force the alanine aminotransferase reaction toward the formation of TCAI. Notably, there was an accumulation of muscle lactate after 1 min of exercise in both trials (main effect for time, P ≤ 0.05), as well as an increase in muscle pyruvate, although the latter was not significant until 15 min of exercise (main effect for time, P ≤ 0.05). In addition, it should be recognized that flux through alanine aminotransferase is also dependent on the muscle concentration of glutamate, which typically shows a large decrease during the initial minutes of exercise (10, 22, 29). Although we did not measure muscle amino acids in the present study, recent observations (unpublished data) from this laboratory suggest that the initial resting concentration of glutamate, provided there is sufficient pyruvate flux, may determine the net increase in muscle TCAI during exercise.

A second possible explanation for the higher rate of increase in muscle TCAI during the DCA trial could be related to an increase in the resting acetyl-CoA-to-CoASH ratio. As alluded to previously, Timmons and co-workers (26–28) have demonstrated a marked accumulation of acetyl-L-carnitine at rest after DCA administration, due to the large increase in PDH activity. This would subsequently be expected to increase the acetyl-CoA-to-CoASH ratio, thereby augmenting one of the main inhibitors of the PDH enzyme complex at rest (30). In vivo regulation of PDH flux is extremely complex and subject to many numerous controlling factors; moreover, it is not clear which factors predominate under various situations, and particularly in skeletal muscle during the rest-to-work transition. Nonetheless, it clearly appears that PDH₃ and PDH flux were increased at rest after DCA infusion in the present study, despite an apparent increase in the acetyl-CoA-to-CoASH ratio.

We speculate, however, that the net effect during exercise might have been that PDH flux was actually lower during the initial seconds of contraction in the DCA trial, despite near-complete transformation of the enzyme to its more active form. Consequently, a transiently higher muscle pyruvate may have briefly increased flux through alanine aminotransferase and contributed to the higher rate of TCAI pool expansion during the first minute of exercise.

Last, it should be noted that DCA may influence the activity of other potential anaplerotic enzymes (25), such as the stimulation of malic enzyme (pyruvate + CO₂ + NAD(P)H ↔ malate − NAD(P)+) in both liver (1) and kidney (18). However, these latter examples in-

### Table 1. Intramuscular metabolites at rest and during exercise

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control Trial</th>
<th>DCA Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>1 min</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.28 ± 0.05</td>
<td>0.30 ± 0.03</td>
</tr>
<tr>
<td>Lactate</td>
<td>7.9 ± 2.0</td>
<td>28.8 ± 7.5</td>
</tr>
<tr>
<td>PCr</td>
<td>77.9 ± 5.6</td>
<td>59.6 ± 8.3</td>
</tr>
<tr>
<td>Creatine</td>
<td>41.8 ± 4.1</td>
<td>60.0 ± 9.4</td>
</tr>
<tr>
<td>Citrate</td>
<td>0.38 ± 0.05</td>
<td>0.58 ± 0.08</td>
</tr>
<tr>
<td>Isocitrate</td>
<td>0.07 ± 0.01</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>Succinate</td>
<td>0.52 ± 0.12</td>
<td>0.99 ± 0.22</td>
</tr>
<tr>
<td>Fumarate</td>
<td>0.03 ± 0.02</td>
<td>0.45 ± 0.12</td>
</tr>
<tr>
<td>Malate</td>
<td>0.54 ± 0.10</td>
<td>1.97 ± 0.41</td>
</tr>
</tbody>
</table>

Values are means ± SE for 6 subjects, expressed in mmol/kg dry wt. Exh, exhaustion; PCr, phosphocreatine; DCA, dichloroacetate. For all such that Exh > 1 min, Exh > rest and 15 min > rest. There were no significant interactions between trials, except for muscle citrate as indicated (*P ≤ 0.05 vs. control at same time point).

![Fig. 3. Net increase in 5 measured TCAI during 1st min of exercise after saline or DCA infusion. Values are means ± SE; n = 6. *P ≤ 0.05 vs. saline control trial.](http://ajpendo.physiology.org/Downloadedfromhttp://ajpendo.physiology.org)
volve increased expression of the enzymes, which therefore precludes any possible involvement of this mechanism in the present study. Regardless of the precise mechanisms involved, however, the present data illustrate that DCA does not alter the steady-state level of TCAI during submaximal exercise or peak TCAI pool size after intense leg extensor work.

Finally, although performance was not the focus of the present investigation, subjects did perform intense exercise until volitional fatigue. DCA has previously been shown to enhance peak pulmonary O₂ uptake and maximal work capacity during incremental cycling in humans (17). This ergogenic effect was primarily attributed to an enhanced cardiac output (16), because DCA appears to exert a direct inotropic effect on the myocardium in addition to altering substrate utilization (25). We did not observe any effect of DCA on time to fatigue; however, the conflicting results between studies may be attributed to the different modes of exercise employed. During small muscle mass exercise, such as the isolated knee extensor work performed in the present study, peak power output and O₂ uptake are probably not limited by muscle blood flow (21). Thus, although a DCA-mediated increase in peak cardiac output could have contributed to the higher maximal exercise capacity observed during whole body cycling exercise (17), this would not be expected to extend time to fatigue during intense leg extensor exercise.

In summary, the results from the present investigation demonstrate that DCA infusion markedly stimulates PDH activity and reduces the total concentration of TCAI in resting human skeletal muscle. However, on contraction, the rate of increase in muscle TCAI was higher after DCA administration, and consequently, there were no differences between trials in TCAI pool size during exercise. The reason for the higher rate of increase in muscle TCAI during DCA trial is not clear but may be related to an initial mismatch between glycolytic flux and PDH flux during both trials, which permitted a sufficient flux of pyruvate to force the alanine transaminase reaction toward the formation of TCAI. This explanation also implies that the availability of muscle glutamate, the cosubstrate for the alanine transaminase reaction, may be an important determinant of the net increase in TCAI during exercise. Alternatively, a transient inhibition of PDH flux in the DCA trial, due to an elevated resting acetyl-CoA-to-CoASH ratio, may have briefly increased flux through alanine transaminase and permitted a more rapid increase in TCAI during exercise.

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