Monocarboxylate transporters, blood lactate removal after supramaximal exercise, and fatigue indexes in humans


Monocarboxylate transporters, blood lactate removal after supramaximal exercise, and fatigue indexes in humans. J Appl Physiol 98: 804–809, 2005. First published November 5, 2005; doi:10.1152/japplphysiol.01057.2004.—The present study investigated whether muscular monocarboxylate transporter (MCT) 1 and 4 contents are related to the blood lactate removal after supramaximal exercise, fatigue indexes measured during different supramaximal exercises, and muscle oxidative parameters in 15 humans with different training status. Lactate recovery curves were obtained after a 1-min all-out exercise. A biexponential time function was then used to determine the velocity constant of the slow phase ($\gamma_2$), which denoted the blood lactate removal ability. Fatigue indexes were calculated during 1-min all-out (F1,40) and repeated 10-s (F1,840) cycling sprints. Biopsies were taken from the vastus lateralis muscle. MCT1 and MCT4 contents were quantified by Western blots, and maximal muscle oxidative capacity ($V_{\text{max}}$) was evaluated with pyruvate + malate and glutamate + malate as substrates. The results showed that the blood lactate removal ability (i.e., $\gamma_2$) after a 1-min all-out test was significantly related to MCT1 content ($r = 0.70, P < 0.01$) but not to MCT4 ($r = 0.50, P > 0.05$). However, greater MCT1 and MCT4 contents were negatively related with a reduction of lactate concentration at the end of 1-min all-out exercise ($r = -0.56$, and $r = -0.61, P < 0.05$, respectively). Among skeletal muscle oxidative indexes, we only found a relationship between MCT1 and glutamate + malate $V_{\text{max}}$ ($r = 0.63, P < 0.05$). Furthermore, MCT1 content, but not MCT4, was inversely related to F1,40 ($r = -0.54, P < 0.05$) and F1,840 ($r = -0.58, P < 0.05$). We concluded that skeletal muscle MCT1 expression was associated with the velocity constant of net blood lactate removal after a 1-min all-out test and with the fatigue indexes. It is proposed that MCT1 expression may be important for blood lactate removal after supramaximal exercise based on the existence of lactate shuttles and, in turn, in favor of a better tolerance to muscle fatigue.

DURING SUPRAMAXIMAL EXERCISE contracting skeletal muscles produce and accumulate lactate and proton ions. Lactate is either removed by oxidation in the muscle fibers or is released to the blood and removed by other cells according to the cell-cell lactate shuttle, which is facilitated by membrane-bound monocarboxylate transporters (MCTs; see Refs. 6, 14, 19, 30). In human skeletal muscle, two isoforms (MCT1 and MCT4) with different kinetic properties have been described (36). MCT1 has been found predominantly in oxidative muscles and only in small amounts in glycolytic muscles, whereas MCT4 has been demonstrated with a large fiber type variability (14, 29).

MCT1 and MCT4 contents in human skeletal muscle have been shown to be elevated after a period of endurance (7, 14) and high-intensity training (7, 14, 25, 28). Only a few studies have investigated expression of MCTs and their responses during exercise in humans (14, 21). Both MCT1 and MCT4 were found to be associated with net muscle lactate release during submaximal exercises (7, 14). Although MCT1 and MCT4 are coexpressed in mitochondria and sarcoplasmic membrane (4, 14), only MCT1 appears to participate in increased lactate oxidation after training, by facilitating intramuscular lactate transport (14).

After supramaximal exercise, blood lactate removal can be described by a biexponential function (16–18), and the low velocity constant of this function ($\gamma_2$) reflects the net blood lactate removal ability. Recently, we reported that maximal muscle oxidative capacity was related to $\gamma_2$ after supramaximal exercise in humans with different training status (34). As MCT1 content is related to muscle oxidative capacity (14, 26), we hypothesized therefore that MCT1 content may also be associated with net blood lactate removal.

In an attempt to verify this hypothesis, we investigated the expression of MCT1 in human skeletal muscle and the velocity constant of net blood lactate removal during recovery from a supramaximal exercise in humans with different training status. Because endurance training is associated with a reduction in muscle (13) and blood lactate concentration (23) and induces an increase in skeletal MCT1 expression (7, 14), we further posited that MCT1 content may play a role in the reduction of blood lactate concentration after supramaximal exercise. Although MCT4 does not seem to be related to the lactate uptake from the blood (14), we also investigated the expression of MCT4.

MATERIALS AND METHODS

Subjects

Fifteen healthy male volunteers (mean ± SE; age: 26.5 ± 1.6 yr, weight: 178.7 ± 1.3 kg, body mass: 74.0 ± 2.7 kg) participated in this study. Informed consent was obtained from all subjects after the nature and risks involved in study participation were explained. The study was approved by the local Ethics Committee and conformed to the Declaration of Helsinki regarding the use of human subjects.

Subjects with different training status were deliberately targeted for this study, and subgroups were determined from the subjects’ responses to a physical activity questionnaire and their maximal oxygen

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upstroke (V\textsubscript{O\textsubscript{2 max}}) values. The group consisted of untrained subjects (n = 4, V\textsubscript{O\textsubscript{2 max}} of 52.6 \pm 5.4 ml·min\textsuperscript{-1}·kg\textsuperscript{-1}), recreational athletes (n = 3, V\textsubscript{O\textsubscript{2 max}} of 58.5 \pm 1.2 ml·min\textsuperscript{-1}·kg\textsuperscript{-1}), and middle-distance (MD; n = 4, V\textsubscript{O\textsubscript{2 max}} of 64.5 \pm 1.1 ml·min\textsuperscript{-1}·kg\textsuperscript{-1}) and long-distance (LD; n = 4, V\textsubscript{O\textsubscript{2 max}} of 66.9 \pm 1.9 ml·min\textsuperscript{-1}·kg\textsuperscript{-1}) runners. The untrained subjects had a normal level of physical activity without any athletic training, whereas the recreational athletes trained at low intensity two to three times per week but did not compete. The MD runners competed in the 800-m and 1,500-m events and trained five to six times per week. Their training included frequent high-intensity sessions alternated with endurance sessions. The LD runners competed in events of 5 km or more. They trained eight to ten times per week and ran an average of 110 km per week, including high-intensity workouts with 400- to 3,000-m intervals and continuous running (10–25 km).

**Experimental Design**

All subjects came to the laboratory for the three exercise sessions, as well as for the skeletal muscle biopsy. At least 48 h separated the exercise sessions, and the muscle biopsy was performed 1 wk after the last exercise. The subjects performed all exercise tests at a laboratory temperature of 22°C. An initial laboratory visit was scheduled to obtain data on physical characteristics and individual maximal oxygen uptake (V\textsubscript{O\textsubscript{2 max}}). During the second visit, the subjects were familiarized with the testing procedure on a cycle ergometer. In the third session, they performed a 1-min all-out exercise followed by 60 min of recovery and then performed 10 short cycling sprints separated by 30-s recovery intervals. Each subject was instructed to refrain from intense physical exercise for 48 h before this third visit. In the final session, a muscle biopsy was taken from the vastus lateralis to determine MCT1 and MCT4 contents, citrate synthase (CS) activity, and maximal muscle oxidative capacity.

**Assessment of V\textsubscript{O\textsubscript{2 max}}**

At the beginning of the study, all subjects underwent an incremental maximal exercise test on a treadmill (LE 200 CE Jaeger, Hoechberg, Germany). During the test, oxygen uptake (V\textsubscript{O\textsubscript{2}}), carbon dioxide production, and minute ventilation were measured breath by breath by means of an open circuit metabolic cart (Oxycon Pro, Jaeger). The test consisted of the repetition of short maximal sprints using different friction loads with 400- to 3,000-m intervals and continuous running (10–25 km).

**Supramaximal Exercises**

The subjects performed all exercise tests on a standard friction-loaded cycle ergometer (Monark type 818 E, Stockholm, Sweden) equipped with both a strain gauge (Interface MFG type, Aldingen, Germany; for details, see Ref. 1) and an optical encoder (Hengstler type RIS IP50, Scottsdale, AZ) and an optical encoder (Hengstler type RIS IP50, Scottsdale, AZ) and an optical encoder (Hengstler type RIS IP50, Scottsdale, AZ) and an optical encoder (Hengstler type RIS IP50, Scottsdale, AZ) and an optical encoder (Hengstler type RIS IP50, Scottsdale, AZ). For each sprint, the friction force was adjusted to the subject's body mass (31). The fatigue index of the all-out test for power (F\textsubscript{IAO}) output was calculated as the percentage of decline from peak to end values for each subject.

The subjects then performed a 6-min warm-up on the cycle ergometer at a moderate power output. After 5 min of rest, the subjects performed 10 consecutive 10-s sprints separated by 30-s recovery intervals against a friction load corresponding to 50% of the optimal value of the friction force for each subject (31). The fatigue index of the sprints for peak power output (F\textsubscript{IAO}) was calculated as the percentage of decline from the first sprint (P\textsubscript{MaxSprint10}) to the tenth sprint (P\textsubscript{MaxSprint10}) (31).

**Blood Lactate**

Blood samples were drawn from a 32-mm catheter placed into a superficial forearm vein. The blood samples were collected in tubes containing heparin; at rest; just after the warm-up; and at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 30, 40, 50, and 60 min of recovery from the all-out test. Blood lactate content was analyzed enzymatically according to the spectrophotometric method of Gutmann and Wahlberg (22). Each individual curve was fitted using the following biexponential function:

\[ y(t) = A_1 \exp(-t/T_1) + A_2 \exp(-t/T_2) \]

where \( A_1 \) and \( A_2 \) are the measured lactate concentrations in blood at time \( t \) after the end of exercise and at the beginning of the recovery; \( T_1 \) and \( T_2 \) (in min) are the amplitudes of the two exponential components; and \( \gamma_1 \) and \( \gamma_2 \) (per minute) are their respective velocity constants. The individual parameters of the biexponential function were fitted by means of an iterative nonlinear technique, using DataFit 6.0 software to determine the values of \( A_1 \), \( A_2 \), \( \gamma_1 \), and \( \gamma_2 \). The percentage of the variance explained by the use of the biexponential curve fit was determined by correlation of the observed and the predicted [La(t)] at each time and by squaring of the Pearson product-moment correlation coefficient.

**Skeletal Muscle Biopsy**

Vastus lateralis muscle biopsies were taken by the percutaneous Bergström technique after local anesthesia (Xylocaine) 1 wk after all cycling exercise tests. The muscle samples were divided into two portions. One portion was immediately frozen in liquid nitrogen and stored at \( -80°C \). The other portion was used for the in situ respiration studies (35). As we previously reported, maximal muscle oxidative capacity, reflected by maximal ADP-stimulated mitochondrial respiration, was evaluated in an oxygraph cell on saponin-permeabilized
are mean, peak, and end power outputs, respectively. FIAO is the fatigue index. Sample preparation for Western blotting. Proteins were isolated from muscles for Western blotting by a method previously described by McCullagh et al. (26) and previously used in our laboratory (15). Muscle protein concentrations were determined in duplicate by bicinchoninic acid assay (Pierce, Interchim, Montluçon, France) with the use of BSA as a standard. 

Western blotting of MCT1 and MCT4. Affinity polyclonal antibodies directed against the carboxy terminus of human MCT1 and MCT4 were produced with the synthetic peptide C-Ahx-KDEGGPKREEESPV-OH for MCT1 and CAhx-GEVHPTETSV-OH for MCT4, like the sequence used by Brooks and coworkers (12, 14) and by Halestrap and associates (26, 36). Such peptides were linked with keyhole limpet hemocyanin-activated carrier protein according to a protocol recommended by the protocol recommended (Pierce), and the resulting solutions were injected as specific antigens for immunization of New Zealand White rabbits. Western blotting was probed with these antibodies and Chemicon International antibodies (Temecula, CA; rabbit anti-MCT1: AB3538P and rabbit anti-MCT4: AB3316P). Polyclonal antibodies yielded a single band on a Western blot that corresponded to 43 kDa, consistent with the molecular mass reported earlier (26). Antibody specificities were confirmed in preliminary experiments in which the peptides blocked the detection of MCT1 and MCT4. Samples of muscle homogenates (12 μg protein) and prestained molecular mass markers (Bio-Rad), were separated on 10% Bis-Tris-acrylamide gels (200 V for ~60 min) with the Novex system (Invitrogen, Groningen, The Netherlands). We prepared a large sample of a human biopsy. At each time, we used this sample to serve as control for all gels. Proteins were then transferred from the gels to polyvinyliden difluoride membranes (30 V, 180 min), and the membranes were incubated on a shaker overnight at 4°C temperature in buffer D [20 mmol/l Tris base, 137 mmol/l NaCl, 0.1 mol/l HCl, adjusted to pH 7.5, 0.1% (vol/vol) Tween 20, and 5% (wt/vol) nonfat dried milk]. The membranes were then incubated with diluted carboxy terminus of either MCT1 antibody (1:90,000) or MCT4 (1:90,000) in buffer D for 2 h at room temperature, followed by four washes in buffer E (i.e., buffer D without dried milk; 4 × 5-min washes), and then incubated for 50 min with goat anti-rabbit immunoglobulin G horseradish peroxidase-conjugated secondary antibody (1:10,000, BI 2407, BioSys, Compiègne, France) in buffer E. Membranes were washed as previously described, and MCT1 or MCT4 expression was detected by enhanced chemiluminescence (Biomax MR films, Kodak, Reuil-Malmaison, France). Films were developed and fixed using a Hyperprocessor (RNP 1700, Amersham, Les Ulis, France). MCT1 and MCT4 protein band densities were determined by scanning the blots on a scanner (AGFA Duo Scan T1200, New York, NY) and Scion Image software (Scion, Frederick, MD). Results were expressed in arbitrary optical density units as used by others (15). 

CS activity. Homogenates for CS activity were prepared in buffer containing (in mM) 210 sucrose, 2 EGTA, 40 NaCl, 30 HEPES, 5 EDTA, and 2 phenylmethylsulfonyl fluoride (pH 7.4) and stored at –80°C. CS activity was assayed by a spectrophotometric method according to Srere (33). Changes in absorbance were recorded over 3 min at 412 nm at 25°C.

Table 1. Values of mechanical power output and the fatigue index during the 1-min all-out test

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<tr>
<td>PMeanAO, W</td>
<td>448±13</td>
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<tr>
<td>PMaxAO, W</td>
<td>1,203±55</td>
</tr>
<tr>
<td>PMeanAO, W</td>
<td>348±24</td>
</tr>
<tr>
<td>FIAO, %</td>
<td>69±5</td>
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</table>

Values are means ± SE for n = 14 subjects. PMeanAO, PMaxAO, and PMeanAO are mean, peak, and end power outputs, respectively. FIAO is the fatigue index.

Table 2. Values of peak power output for sprints 1 and 10 and the fatigue index during the repeated short sprints

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<table>
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<tr>
<td>PMaxSprint1, W</td>
<td>1,275±47</td>
</tr>
<tr>
<td>PMaxSprint10, W</td>
<td>1,044±34</td>
</tr>
<tr>
<td>FISprint, %</td>
<td>17±3</td>
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Values are means ± SE for n = 15 subjects. PMaxSprint1 and PMaxSprint10 are the maximal power output during the first and the ten sprints. FISprint is the fatigue index.

Statistical Analysis

Descriptive statistics are expressed as means ± SE. Student’s t-test was performed to compare MCT1 and MCT4 contents determined with different antibodies and to compare the level of MCTs between the well-trained group (n = 8: MD and LD runners) and less-trained subjects (n = 7: recreational and untrained subjects). Individual relationships between variables were studied by means of linear regressions using Statview software. The level of significance was set at P ≤ 0.05.

RESULTS

Some data were reported previously (34) but are repeated for convenience.

Mechanical Performance

The values for peak power output and mean power output during the 1-min all-out test and the 10 short repeated sprints are given in Tables 1 and 2.

Peak Blood Lactate Concentrations

Peak blood lactate concentrations measured during the recovery of all-out exercise were 13.4 ± 0.9 mmol/l for the untrained subjects, 13.8 ± 1.6 mmol/l for the recreational subjects, and 13.8 ± 1.5 mmol/l for the MD runners, and 9.7 ± 0.7 mmol/l for the LD runners.

Blood Lactate Recovery Curves

As previously described (34), the biexponential model accurately fit the individual recovery curves observed after the 1-min all-out exercise. The mean values of the parameters of the fit from the biexponential equation to the lactate recovery curves are reported in Table 3. In most cases, the biexponential equation accounted for >98% of the variance in the measured lactate recovery curves. In addition, the fit accuracy for all subjects was comparable to that obtained previously by other authors (17).

Table 3. Parameters of the biexponential curves fitted to blood lactate recovery curves obtained from 1-min supramaximal cycling exercise

<table>
<thead>
<tr>
<th>[La(0)], mmol/l</th>
<th>A1, mmol/l</th>
<th>γ1, min⁻¹</th>
<th>A2, mmol/l</th>
<th>γ2, min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SE</td>
<td>5.2±0.6</td>
<td>20.0±7.3</td>
<td>0.266±0.028</td>
<td>-28.0±7</td>
</tr>
</tbody>
</table>

Values are means ± SE for n = 15 subjects. A1, A2, γ1, and γ2 refer to the definition of the biexponential equation in lactate kinetics analysis. [La(0)], blood lactate concentration at the end of exercise.
MCT1 and MCT4 Contents

No statistical difference was found in muscle MCT1 and MCT4 contents determined both with our antibodies and those of Chemicon (Fig. 1A). As shown in Fig. 1B, MCT1 content was significantly higher \((P < 0.05)\) in well-trained subjects [MD and LD runners: 79 ± 6 arbitrary units (AU)] than in less-trained subjects (recreational and untrained: 55 ± 6 AU). Surprisingly, there was no significant difference \((P = 0.1)\) in MCT4 content between the well-trained group (71 ± 7 AU) and less-trained subjects (55 ± 6 AU).

Relationships Between MCT1 and MCT4 Contents and Other Variables

As shown in Fig. 2, A and B, the velocity constant of net blood lactate removal \((\gamma_2)\) was positively correlated with the MCT1 content \((r = 0.70, P < 0.01)\) but not with MCT4 content \((r = 0.50, P > 0.05)\). Blood lactate concentration at the end of 1-min all-out exercise was negatively correlated to the MCT1 \((r = 0.56, P < 0.05)\) and MCT4 contents \((r = 0.61, P < 0.05)\).

MCT1 was correlated with maximal ADP-stimulated mitochondrial respiration measured with glutamate \((r = 0.63, P < 0.05)\) but not with pyruvate + malate as substrates \((r = 0.38, P > 0.05)\). Surprisingly, MCT1 was not correlated with CS activity \((r = 0.43, P > 0.05)\). MCT4 was not statistically correlated with either maximal ADP-stimulated mitochondrial respiration or with CS activity.
In addition, MCT1 content was negatively correlated with the fatigue indexes during the 1-min supramaximal all-out exercise test \( (r = -0.54, P < 0.05) \) and during the 10 short cycling sprints \( (r = -0.58, P < 0.05) \). No statistical correlation was found between MCT4 and both fatigue indexes.

**DISCUSSION**

The main result of the present study was that the blood lactate removal ability (i.e., \( \gamma_2 \)) after a 1-min all-out test was related to MCT1 content but not to MCT4. However, greater MCT1 and MCT4 contents were associated with a reduction of blood lactate concentration at the end of 1-min all-out exercise. Among indexes of skeletal muscle oxidative capacity, we only observed a relationship between MCT1 and maximal ADP-stimulated respiration with glutamate + malate as substrates. Furthermore, MCT1 content, but not MCT4, was inversely related to the fatigue indexes measured during the 1-min all-out test and 10 successive cycling sprints.

Well-trained subjects presented significantly higher MCT1 expression compared with less-trained subjects, but no significant difference was found for MCT4, although a trend existed \( (P = 0.1) \). These results agreed with past studies on endurance training effects on MCT1 and MCT4 expression \( (7, 14) \). Therefore, training status may influence the relationships between MCT1 content and net blood lactate removal ability, although some disparities for MCT1 expression appeared among well-trained subjects (Fig. 2A). In MD and LD runners, greater MCT1 content could facilitate lactate uptake at the sarcolemmal \( (2, 7, 14) \) and mitochondrial membranes \( (11, 14) \). Furthermore, when arterial lactate concentration and muscle blood flow are high \( (3) \), resting and previously active muscle fibers can uptake lactate during recovery because of the existence of lactate shuttles \( (9, 11) \).

In the present study, significant negative relationships were observed between both MCT1 and MCT4 contents and blood lactate concentration at the end of supramaximal exercise as a function of training status. This lower blood lactate in highest-trained subjects was in agreement with the fact that endurance training has been shown to decrease muscle lactate concentration by increasing lactate clearance \( (13) \) and by decreasing lactate production at low but not high power outputs \( (5) \). Indeed, based on some relationships presented here, we postulate that the lower lactate concentration was also due to an increased removal capability subsequent to increased expression of MCTs. This interpretation is in agreement with Green et al. \( (21) \). Indeed, they proposed that, after another type of exercise stimulus (submaximal prolonged cycle exercise), the observed lower lactate concentration was likely a result of an increased lactate removal dependent on increases in blood flow and MCT levels. By facilitating blood and intramuscular lactate exchange and oxidation, MCT1 could depress net muscle lactate release \( (14) \).

In human cross-sectional studies \( (27, 29) \), lactate transport capacity and MCT1 content were positively correlated with the percentage of slow-twitch oxidative fibers, whereas MCT4 content presented a large fiber type variability \( (29) \). Because maximal muscle oxidative capacity is related to the percentage of slow-twitch fibers, we hypothesized that MCT1 content, but not MCT4, measured in this study would be positively correlated with maximal muscle oxidative capacity \( (34) \). The absence of relationship between MCT4 expression and either maximal muscle oxidative capacity or CS activity corroborates previous results in rats \( (10, 36) \) and humans \( (14) \). Surprisingly, MCT1 content was only related to the maximal muscle oxidative capacity measured with glutamate + malate but not with pyruvate + malate or with CS activity. These last results contrasted to previous well-established studies in rats \( (26) \) and in humans \( (14) \). This trend of a dissociation between oxidative metabolism and lactate transport system was, however, in agreement with results obtained after high-intensity training \( (25, 28, 32) \), endurance training \( (32) \), or a single prolonged exercise bout in humans \( (21) \). It is difficult to know which metabolic adaptations are associated with the improved blood lactate removal, but nevertheless, it has been reported that the lactate transport is far higher than the metabolic clearance after intense exercise \( (24) \). So, metabolism appears to be a crucial factor and limitations in the membrane transport capacity are of minor importance \( (20) \), although we cannot rule out a regulatory role for lactate carrier with training.

Finally, removal of lactate and protons from skeletal muscle seems to be important to maintain force production. We found that the fatigue indexes measured during the continuous and intermittent supramaximal exercise were inversely related to MCT1 expression. We hypothesized therefore that high lactate and proton transport can prevent fatigue during different types of supramaximal exercises, because the beneficial effects of lactate anions providing oxidizable substrate and gluconeogenic precursors have been recognized \( (8) \).

In conclusion, the present study demonstrated that skeletal muscle MCT1 expression was correlated with the velocity constant of net blood lactate removal after a 1-min all-out test and inversely with the fatigue indexes measured during continuous and intermittent supramaximal all-out exercises. It is proposed that MCT1 expression may be important for blood lactate removal after supramaximal exercise and, in turn, in favor of a better tolerance to muscle fatigue.

**ACKNOWLEDGMENTS**

The authors thank Fabien Junius, Magali Bonnet, Marie-Chantal Granat, and Anne Levasseur for technical assistance. We thank also David Bishop for English correction.

**GRANTS**

The present study was supported by a Research Grant from Laboratoire MERCK-MF Richelet (Paris, France).

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LACTATE TRANSPORT AND SUPRAMAXIMAL EXERCISE 809


