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Endurance training, expression, and physiology of LDH, MCT1, and MCT4 in human skeletal muscle

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Dubouchaud, Hervé, Gail E. Butterfield, Eugene E. Wolfel, Bryan C. Bergman, and George A. Brooks. Endurance training, expression, and physiology of LDH, MCT1, and MCT4 in human skeletal muscle. *Am J Physiol Endocrinol Metab* 278: E571–E579, 2000.—To evaluate the effects of endurance training on the expression of monocarboxylate transporters (MCT) in human vastus lateralis muscle, we compared the amounts of MCT1 and MCT4 in total muscle preparations (MU) and sarcolemma-enriched (SL) and mitochondria-enriched (MI) fractions before and after training. To determine if changes in muscle lactate release and oxidation were associated with training-induced changes in MCT expression, we correlated band densities in Western blots to lactate kinetics determined in vivo. Nine weeks of leg cycle endurance training [75% peak oxygen consumption ($\dot{V}O_{2peak}$)] increased muscle citrate synthase activity (+75%, $P < 0.05$) and percentage of type I myosin heavy chain (+50%, $P < 0.05$); percentage of MU lactate dehydrogenase-5 (M4) isozyme decreased (−12%, $P < 0.05$). MCT1 was detected in SL and MI fractions, and MCT4 was localized to the SL. Muscle MCT1 contents were consistent among subjects both before and after training; in contrast, MCT4 contents showed large interindividual variations. MCT1 amounts significantly increased in MU, SL, and MI after training (+90%, +60%, and +78%, respectively), whereas SL but not MU MCT4 content increased after training (+47%, $P < 0.05$). Mitochondrial MCT1 content was negatively correlated to net leg lactate release at rest ($r = -0.85$, $P < 0.02$). Sarcolemmal MCT1 and MCT4 contents correlated positively to net leg lactate release at 5 min of exercise at 65% $\dot{V}O_{2peak}$ ($r = 0.76$, $P < 0.03$ and $r = 0.86$, $P < 0.01$, respectively). Results support the conclusions that *1*) endurance training increases expression of MCT1 in muscle because of insertion of MCT1 into both sarcolemmal and mitochondrial membranes, *2*) training has variable effects on sarcolemmal MCT4, and *3*) both MCT1 and MCT4 participate in the cell-cell lactate shuttle, whereas MCT1 facilitates operation of the intracellular lactate shuttle.

exercise; exertion; lactate; lactate shuttle; lactate transport; glucose transport; lactate dehydrogenase; mitochondria; monocarboxylate transporter

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ACCORDING TO THE CELL-CELL lactate shuttle hypothesis, lactate is more a metabolic intermediate than an end product (7, 8). Lactate is continuously formed in and released from diverse tissues such as skeletal muscle, skin, and red blood cells (RBCs). Lactate also serves as an energy source in highly oxidative tissues such as the heart and a gluconeogenic precursor for the liver. Lactate exchanges between these tissues appear to occur under various conditions ranging from postprandial to sustained exercise (11, 13, 18, 36). In humans, arterial lactate concentration is low at rest and increases little during moderate exercise because lactate oxidation and gluconeogenesis match production (5, 11, 15, 36). The cell-cell lactate shuttle is therefore a central means by which the intermediary metabolism in diverse tissues is coordinated (8).

Cell-cell lactate exchanges are facilitated by membrane-bound monocarboxylate transporters (MCT; see Refs. 17, 30, 33). In skeletal muscle, two MCT isoforms (MCT1 and MCT4) with different kinetic properties have been described (17, 37). Recently, the existence of an intracellular lactate shuttle was hypothesized and demonstrated (9, 12), suggesting that cytosolic lactate produced in myocytes and hepatocytes can be transported and oxidized in mitochondria of the same cell. Direct lactate oxidation by mitochondria is dependent on the presence of mitochondrial lactate dehydrogenase (LDH) and a pyruvate/lactate transporter (9).

Endurance training decreases muscle lactate concentrations by increasing lactate clearance (15) and by decreasing lactate production at low but not high power outputs (5). MCT1 expression increases after short-term training in humans (6) and chronic electrical stimulations in rats (25) and decreases after denervation in rats (37).

We hypothesized that during endurance training, regulation of MCT expression would occur to adapt membrane lactate transport capacity to maintain lactate exchanges despite attenuated lactate concentrations. For that purpose, we determined the effect of 9 wk of leg cycle endurance training on expressions of MCT1 and MCT4 in human vastus lateralis muscle. Also, we evaluated the effects of training on glucose and lactate fluxes and muscle exchange (4, 5). Because the mitochondrial lactate/pyruvate transporter has been shown to be MCT1 (10), and because training has been

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shown to increase muscle mitochondrial mass (14, 20), muscle mitochondrial MCT1 content was evaluated. Furthermore, we determined if changes in muscle lactate release and oxidation were associated with training-induced changes in MCT expression.

METHODS

Subjects. Nine healthy sedentary males (age: 27.4 ± 2 yr, height: 178 ± 2.5 cm) participated in the study. Subjects were considered untrained if they engaged in no more than 2 h of physical activity per week for the previous year and had a peak oxygen consumption ($\dot{V}O_{2\,peak}$) of <45 ml·kg^{-1}·min^{-1}. Subjects were included in the study if they had <25% body fat, were nonsmokers, were diet and weight stable, had a 1-s forced expiratory volume (FEV_1) of 70% or more of vital capacity, and were injury/disease free as determined by physical examination. Subjects gave their informed consent, and the study was approved by the Committee for the Protection of Human Subjects at Stanford University and the University of California, Berkeley (CPHS 97–6–34).

Preliminary testing. Determinations of $\dot{V}O_{2\,peak}$ were carried out on an electronically braked cycle ergometer. Exercise testing started at a power output of 50 watts that increased by 25 or 50 watts every 3 min until exhaustion (3–5). Respiratory gases were analyzed via an indirect open-circuit system and were recorded on an on-line, real-time PC-based system. FEV_1 were determined using a 9-liter spirometer.

Training protocol and muscle biopsies. Training was performed on stationary cycle ergometers 6 days/wk with workloads adjusted to elicit heart rates corresponding to 75% of $\dot{V}O_{2\,peak}$. Subjects were exercising at 75% of their $\dot{V}O_{2\,peak}$ for 1 h by the end of the second week of training. After 4 wk of training, subjects performed another maximal exercise test to quantify increases in $\dot{V}O_{2\,peak}$, and training workloads were adjusted to maintain relative training intensity at 75% $\dot{V}O_{2\,peak}$. Two weeks before posttraining testing, subjects began interval training during the last 10 min of each 1-h workout to develop recruitment patterns conducive to reaching maximal power outputs during posttraining evaluation. Subjects were weighed daily and were asked to increase energy intake to maintain weight during the training program without changing normal macronutrient composition. The day before exercise testing, subjects rested to avoid any acute effect of exercise. On the day of exercise testing, one vastus lateralis was prepared for percutaneous needle biopsy. Muscle biopsies (40–150 mg) were taken at rest before and after the training period, immediately plunged in liquid nitrogen, and subsequently stored in liquid nitrogen until analysis.

Biopsy preparation. Muscle homogenates (1:33) were prepared at 4°C in *buffer A* composed of 210 mM sucrose, 2 mM EGTA, 40 mM NaCl, and 30 mM HEPES, pH 7.4, supplemented with 0.15% protease inhibitor cocktail (Sigma P-8340) by means of a motor-driven glass homogenizer. Aliquots were stored at −78°C for myosin heavy chain (MHC) analysis and biochemical assays. To give a total muscle (MU) preparation, homogenates were centrifuged at 600 *g* for 10 min at 4°C to eliminate RBC material. The supernatant was diluted with 0.75 vol of *buffer B* (1.167 M KCl and 58.3 mM $Na_4P_2O_7 \cdot 10\ H_2O$, pH 7.4) and centrifuged at 230,000 *g* for 90 min to remove contractile proteins. The resulting pellet was resuspended with a Polytron set at 50% of maximal speed (2 × 15 s at 4°C) with *buffer* C composed of 1 mM EDTA and 10 mM Tris, pH 7.4. The suspension was then mixed with 0.33 vol of 16% SDS and centrifuged at room temperature for 25 min at 1,100 *g* to remove insoluble materials. Supernatants were divided into aliquots and were stored at −78°C for protein assays and immunoblotting. For four subjects, the size of the biopsy also permitted preparation of mitochondria-enriched (MI) and sarcolemma-enriched (SL) fractions. Separation was achieved by centrifuging a part of the 600-*g* muscle homogenate supernatant at 5,000 *g* for 10 min at 4°C. The supernatant containing the SL fraction was diluted with 0.75 vol of *buffer B* and processed as for the MU preparation. The pellet containing mitochondrial membrane material was resuspended with *buffer C*, mixed with 0.33 vol of 16% SDS, and centrifuged as described above. The supernatant containing the MI fraction was separated into aliquots and stored at −78°C for protein assay and immunoblotting.

Biochemical assays. Proteins were determined by the bicinchoninic acid assay (Pierce) with BSA as a standard. Citrate synthase (CS) activity was measured on muscle homogenates according to the kinetic procedure of Srere (35) by use of a Beckman DU-640 spectrophotometer. Muscle homogenates were thawed at room temperature and sonicated for 30 s before enzymatic assay. Results were expressed in micromoles per minute per gram. LDH isozymes present in the MU preparation and in the MI fraction were separated as previously described (12). Typically, 1–2 µg protein were loaded on 1% agarose gels and separated for 45 min at 90 volts. Separated LDH isozymes were visualized with a commercial kit (procedure 105; Sigma). Gels were scanned with a Bio-Rad GS-700 densitometer, and bands were quantified by software analysis with Molecular Analyst (Bio-Rad Laboratories). Results were expressed as percentage of the total LDH content.

MHC isoform analyses. Myofibrils were purified as previously described (16). Briefly, samples of muscle homogenates (corresponding to 0.5–1 mg of muscle) were pelleted by centrifugation at 10,000 *g* for 20 min at 4°C. Pellets were resuspended in 10 vol of 175 mM KCl, 5 mM EDTA, 0.5% Triton X-100, and 20 mM Tris, pH 6.8, and were centrifuged at 10,000 *g* for 20 min at 4°C. The resulting pellets were washed two times in 10 vol of 150 mM KCl, 5 mM EDTA, and 20 mM Tris, pH 7, and were resuspended in the same buffer to a final concentration of 0.25 mg/ml. Myosin extracts were further diluted to 0.125 mg/ml with sample buffer (22) before being boiled for 5 min. The stacking gels were composed of 30% glycerol, 4% acrylamide-*N,N'*-methylene-bis-acrylamide (bis-acrylamide), 4 mM EDTA, 0.4% SDS, and 70 mM Tris, pH 6.7. The separating gels were composed of 30% glycerol, 7.5% bis-acrylamide, 0.4% SDS, 0.1 M glycine, and 0.2 M Tris, pH 8.8. The amount of protein run on the gel varied between 0.5 and 1.0 µg/lane. Electrophoresis was carried out with a Bio-Rad Mini Protean II cell at 90 volts (constant voltage) for 30 h at 4°C. Gels were stained with Coomassie blue, and the MHC isoforms were analyzed as described above. Results were expressed as percentage of the total MHC content.

Immunoblotting. Affinity-purified polyclonal antibodies to MCT1 and MCT4 were produced by immunizing rabbits with synthetic peptides as described elsewhere (29). The peptides corresponded to amino acids 483–500 of human MCT1 (SPDQKDTEGGPKEEESPV) and to amino acids 440–455 of human MCT4 (LREVEHFLKAEPEKNG; see Ref. 30). Antibody specificities were confirmed in preliminary experiments where the peptides blocked the detection of MCT1 and MCT4. Human RBC membranes were used as a positive control for MCT1 and to fix an arbitrary unit to allow comparison between experiments (1 equals the MCT1 signal generated by 5 µg of RBC proteins). The rabbit polyclonal antibodies to GLUT-1 and GLUT-4 used in this study were gifts (M. Kern and G. L. Dohm; see Ref. 21). The anti-cytochrome oxidase (Cox) antibody was a mouse monoclonal antibody (20E8-C12) against the subunit IV of the Cox (Molecular Probes). The

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secondary antibodies used were a donkey anti-rabbit immunoglobulin horseradish peroxidase-linked monoclonal antibody and a sheep anti-mouse immunoglobulin horseradish peroxidase-linked monoclonal antibody (Amersham). Protein contents of membrane preparations were determined as described above, and equal amounts of proteins were solubilized in sample buffer (22). The proteins along with molecular weight markers were separated on an 11% SDS-PAGE and were transferred by electroblotting onto polyvinylidene difluoride (PVDF) membranes. For each experiment, a preliminary electrophoresis was run, and the gel was stained with Coomassie blue to compare the protein band intensities to verify that equal amounts of proteins were effectively loaded on gels. PVDF membranes were incubated overnight at 4°C in blocking buffer (150 mM NaCl, 10% nonfat dried milk, 0.1% Tween 20, and 50 mM Tris, pH 7.5) and then incubated with either anti-MCT1 antibody (0.1 μg/ml) or anti-MCT4 antibody (0.2 μg/ml) for 2 h at room temperature in blocking buffer. After a 15-min wash followed by two 5-min washes in 150 mM NaCl, 0.1% Tween 20, and 50 mM Tris, pH 7.5 (TTBS), membranes were incubated for 60 min at room temperature with anti-rabbit secondary antibody in TTBS. Membranes were then washed as described above, and MCT1 or MCT4 expression was detected by enhanced chemiluminescence (ECL) detection according to the manufacturer's instructions (Renaissance; NEN). Autoradiography films (Reflection; NEN) were exposed to membranes for appropriate times and were developed with a film processor (Konica QX-130A Plus). Films were scanned, and band intensities were determined as described above. In a preliminary set of experiments, we found that the detection of MCT1 and MCT4 was linear from 1.25 to 30 μg RBC proteins. After being probed, the blots were incubated in 2% SDS, 100 mM β-mercaptoethanol, and 60 mM Tris, pH 6.8, for 30 min at 50°C and were washed with TTBS, as described above, to remove the MCT signal. After blocking for 1 h at room temperature, the membranes were cut into two parts at the 30-kDa region. Top sections were incubated with anti-GLUT-1 (1 μg/ml) or anti-GLUT-4 antibodies (0.5 μg/ml) for 2 h at room temperature. Bottom sections were incubated with anti-Cox antibodies (0.2 μg/ml) for 5 h at room temperature. After being washed as described above and after being incubated with appropriate secondary antibodies, GLUT-1, GLUT-4, and Cox contents were visualized by ECL detection and analyzed as described earlier.

Lactate exchange and metabolism in vivo. In a previous report from the same investigation (5), we quantitated active limb lactate metabolism and exchanges by using both tracers and arteriovenous differences of lactate. To evaluate the roles of MCT1 and MCT4 in lactate metabolism and exchange, we correlated parameters of lactate kinetics and the results on MCT expression.

Statistical analyses. Complete sets of pre- and posttraining biopsies were preserved from seven subjects; of these, sample sizes were sufficient to separate pre- and posttraining MI and SL fractions from four subjects. Data are presented as means ± SE. Western blot data are expressed in arbitrary units as defined earlier. Statistical significance was assessed by paired Student's *t*-tests to examine the effect of leg cycle endurance training on the variables studied. Pearson product-moment regression coefficients were calculated by the least-squares method to fit data to a linear model. A probability level of $P < 0.05$ was used throughout.

RESULTS

Subject characteristics. After 9 wk of leg cycle endurance training, $\dot{V}O_{2peak}$ and peak power output significantly increased by 15 and 23%, respectively, whereas body weight remained unchanged (Table 1). We previously reported that the power output corresponding to the lactate threshold increased by 22% (5). In addition, CS activities in whole muscle homogenates increased by 75% due to training ($P < 0.05$; Table 1).

Table 1. *Subject characteristics before and after 9 wk of leg cycle endurance training at 75% $\dot{V}O_{2peak}$*

	Weight, kg	$\dot{V}O_{2peak}$, l/min	Peak Power Output, W	CS Activity, $\mu mol \cdot min^{-1} \cdot g^{-1}$
Unt	81.8 ± 3.3	43.5 ± 1.3	233.3 ± 7.2	13.7 ± 1.4
Tnd	81.3 ± 3.2	50.1 ± 1.6*	286.1 ± 9.4*	23.2 ± 1.7*

Values are means ± SE; n = 9 subjects except for citrate synthase (CS) activity, where n = 8. Unt, untrained; Tnd, trained; $\dot{V}O_{2peak}$, peak oxygen consumption. *Significantly different from Unt values, $P < 0.05$.

MHC isoform distributions. Three different MHC isoforms were separated from purified myofibrils from vastus lateralis muscle before and after training. Representative electrophoresis results from four subjects are shown in Fig. 1*A*. The relative MHC isoform distribution calculated from gel densitometry is shown in Fig. 1*B*. There was a significant increase in the percentage of type I MHC after training (+50%) and a tendency for the relative proportions of type IIx and type IIa

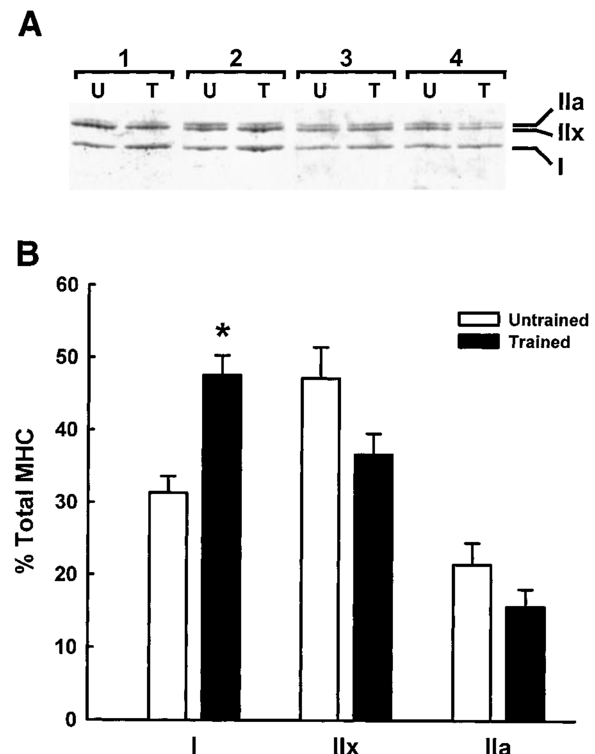

Fig. 1. Effect of leg cycle endurance training on the relative myosin heavy chain (MHC) isoform distribution in human vastus lateralis muscle. *A*: representative electrophoretic MHC separation obtained from 4 subjects before (U) and after (T) training. Three MHC isoforms (I, IIa, and IIx) were named according to their electrophoretic mobility; 1–4, *subjects 1–4*. *B*: densitometric analysis showing relative MHC distribution before and after training. *Significantly different after training, $P < 0.05$. Values are means ± SE of 8 subjects.

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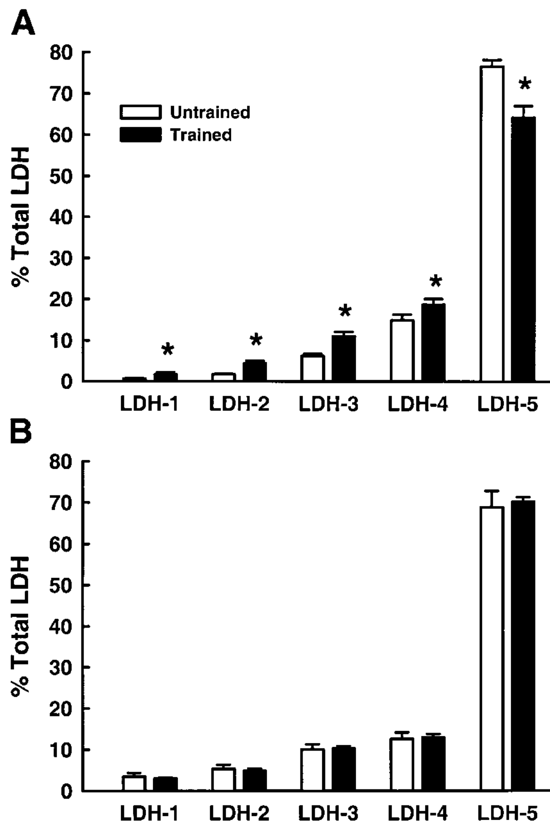

Fig. 2. Effect of leg cycle endurance training on the relative distribution of the lactate dehydrogenase (LDH) isozymes in human vastus lateralis. *A*: LDH distribution in total muscle homogenate expressed as percentage of the total LDH content. *B*: LDH distribution in mitochondria-enriched fraction expressed as percentage of the total LDH content. LDH isozymes are identified as LDH-1, LDH-2, LDH-3, LDH-4, and LDH-5. *Significantly different after training, $P < 0.05$. Values are means ± SE of 7 subjects.

MHC to decrease (−25%, $P = 0.09$ and −32%, $P = 0.06$, respectively).

LDH isozymes. Five LDH isozymes were detected in vastus lateralis muscle (MU) homogenates and MI fractions. The relative amounts of each isozyme in MU and in MI fractions are shown on Fig. 2, *A* and *B*, respectively. LDH-5 was the major isozyme detected in both fractions before and after training. In the MU homogenate, LDH-5 percentage decreased significantly after training (from 76 to 64%), whereas the relative percentage of all other isozymes increased significantly (Fig. 2*A*). In the MI fraction, no significant changes were detected in LDH isozyme distribution with training (Fig. 2*B*).

MCT expression. A representative Western blot showing MCT1 and MCT4 contents in MU preparations obtained from four subjects is shown in Fig. 3*A*. Both MCT1 and MCT4 were expressed in all biopsies studied. Densitometric analysis of blots from seven subjects revealed that MCT1 content increased by ~90% (6.83 ± 0.64 vs. 13.15 ± 0.81 arbitrary units, $P < 0.05$) in MU preparations after training (Fig. 3*B*). Muscle MCT1 contents were consistent among subjects. In contrast, muscle MCT4 contents showed large interindividual variations before training and changed by variable amounts due to training. Muscle MCT4 content declined in *subject 4* (Fig. 3*A*, *right*) after training, whereas as in other subjects the muscle MCT1 content increased. As a result of these variable changes in muscle MCT4 content, the mean value from muscle MCT4 content in seven subjects did not change significantly due to training (5.12 ± 1.05 vs. 7.21 ± 1.81 arbitrary units, $P = 0.36$; Fig. 3*B*).

Muscle CS activity correlated significantly with muscle MCT1 content ($r = 0.83$, Fig. 4*A*) but not with muscle MCT4 content (Fig. 4*B*). Furthermore, muscle CS activity correlated equally well with muscle Cox content ($r = 0.83$, data not shown). The GLUT-4 content in MU preparations and in the SL fraction increased significantly after 9 wk of training (5.08 ± 0.50 vs. 10.29 ± 0.88 and 5.96 ± 0.99 vs. 11.82 ± 1.31 arbitrary units, respectively; data not shown).

Cellular localization of MCT1. Western blots revealed the presence of MCT1 in the MI fraction in addition to its presence in the MU preparation and the SL fraction (Fig. 5). GLUT-1, used as marker of sarcolemmal membrane, was present in the MU preparation and the SL fraction but was hardly detected in the MI fraction either before or after training (Fig. 5). A small

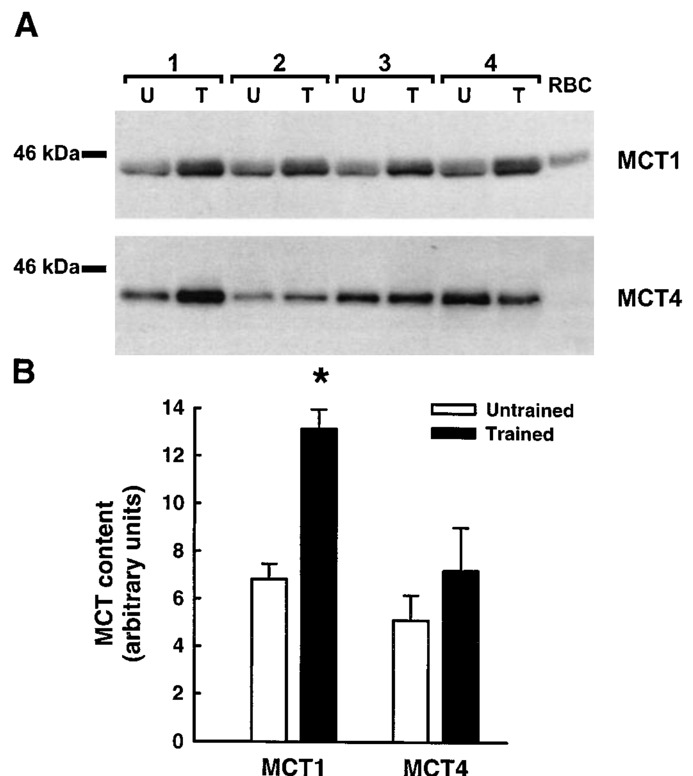

Fig. 3. Effect of leg cycle endurance training on the monocarboxylate transporter (MCT) 1 and MCT4 expression. *A*: representative Western blots showing the expression of MCT1 and MCT4 in total muscle homogenates from 4 subjects before and after training. RBC, human red blood cells. *B*: comparison of the MCT1 and MCT4 contents in total muscle homogenates before and after training. *Significantly different after training, $P < 0.05$. Values are means ± SE of 7 subjects. Results are expressed in arbitrary units (1 = MCT1 signal measured on 5 μg of human RBCs in the same conditions).

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amount of GLUT-4 and MCT4 was detected in the MI fraction before and after training. The Cox protein was expressed in the MU preparation and in the MI fraction and to a lesser extent in the SL fraction (Fig. 5). Relative comparison showed that the ratio of MCT1 in the MI fraction to that of MCT1 in the SL fraction was 12 times larger than the ratio of GLUT-1 in the MI fraction to GLUT-1 in the SL fraction. Similarly, the ratio of MCT1 in the MI fraction to MCT1 in the SL fraction was 6.8 times larger than the ratio of MCT4 in the MI fraction to MCT4 in the SL fraction.

MCT expression in SL fractions. The contents of MCT1 and MCT4 in the SL fractions are shown in Fig. 6*A*. Analyses conducted on four subjects revealed that both MCT1 and MCT4 contents in the SL fraction were significantly increased after training (5.23 ± 0.76 vs. 8.40 ± 0.64 and 6.97 ± 0.70 vs. 10.32 ± 0.85 arbitrary units, respectively; Fig. 6*B*). As a marker of mitochondria, Cox was negligible in the SL fractions and remained unchanged before and after training (Fig. 6*A*, *bottom).*

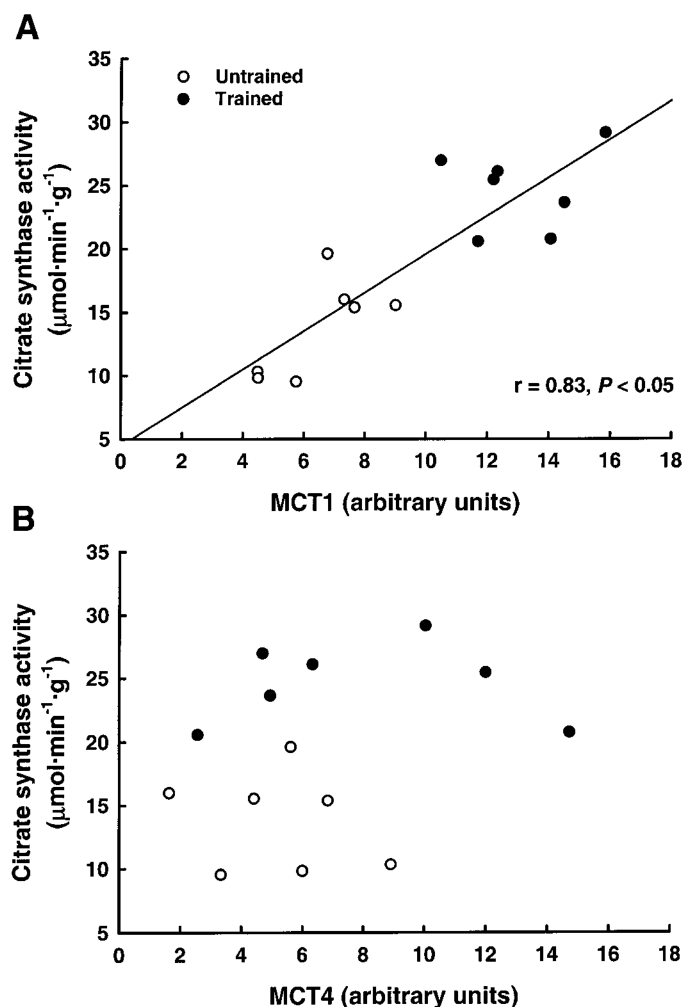

Fig. 4. Relationship between muscle citrate synthase activity and muscle MCT1 content (*A*) and muscle MCT4 content (*B*). MCT contents are expressed in arbitrary units as described in the legend to Fig. 3; $n = 7$ subjects.

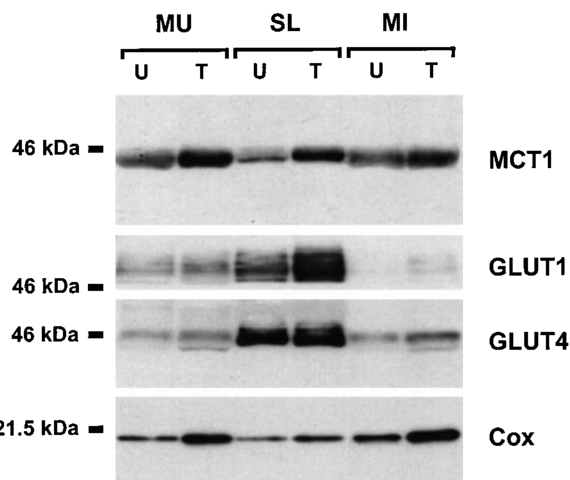

Fig. 5. Representative Western blots showing the amount of MCT1, GLUT-1, GLUT-4, and cytochrome oxidase (Cox) in 3 different muscle fractions before and after endurance training. The fractions studied were total muscle homogenate (MU), sarcolemma-enriched fraction (SL), and mitochondria-enriched fraction (MI).

MCT and Cox expression in MI fractions. Contents of MCT1, MCT4, and Cox in MI fractions are shown in Fig. 7. Densitometric analysis conducted on four subjects revealed that the mitochondrial MCT1 amount was increased by 78% after training (4.05 ± 0.99 vs. 7.28 ± 0.76 arbitrary units). MCT4 contents in the MI fraction remained unchanged after training. Like MCT1, Cox density per amount of mitochondrial protein increased significantly due to endurance training (4.71 ± 0.66 vs. 9.67 ± 1.10 arbitrary units, Fig. 7, *bottom*).

MCT expression and lactate kinetics. In this study, systemic lactate kinetics as well as muscle lactate uptake, oxidation, and release were determined over the course of moderate (45% $\dot{V}O_{2peak}$) and hard (65% $\dot{V}O_{2peak}$) exercise, before and after training (5). In an effort to assess the physiological roles of muscle MCTs, we performed multiple correlations among MCT contents and parameters of muscle lactate metabolism. Analyses on the four subjects from whom pre- and posttraining MI fractions were obtained revealed that the mitochondrial MCT1 content correlated negatively with the net leg lactate release at rest ($r = -0.85$, $P < 0.02$; Fig. 8*A*). In contrast, sarcolemmal MCT1 and MCT4 contents in the same subjects correlated positively with the net lactate release measured at 5 min of exercise at 65% $\dot{V}O_{2peak}$ ($r = 0.76$, $P < 0.03$ and $r = 0.86$, $P < 0.01$, respectively; Fig. 8, *B* and *C*).

DISCUSSION

We report results of the first longitudinal study of long-term endurance training effects on contents of lactate transporter isoforms MCT1 and MCT4 in human vastus lateralis muscle. MCT1 content increased significantly in MU, SL, and MI fractions, whereas MCT4 content increased significantly only in the SL fraction. The presence of MCT1 in both MI and SL fractions and its sensitivity to endurance training imparts a role of this isoform as a mitochondrial and sarcolemmal lactate transporter necessary for the cell-

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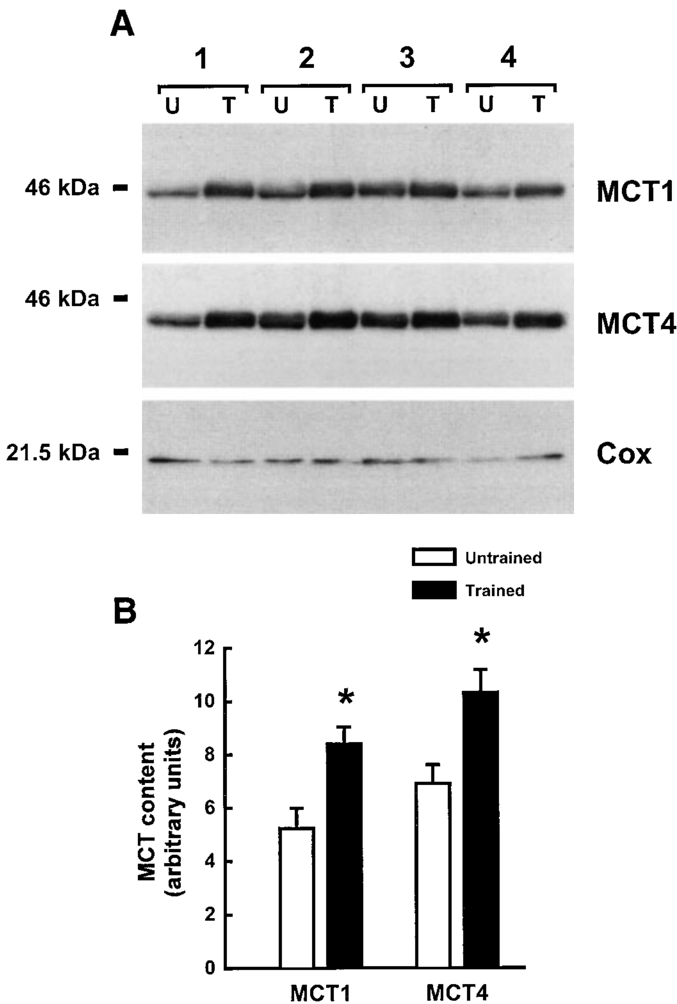

Fig. 6. Effect of leg cycle endurance training on the MCT1 and MCT4 expression in sarcolemma-enriched fractions. *A*: representative Western blots showing the MCT1 and MCT4 contents in the sarcolemma-enriched fractions before and after leg cycle endurance training in 4 subjects. *B*: comparison of the MCT1 and MCT4 contents in sarcolemma-enriched fractions before and after training. *Significantly different after training, $P < 0.05$. Values are means ± SE of 4 subjects. Results are expressed in arbitrary units as described in the legend to Fig. 3.

cell and intracellular lactate shuttles. MCT4 appears to be a constitutive sarcolemmal MCT isoform more likely involved in cell-cell lactate exchange than in intracellular lactate oxidation.

The protocol employed in the present study induced metabolic adaptations with a 15% increase in $\dot{V}O_{2peak}$ and a 23% increase in peak power output. These adaptations were associated with increases in the muscle CS activity. Also, other enzymes and proteins usually associated with endurance training changed as well.

Leg cycle endurance training induced transition from type II to type I myosin isoforms instead of transition within type II isoforms. With the use of histological fiber-typing techniques, increases in the percentage of type I fibers after 8 wk of high-intensity endurance training in human quadriceps (2) have been reported. Also, similar changes have been observed in deltoid muscles of paraplegics and tetraplegics (34). Thus the present data appear to be consistent with previously observed effects of training on myosin isoform composition in human mixed muscle.

LDH-5 decreased in muscle after training in association with an increase in the proportions of other LDH isozymes. Our findings about the proportions of LDH isozymes are in accordance with previous studies reporting that LDH-5 accounts for ~80% of total LDH in human (23) and rat (24) muscles. Limited data from York et al. (38) showed a decrease in the M-subunit percentage in rats after a 12-wk running program. In aggregate, the muscles of the men we studied appeared to acquire a higher-oxidative, slow-twitch pattern as the result of chronic exercise.

Because of the small sample sizes, we could not determine the enzymatic activities of LDH in cell fractions. However, we were successful in separating and quantitating LDH isoforms in cell fractions. Because muscle mitochondrial content increased significantly due to training, we can assume that training induced increases in the mitochondrial LDH activity. The detection of LDH in the mitochondria confirmed our previous observations in rats (12) and supports the presence of the intracellular lactate shuttle in human muscle. Before training, LDH isoforms containing at least two heart-type subunits were present in a higher relative amount in mitochondria than in the MU preparation (Fig. 2, *A* and *B*). These differences were attenuated after training due to increases in the relative amounts of LDH-1–4 in the MU preparations. Our results suggest that lactate shuttle capacity is sensitive to endurance training.

In previous studies on MCT expression in skeletal muscle, Western blots were carried out on MU homogenates from which there was no attempt to remove contaminating RBCs or distinguish between sarcolemmal and mitochondrial MCT locations (1, 6, 24, 25, 27, 28). It has been shown that MCT1 is highly expressed in cell membranes of RBCs, and early attempts to isolate the lactate transporters focused on RBC mem-

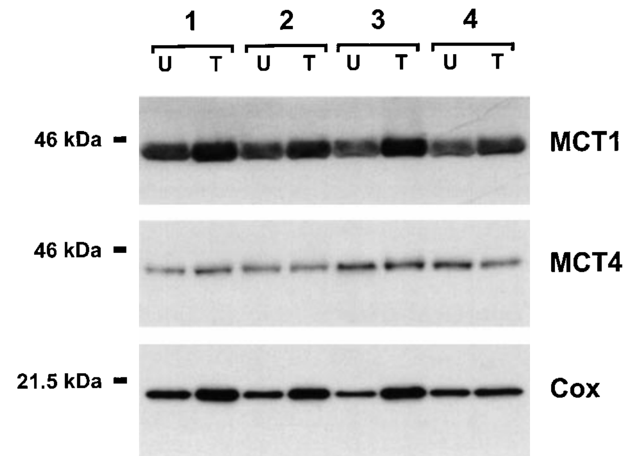

Fig. 7. Representative Western blots showing the MCT1 and MCT4 contents in the mitochondria-enriched fractions before and after leg cycle endurance training in 4 subjects.

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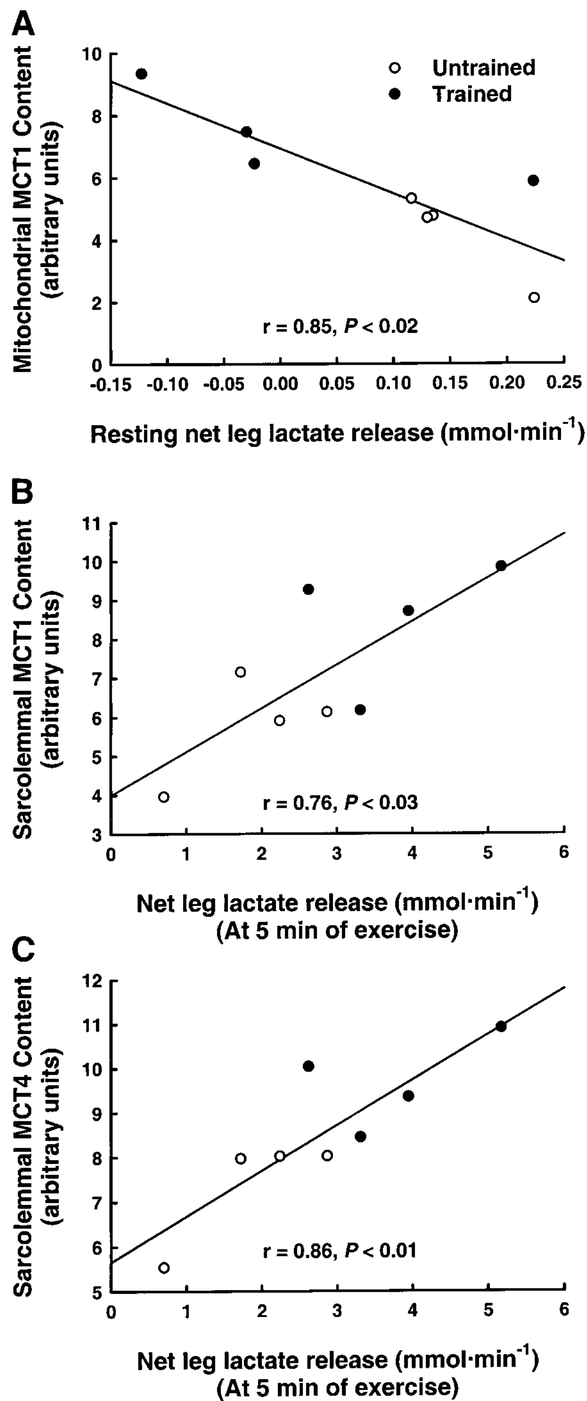

Fig. 8. Relationships between lactate kinetic values and MCT contents. *A*: correlation between net leg lactate release at rest and mitochondrial MCT1 content. *B*: correlation between net leg lactate release at 5 min of exercise and sarcolemmal MCT1 content. *C*: correlation between peak net lactate release from active muscles at 5 min of exercise and sarcolemmal MCT4 content. Lactate data are from Ref. 5; $n = 4$ subjects.

branes (29). To limit nonspecific variations in our data due to increased muscle capillarization after training (31), care was taken to eliminate RBCs from the preparations. Because muscle MCT1 content has been reported to be highly correlated to the mitochondrial function (1, 24), we also separated an MI and a SL fraction from MU homogenate by using differential centrifugation. In this process, we avoided use of Nagarse or trypsin on muscle homogenates (12) to minimize artifactual loss of protein due to the enzymatic digestion of the muscle. To assess cross-contamination of the membrane fractions, we used Cox as a marker of the mitochondrial membrane and GLUT-1 and GLUT-4 as markers of the SL fraction. Contamination of the SL fraction by mitochondrial remnants was negligible because of the relatively lower amount of Cox measured in the SL fraction, as suggested by the low ratios of Cox in the SL fraction to Cox in the MI fraction obtained before and after training (0.25 and 0.09, respectively). On the other hand, the abundance of GLUT-1 and GLUT-4 in the SL fraction confirms that plasma membrane proteins were concentrated in that fraction. Although the SL fraction was not completely free of mitochondrial material, we believe that the cross-contamination of the SL fraction did not interfere in the increases in MCT1 and MCT4 contents because the Cox content in this particular fraction was similar before and after training. The high Cox content in the MI fraction along with the absence of any GLUT-1 confirmed the mitochondrial origin of the MI fraction. GLUT-1 was judged to be a better sarcolemmal marker than GLUT-4 because some GLUT-4 was detected in the MI fraction. Comparison of the ratios of the relative signals for MCT1, GLUT-1, and MCT4 confirmed that assumption. The presence of GLUT-4 in the MI fraction could be the result of contamination of the fraction by intracellular membrane material as it is known that GLUT-4 is located at the plasma membrane level and intracellular depots (26).

In a previous report, we described lactate kinetics measured in the same subjects before and after the 9-wk training protocol (5). Figure 4 in that report shows a hyperbolic relationship between the leg lactate oxidation and the arterial lactate concentration, suggesting an involvement of transporters facilitating lactate metabolism. To determine the respective roles of MCT1 and MCT4 in the muscle lactate exchanges and metabolism, we correlated MCT1 and MCT4 contents with parameters of lactate metabolism, including net muscle (limb) lactate release and intramuscular lactate production, oxidation, and clearance. No correlations were found between muscle MCT contents and lactate oxidation or clearance. Failing to find such correlations requires us to conclude that lactate transport is not the only factor involved in lactate oxidation and clearance. We found that the sarcolemmal MCT1 and MCT4 contents are related to the net leg lactate release occurring during the first 5 min of exercise (5) when peak release occurs. We also found mitochondrial MCT1 content to be correlated negatively with the net leg lactate release in resting conditions (5). Such differences suggest that MCT1 and MCT4 have different functions in muscle. Sarcolemmal MCT1 and MCT4 would accelerate efflux of protons and lactate during exercise from fast glycolytic fibers to neighboring oxidative fibers or the blood for disposal (7). In contrast, mitochondrial MCT1 would facilitate lactate uptake

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and removal by oxidative fibers during and after exercise, especially after endurance training that enhances muscle mitochondrial mass (5, 7, 15). These conclusions are supported by the observed increases in MCT1 content and in lactate oxidation and clearance during exercise (5). Thus, by facilitating intramuscular lactate exchange and oxidation, MCT1 depresses net muscle lactate release.

In our study, expression of MCT1 was correlated to the CS activity, and this reflects the increase in muscle mitochondrial mass due to training. The absence of a relationship between MCT4 expression and either the Cox content or CS activity corroborated previous results on rats (10, 37). After training, the content of MCT1 increased significantly in all of the compartments, whereas the MU content of MCT4 was not significantly modified. These observations were associated with a 100% increase in the sarcolemmal GLUT-4 content and reflect membrane adaptations to endurance training that were not restricted to lactate transporter isoforms. The increases in GLUT-4 expression observed were similar to those reported earlier (32). Increases in muscle MCT1 and MCT4 expression in human muscle have been reported after short-term high-intensity training (6) or after one-legged knee-extensor exercise training (27). Those training programs affected only the working muscles but did not induce the physiological modifications observed in the present study. Differences in MCT expression after training could, therefore, be dependent on the intensity of training. Moreover, the muscle MCT1 contents that we observed appeared to be similar among the subjects, whereas MCT4 contents showed a great intersubject variability both in the muscle preparations and in the SL fractions. Such differences were described previously and were attributed to the heterogeneity of the population studied (28). Interindividual differences in MCT4 expression and the absence of MCT4 in the mitochondria suggest that MCT4 is a constitutive sarcolemmal lactate transporter isoform and is less sensitive to endurance training than the MCT1 isoform.

The mitochondrial MCT1 pool is sensitive to endurance training because a 78% increase was measured after leg cycle training (Fig. 7). Because similar amounts of mitochondrial proteins were loaded on the gels, the increase in mitochondrial MCT1 content after training is due to specific mitochondrial adaptations to training, independent of increases in the total mitochondrial density. Based on the CS activity results, we can also assume that the mitochondrial density increased by 75% after training, a result that is consistent with animal studies (14). Although increases in the muscle mitochondrial content have been observed to occur with minor changes in specific activities of mitochondrial constituents (14), significant increases in the surface density of cytochrome *c* oxidase-positive mitochondria were also observed after endurance training in humans (19). The increase in the absolute mitochondrial MCT1 content is therefore the result of an increase in both mitochondrial density and intrinsic mitochondrial MCT1 content. The mitochondrial MCT1 could participate in increased lactate oxidation after training (5) by facilitating intramuscular lactate oxidation (9, 12). Lactate uptake in mitochondria is facilitated by the mitochondrial MCT1, leading to an increase in lactate oxidation and a decrease in lactate release and resulting in the negative correlation between mitochondrial MCT1 content and the net leg lactate release reported above.

In summary, our results suggest that endurance training increases expression of MCT1 in muscle because of insertion of MCT1 into both SL and MI membranes. In contrast, training of the type that shifts the profile of contractile and metabolic proteins toward slow-twitch, high-oxidative fibers has variable effects on expression of the sarcolemmal lactate transporter MCT4. Both MCT1 and MCT4 appear to participate in the lactate exchange among cells, tissues, and organs, whereas MCT1 facilitates lactate uptake and oxidation in cells with high mitochondrial densities.

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