Distribution of the lactate/H⁺ transporter isoforms MCT1 and MCT4 in human skeletal muscle

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Pilegaard, Henriette, Gerasimos Terzis, Andrew Halestrap, and Carsten Juel. Distribution of the lactate/H⁺ transporter isoforms MCT1 and MCT4 in human skeletal muscle. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E843–E848, 1999.—The profiles of the lactate/H⁺ transporter isoforms [monocarboxylate transporter isoforms (MCT)] MCT1 and MCT4 (formerly MCT3 of Price, N. T., V. N. J. Jackson, and A. P. Halestrap. Biochem. J. 329: 321–328, 1998) were studied in the soleus, triceps brachii, and vastus lateralis muscles of six male subjects. The fiber-type compositions of the muscles were evaluated from the occurrence of the myosin heavy chain isoforms, and the fibers were classified as type I, IIA, or IIX. The total content of MCT1 and MCT4 was determined in muscle homogenates by Western blotting, and MCT1 and MCT4 were visualized on cross-sectional muscle sections by immunofluorescence microscopy. The Western blotting revealed a positive, linear relationship between the MCT1 content and the occurrence of type I fibers in the muscle, but no significant relation was found between MCT4 content and fiber type. Moreover, the interindividual variation in MCT1 content was much larger than the interindividual variation in MCT1 content in homogenate samples. The immunofluorescence microscopy showed that within a given muscle section, the MCT4 isoform was clearly more abundant in type II fibers than in type I fibers, whereas only minor differences existed in the occurrence of the MCT1 isoform between type I and II fibers. Together the present results indicate that the content of MCT1 in a muscle varies between different muscles, whereas fiber-type differences in MCT1 content are minor within a given muscle section. In contrast, the content of MCT4 is clearly fiber-type specific but apparently quite similar in various muscles.

immunofluorescence microscopy; Western blotting; monocarboxylate transporter; fiber type

DURING HIGH-INTENSITY exercise, large amounts of lactate and H⁺ can be produced in skeletal muscle, and the resulting accumulation of lactate and lowering of pH in the muscle can impair the ability of the muscle to maintain force (4). The capacity to transport lactate and H⁺ out of the muscle fibers may, therefore, be expected to affect the ability to perform sustained high-intensity exercise. In addition, the capacity of skeletal muscle to take up lactate constitutes an important step in the redistribution and reuse of lactate after high-intensity exercise.

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tions of the muscles were evaluated from determinations of myosin heavy chain isoforms. The total muscle contents of the MCT1 and the MCT4 isoforms were measured by Western blotting, and MCT1 and MCT4 were visualized on cross-sectional muscle sections by immunofluorescence microscopy.

MATERIALS AND METHODS

Subjects. Six human male subjects age 21–27, with an average height and weight of 188.2 cm (range 183–193) and 84.2 kg (68–96), respectively, participated in the study. The habitual physical activity of the subjects varied from one subject performing low-intensity activities a few times per week to a well-trained subject, who trained everyday (Table 1). The subjects were informed about the experimental procedure before they gave their consent, and the study was approved by the Copenhagen Ethics Committee.

Muscle biopsies. Needle muscle biopsies were obtained from the m. soleus, m. triceps brachii, and m. vastus lateralis of each subject. A fraction of each biopsy was embedded in TISSUE-TEK and frozen, and the rest of the sample was frozen directly in liquid nitrogen. All samples were stored at −80°C until they were analyzed.

Fiber-type composition. The fiber-type compositions of the muscles were determined from the occurrence of the myosin heavy chain (MHC) isoforms in the samples (5). With the use of SDS-PAGE, three different MHC bands, corresponding to the MHC isoforms I, IIA, and IIX, can be separated. We have chosen to use the term IIX (instead of IIB) for the fast-contracting human MHC isoform in accordance with the findings of high homology between this human fast-twitch isoform and the rat IIX isoform (21). Muscle samples were placed in 200–400 µl of lysis buffer at 60°C for 10 min, and thereafter 1–3 µl of the myosin-containing samples were loaded on a SDS-PAGE gel containing 6% polyacrylamide and 37.5% glycerol (21). Gels were run overnight at 70 V, followed by 3–4 h at 200 V. Subsequently, the gels were silver stained and the relative MHC isoform content was determined with a densitometric system (Cream 1-D, Kem-En-Tec Aps, Copenhagen, Denmark).

MCT antibodies. Quantification and visualization of MCT isoforms were carried out with polyclonal antibodies that had been raised to human MCT1 and MCT4 in rabbits as described by Price et al. (19). The peptides used contained the COOH-terminal 16 residues of MCT1 (8) and MCT4 (19), respectively, supplemented with an NH2-terminal cysteine for coupling to keyhole limpet hemocyanin.

MCT isoforms: Western blotting. Muscle samples (20–35 mg) were homogenized in 1 ml of buffer (250 mM sucrose, 30 mM HEPES, 2 ml EGTA, 40 mM NaCl, 2 ml phenylmethylsulfonyl fluoride, pH 7.4) with a Polytron 2100 (2 × 30 s on, setting 6). After centrifugation at 200,000 g for 90 min at 4°C, the pellet was resuspended in 250–400 µl Tris-SDS (10 mM Tris, 4% (wt/vol) SDS, 1 mM EDTA, pH 7.4) and was homogenized with a Polytron 1200 (2 × 15 s, setting 6). After protein determinations (Bio-Rad, detergent compatible protein assay), samples were diluted to 2 mg/ml and subjected to SDS-PAGE (Excell 8–18% gradient gel; Ref. 22). Proteins were transferred to an Immobilon P-polyvinylidene difluoride membrane (Millipore IPVH 00010), which was then incubated in the primary antibody and thereafter in horsedarsh peroxidase labeled goat anti-rabbit antibody. Finally, anti-antigen complexes were visualized by the enhanced chemiluminescence detection method. All samples were run on the same gel to ensure that the MCT densities could be compared. The contents of MCT1 and MCT4 were quantified by densitometric scanning (SigmaGel) of the gel with the spot-measuring mode and by subtracting the background quantified from a measurement outside the lanes.

MCT isoforms: immunofluorescence microscopy. The antibodies directed toward MCT1 and MCT4 were used to localize the lactate/H+ transporter isoforms, and an antibody toward fast MHC was used to identify type I fibers in cross-sectional sections of the muscle biopsies. Thus fiber type and one of the MCT isoforms could be determined in the same section. The fraction of the muscle biopsy that was embedded in TISSUE-TEK was cut in a cryostat into 9-µm thick cross-sectional sections that were put onto glass slides. The following protocol was carried out at room temperature in Tris-base buffered saline (TBS) at pH 7.4. Between each of the incubations, the muscle sections were washed for 10 min in TBS supplemented with 1% (wt/vol) bovine serum albumin (BSA; Sigma), and primary and secondary antibodies were also diluted in TBS with 1% BSA. The sections were fixed with 2% paraformaldehyde for 30 min and thereafter were permeabilized with 0.3% (vol/vol) Triton X-100 for 45 s. Nonspecific binding of antibodies was reduced by blocking for 60 min with normal swine immunoglobulin (20 mg/ml; DAKO A/S, Copenhagen, Denmark), and thereafter the samples were incubated for 50 min with primary antibodies directed toward both fast MHC (1:400; Sigma) and either MCT1 or MCT4 (1:100). Fluorescein-conjugated goat anti-mouse IgG (DAKO A/S) and biotinylated goat anti-rabbit IgG (DAKO A/S) secondary antibodies were then added for 50 min. Finally, streptavidin-sulfhorhodamin (Boehringer Mannheim, Mannheim, Germany) was added for 60 min to amplify the MCT signal. After a final 5-min wash in TBS, the sections were mounted with Vectorshield and examined in a fluorescent microscope. The samples were stored at −20°C until photography.

Statistics. The software program SigmaStat was used for the linear regression analysis based on the least-squares regression method.

RESULTS

Fiber-type composition. The fiber-type compositions of the three different muscles covered a broad range of values with large interindividual differences (Table 2). As expected, the soleus muscles were characterized by a high percentage (range 67–95%) of type I fibers and a small fraction of type IIA fibers. The occurrence of type I and type IIA fibers was more equal in the vastus lateralis and triceps brachii muscles, although large interindividual variations were apparent. Type IIX fibers were present in m. triceps brachii in four of the subjects, only one subject expressed IIX in m. vastus lateralis, and IIX fibers were absent in all soleus muscles.

Table 1. Physical activity profile of subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Physical Activity Profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>Low-intensity activities 2 times/wk</td>
</tr>
<tr>
<td>NL</td>
<td>Recreational volleyball 1 time/wk</td>
</tr>
<tr>
<td>JK</td>
<td>Jogging 2 times/wk, 5–10 km</td>
</tr>
<tr>
<td>SH</td>
<td>Running, 10 km, 1 time/wk + recreational volleyball 1 time/wk</td>
</tr>
<tr>
<td>MB</td>
<td>Heavy resistance training 5 times/wk</td>
</tr>
<tr>
<td>PC</td>
<td>Heavy resistance training 5 times/wk + 5 km high-intensity running 2 times/wk + long-distance walking 2–3 times/month</td>
</tr>
</tbody>
</table>
MCT isoforms: Western blotting. MCT1 was clearly expressed in all muscle homogenates examined. In general, a high and quite similar amount of MCT1 was found in the soleus muscles and a low MCT1 content was observed in the triceps brachii muscles. The vastus lateralis muscles that showed marked differences in fiber-type distribution demonstrated also large interindividual differences in the MCT1 content. The total MCT1 content was positively correlated (r = 0.66, P < 0.01) with the percentage of type I fibers in the muscle (Fig. 1). Extrapolation of the regression line revealed that the MCT1 content in a muscle with no type I fibers can be expected to have ~40% of the MCT1 content in a muscle composed exclusively of type I fibers. Furthermore, there was a negative correlation (r = 0.73, P < 0.01, not shown) between MCT1 content and percentage of type IIX fibers in a muscle, but no relation between MCT1 content and percentage of type IIA fibers.

The MCT4 isoform was also found in all muscle homogenates examined. Strikingly, the variation in the MCT4 content among the three muscles from a given subject was less than the interindividual variation. Thus the subjects with the lowest MCT4 muscle content had only ~10% of that found in the subject with the highest amount of MCT4 (Fig. 2). There was no correlation (P > 0.05) between the MCT4 content and the percentage of any of the fiber types.

In addition, there was no relation between the contents of MCT1 and MCT4 in the muscles.

MCT isoforms: immunofluorescence microscopy. The pictures demonstrated that both MCT1 and MCT4 were uniformly localized in the surface membrane of the muscle fibers. A common finding for all subjects and muscles was a rather similar occurrence of MCT1 in type I and type II fibers (Fig. 3). Hence, only a minor difference was apparent in MCT1, with somewhat more staining of the membrane in type I fibers than in the membrane of type II fibers.

In contrast, MCT4 showed for all subjects and muscles a clear fiber-type difference, with more staining in the membrane of type II fibers than in the membrane of type I fibers (Fig. 4). As for the homogenate samples, the interindividual variation in MCT4 staining was also considerable when immunofluorescence microscopy was used. Interestingly, one of the subjects had an almost complete absence of MCT4 in type I fibers in all three muscles, whereas the distribution of MCT1 was similar to that found for the other subjects. It should be noted that this subject, who performed heavy resistance training, also had the lowest MCT4 content in the homogenate samples.

DISCUSSION

This study shows for the first time the profiles of the lactate/H\(^+\) transporter isoforms MCT1 and MCT4 in various human skeletal muscle.

The present finding of a positive correlation between the total content of MCT1 and the percentage of occurrence of type I fibers in human muscle is in accordance with the results obtained with rat skeletal muscle (12, 22), although the regression line for the human muscle appears less steep than previously found in rats. Moreover, the lack of any detectable relation between the total muscle content of MCT4 and the fiber-type composition is in agreement with the nearly identical MCT4 content in various rat skeletal muscle except for a very low MCT4 content in rat soleus.
Calculations reveal that the total MCT1 content in a hypothetical human muscle composed exclusively of glycolytic fibers (IIA + IIX) can be estimated to be ~40% of the MCT1 content in a muscle entirely composed of oxidative fibers (type I). Similar determinations in rat skeletal muscle (12, 22) revealed 25 and 10% lower relative MCT1 content in muscles composed of glycolytic fibers (type IIB) than in muscles with only oxidative fibers (type I + IIA). However, direct comparisons between rat and human skeletal muscle are complicated because of the different properties of the various fiber types in rats and humans. Thus oxidative fibers in rat skeletal muscle include both type I and type IIA fibers (3), but only type I fibers are generally described as oxidative fibers in humans (20).

Moreover, the existence of rat skeletal muscle with almost uniform fiber-type distributions makes it possible experimentally to determine fiber-specific MCT contents, whereas estimations are needed to obtain such values for human skeletal muscle when muscle homogenates are used.

When only homogenate data are used, interpretations about relations between MCT isoforms and fiber type assume that each of the individual fiber types is identical in different muscles. Hence, it cannot be evaluated whether inherent differences among the soleus, triceps brachii, and vastus lateralis muscles have influenced the findings. However, immunofluorescence microscopy enables differentiation between the MCT content in type I and type II fibers in a given muscle section. Thus the use of the latter method showed that within a given muscle section only minor fiber-type differences existed in the distribution of MCT1, whereas MCT4 exhibited a clear fiber-type dependency with markedly more staining of type II fibers than of type I fibers. These general patterns of MCT isoform profiles in human skeletal muscle applied to all three muscles and all subjects examined. Moreover, these observations are in accordance with the results obtained for rat skeletal muscle (22), although the fiber-type occurrence is different in rat and human muscle. Thus fiber-type determinations based on MHC isoforms reveal three major fiber types in humans (type I, IIA, and IIX).
I, IIA, and IIX; Ref. 21), but an additional fiber type (IIb) is frequent in rat muscle (3). Interestingly, the present observations that type I fibers are low in MCT4 content and that both MCT1 and MCT4 seem to be present in type IIA and type IIX fibers in human skeletal muscle are consistent with the findings in rats (22). In addition, it appears that type IIB fibers in rat skeletal muscle lack or almost lack MCT1 (22).

The findings that the total MCT1 content is positively related to the percentage of type I fibers in the muscle and that only minor fiber-type differences exist in the MCT1 content in a given muscle section imply that the MCT1 content in a given fiber type in one muscle is different from the MCT1 content in the same fiber type in another muscle. Similarly, the clear difference in MCT4 content between type I and type II fibers in a given muscle section together with the lack of a significant relation between the total MCT4 content and fiber type suggests that the MCT4 content in a given fiber type is also different in various muscles. The idea of such a diversity of the MCT isoforms within a given fiber type is in accordance with the statements that enzyme activities can vary considerably within each of the fiber types and that a metabolic continuum may exist in skeletal muscle fibers (13, 20). The different kinds of activities that the various muscles take part in can be expected to be of major importance in this matter, and it may be speculated that differences in lactate concentrations and/or pH could play a role in the existence of a diversity of the MCT isoforms in a given fiber type in different muscles.

The MCT4 content exhibited a much larger interindividual variation than the MCT1 content, which could be due to inborn properties. An alternative possibility is that the variation is related to the difference in the level and/or kind of physical activity that the subjects took part in, although the number of subjects in the present study is too low to allow for a final decision. However, the idea that physical activity plays a role for the content of MCT4 isoforms in a muscle is supported by recent human training studies that reported an increase in MCT1 after 7 days of endurance training (1) and an enhanced content of both the MCT1 (76%) and the MCT4 (32%) isoform after 8 wk of anaerobic training (16). Hence, both isoforms can be affected by training, and the magnitude of change in the contents of the MCT isoforms may differ. Therefore, the present observation of a larger interindividual variation in the muscle content of MCT4 than in the content of MCT1 could be due to differences in the level and/or kind of physical activity. In addition, this difference in extent of interindividual variation in MCT1 and MCT4 content together with the finding that the contents of MCT1 and MCT4 differ in fiber-type dependency is in line with the earlier suggestion that the contents of these isoforms may be subjected to different regulations (22). This proposal is also in accordance with the previous finding of a difference in the magnitude of the training-induced changes in MCT1 and MCT4 content (16), and such distinct regulations may explain that several lactate/H+ transporter isoforms have evolved.

MCT isoforms and transport capacity. Although the relative importance of the MCT1 and MCT4 isoform for the total lactate/H+ transport capacity of a muscle (a muscle fiber) cannot be deduced from the present data, the current observations may help in the understanding and interpreting of earlier findings. We have previously shown in a cross-sectional human study that the sarcolemmal lactate/H+ transport capacity of m. vastus lateralis correlated positively with the percentage of type I fibers in the muscle (15). It can be calculated from the data of that study that a muscle with only type I fibers can be expected to have only one half of the lactate transport capacity of a muscle composed entirely of type I fibers. Interestingly, this estimated fiber-type difference in lactate transport capacity is close to the value calculated here for the MCT1 content (40%) of two such hypothetical muscles. On the basis of the present results, it appears that the correlation previously observed between lactate transport capacity and fiber type could be due to a relation between MCT1 and fiber-type composition. Thus subjects with high percentage of type I fibers in a muscle will be expected to have higher MCT1 content in both type I and type II fibers than subjects with few type I fibers. Moreover, it may be noted that the interindividual variation in sarcolemmal lactate transport capacity was appreciable in the cross-sectional study (15). Because the present data reveal a much larger interindividual variation in MCT4 muscle content than in MCT1 content, it may suggest that the individual differences in lactate transport capacity in the previous investigation (15) primarily can be ascribed to differences in the muscle content of MCT4.

In conclusion, the present results indicate that the total content of MCT1 in human muscle depends on what particular muscle is examined, but only minor differences exist in the MCT1 content between type I and type II fibers in a given muscle section. In contrast, the content of MCT4 in human muscle is fiber-type specific, whereas the total content of MCT4 appears to be quite similar in various muscles. Moreover, it seems that the interindividual variation in MCT4 content is much larger than the difference in MCT1 content between subjects. These findings support the idea that the occurrence of the MCT1 and the MCT4 isoform may be subjected to different regulations.

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