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ORIGINAL ARTICLE

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Current aspects of lactate exchange: lactate/H^+ transport in human skeletal muscle

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Abstract Skeletal muscle is capable of producing and releasing large amounts of lactate and at the same time taking up lactate and using it as a respiratory fuel. The release and uptake of lactate both involve transmembrane transport, which is mediated mainly by a membrane protein called the monocarboxylate transporter (MCT). MCTs mediate membrane transport with an obligatory 1:1 coupling between lactate and H^+ fluxes, and is therefore of great importance for pH regulation, especially during intense muscle activity. The total lactate and H^+ transport capacity is higher in membranes from oxidative fibers than in membranes from more glycolytic fibers. There are two isoforms of MCT present in skeletal muscle, MCT1 and MCT4. In human muscle samples, there is a positive correlation between the proportion of type I fibers and MCT1 density. In contrast, the MCT4 density in human muscle is independent of fiber type and displays a large interindividual variation. Although the two isoforms have identical transport kinetics (K_m), they may have different roles in muscle. MCT1 and MCT4 respond differently to a high-intensity training session, which suggests that these two isoforms are regulated differently.

Keywords Monocarboxylate transporter · MCT · Isoform distribution · pH regulation

Introduction

At the transition from rest to heavy exercise, energy demand rapidly increases, which may induce the accumulation of lactate. Lactate is produced in skeletal muscle for two reasons: firstly, because the acceleration of glycolysis at the onset of muscle activity is fast compared with the ability of the oxidative pathway to accelerate, and secondly, because the maximal glycolytic capacity exceeds the maximal oxidative capacity. In contrast, the production of lactate in muscle is not normally due to insufficient oxygenation (Gladden 1996). Although skeletal muscle is the main producer of lactate in the body, lactate can also be taken up by skeletal muscle and used as a respiratory fuel. Furthermore, lactate released from muscle can be taken up by the heart and even by the brain (Ide et al. 2000). Lactate is, therefore, a useful metabolic intermediate that can be exchanged between different cells within a given muscle, or exchanged between muscle and blood, as well as between muscle and other different tissues. The rapid transport of lactate across the sarcolemma is therefore of fundamental importance for muscle function.

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Measurements of lactate transport and evidence for carrier-mediated transport

It has long been known that lactate cannot move freely from muscle to blood. Experiments with both in situ and isolated muscles have suggested that the translocation of lactate across the plasma membrane is mediated mainly by a transport system that can be inhibited by specific inhibitor compounds (Juel 1988). Transport kinetics (K_m and V_{max}) are difficult to examine owing to cellular lactate metabolism and the complexity of intact muscle. However, studies of transport kinetics can, with advantage, be carried out using membrane vesicles as a model system. The use of small and giant membrane vesicles produced from rat muscles has revealed that lactate and other monocarboxylates cross the sarcolemma of skeletal muscle via a saturable, stereospecific transport system that exhibits an obligatory 1:1 coupling between lactate and H^+. This cotransportation can be blocked reversibly by the competitive inhibitor cinnamate, and irreversibly by mercury-containing com-

pounds. K_m values of 9–41 mM have been reported in most studies with rat muscle. Lactate membrane transport in human muscle has been investigated in only a few studies (Juel et al. 1994), which have demonstrated that lactate transport kinetics in human muscle is similar to the kinetics that have been reported for rat muscle. Furthermore, studies in rats and humans have demonstrated that the lactate/H^+ cotransporter (a monocarboxylate transporter, MCT) mediates the main fraction of any lactate translocation, although simple diffusion of undissociated lactic acid also takes place (Juel 1997; Poole and Halestrap 1993).

Transport capacity in muscle fiber types

The lactate/H^+ transport capacity has been measured in intact rat muscle (Bonen and McCullagh 1994), in vesicles produced from mainly red and white rat muscle (Juel and Pilegaard 1998; Juel et al. 1991) and in vesicles made from rat muscle with a known fiber type composition (Pilegaard and Juel 1995). Taken together, these rat studies have demonstrated that the lactate/H^+ transport capacity in a slow-twitch oxidative fiber is about twice the capacity in a fast-twitch glycolytic fiber.

If the lactate/H^+ transporter is considered to be a system that is specialized for fast lactate release during intense muscle activation, then it is surprising that the highest transport capacity is found in the less glycolytic fibers. However, since oxidative muscles produce lactate during intense exercise and are recruited for longer time periods, it may be important for these fibers to release their lactate at a higher rate. Alternatively, the lactate transporter may be more important for lactate uptake for oxidation. Since lactate uptake normally takes place with a small lactate gradient, the translocation could be rate limiting and the existence of the transporter could therefore be important for facilitating uptake in oxidative fibers.

Lactate/H^+ transport and fiber types in human muscle

Many studies have focused on the effects of exercise on the production, accumulation and disappearance of lactate in human skeletal muscle. In the main, these studies have used blood samples, and sometimes needle biopsy samples, to evaluate lactate movements. In a few studies it has been possible to study directly the membrane transport characteristics using sarcolemmal vesicles produced from needle biopsy material (Juel et al. 1994).

Only one study has focused on the relationship between fiber type and lactate/H^+ transport capacity in human skeletal muscle (Pilegaard et al. 1994). Vesicles were produced from needle biopsy samples obtained from the vastus lateralis muscles of 39 subjects with very different training status, and their membrane transport capacity was measured in tracer flux experiments. There was a positive correlation between the percentage occurrence of type I fibers (as determined by ATPase staining) and membrane transport capacity (Fig. 1A). Interindividual variation was large, which could be ascribed partly to the different training status of the subjects. A linear regression of the data points (not taking training status into account) confirmed that lactate/H^+ transport capacity is dependent upon the fiber type. Thus, the transport capacity in a muscle without type I fibers is only approximately 45% of the transport capacity in a muscle composed exclusively of type I fibers.

The lactate/H^+ transport in muscle is mediated by two isoforms of the MCT protein (MCT1 and MCT4; Juel and Halestrap 1999; Pilegaard et al. 1999b; Wilson et al. 1998). The first isoform investigated, called MCT1,

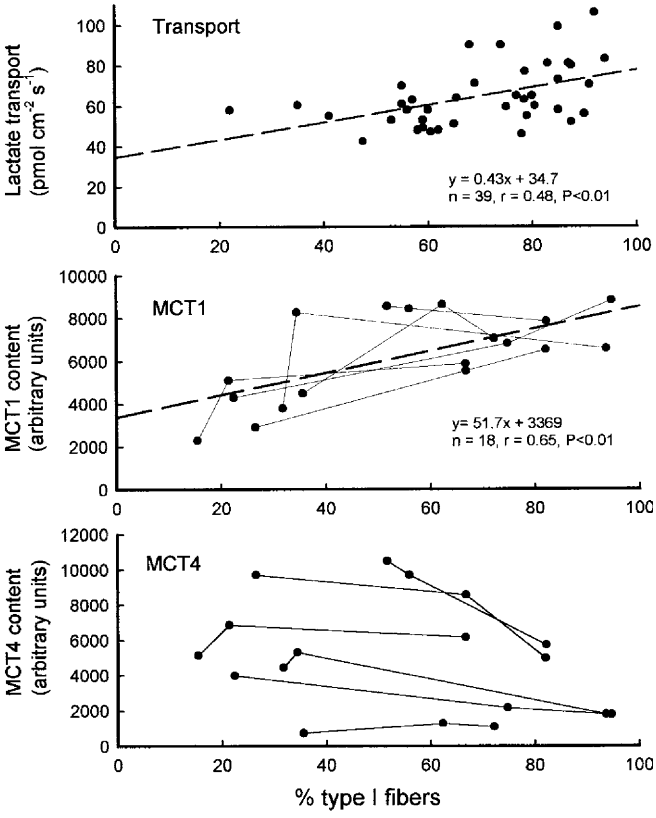

Fig. 1A–C Comparison between lactate/H^+ transport capacity and monocarboxylate transporter (MCT) isoform distribution in different fiber types of human skeletal muscle. **A** Total lactate/H^+ (carrier-mediated + simple diffusion) transport capacity measured in giant vesicles made from needle biopsy samples obtained from the vastus lateralis muscle of 39 subjects. The data is not corrected for the different training status of the subjects. The fiber type distribution was determined by ATPase staining. The *dotted line* represents the best linear fit to the data (data from Pilegaard et al. 1994). **B** MCT1 content (in arbitrary density units) and percentage fiber type. Western blot analysis of needle biopsy material from three muscles (soleus, triceps brachii and vastus lateralis) from six subjects. The fiber type was evaluated from the occurrence of myosin heavy chain isoforms. The lines connect data from the same subject and the linear regression line (*dotted*) for all data is shown (data from Pilegaard et al. 1999b). **C** MCT4 distribution in the same samples as in **B**. There is no correlation between MCT4 content and the percentage occurrence of type I fibers (data from Pilegaard et al. 1999b)

has been localized in all muscle types in man, but with the highest density in type I fibers (Fig. 1B). A linear regression analysis of the data revealed that the MCT1 density in a human muscle without any type I fibers is approximately 40% of the density in a muscle composed exclusively of type I fibers (Pilegaard et al. 1999b). Furthermore, there was a negative correlation between MCT1 content and type IIX fibers (as determined by myosin isoform analysis). There was no relation between MCT1 content and type IIA fibers. The MCT4 isoform was also found in all muscle homogenates analyzed, but the MCT4 present in human skeletal muscle showed a distribution pattern different from that of MCT1 (Fig. 1C). There was a large interindividual variation in MCT4 density; in fact, the subject with the lowest MCT4 density had only 10% of the density in the subject with the highest MCT4 content. In contrast, there was no clear correlation between MCT4 density and the percentage occurrence of type I fibers. It can be seen from Fig. 1B, C, that lactate/H^+ transport in all muscles is mediated by at least two transporter isoforms. The question is which isoform is of greater importance for the transport. The Western blot technique used to quantify the MCT1 and MCT4 density in muscle homogenates does not permit a comparison, in absolute terms, of the density of the two isoforms. Therefore, it cannot be determined from such data which MCT isoform is most important for lactate/H^+ transport in human muscle. If Fig. 1A and B are compared there seems to be a good correlation between total lactate/H^+ transport capacity and MCT1 density. At first sight, this could lead to the conclusion that the main part of the total transport is mediated by MCT1. However, the data cannot be compared directly because two different groups of subjects are included. Furthermore, the data in Fig. 1A does not discriminate between carrier-mediated transport and simple diffusion. The contribution from MCT4 could be larger than evaluated from Fig. 1, since the large variation (ten-fold) in MCT4 protein could be the reason for the large interindividual variation in total transport capacity, which would imply that MCT4 contributes significantly to the total transport. Only a more detailed analysis of lactate/H^+ transport capacity and the distribution of MCT1 and MCT4 can solve the question concerning the relative importance of these two isoforms.

Different roles of MCT isoforms

It has been argued that the high MCT1 density observed in oxidative fibers may explain the need to transport lactate into the cells for oxidation and that MCT1 is specialized for uptake (McCullagh et al. 1996b). In contrast, MCT4 is distributed more uniformly and may therefore be relatively more important in glycolytic fibers, which leads to the suggestion that MCT4 is specialized for lactate efflux from muscle (Wilson et al. 1998). However, these distinct roles proposed for the two MCT isoforms present in muscle are based only on their distribution among different fiber types. If one isoform really was specialized for a distinct type of transport, then other criteria would have to be fulfilled. Firstly, the transporter should be able to direct the substrate in one direction only. However, the direction of lactate/H^+ transport is dependent upon the gradient of both lactate and H^+ (i.e., lactate and H^+ are transported downhill along their concentration gradients). It has never been reported that a MCT isoform is capable of moving lactate selectively in one direction; in fact, one study has demonstrated that the transporters behave symmetrically (i.e., the transport rates from the two sides of the membrane are identical; Juel 1996). Another possibility could be that the K_m values (affinities) of the two isoforms differ, which could be important for directing lactate into particular cell types (oxidative or glycolytic fibers). Studies with cell lines expressing only one isoform have revealed a K_m of 6.4–10.1 mM, with no clear difference in K_m between fiber types (Wilson et al. 1998). In line with this, a study using vesicles obtained from rat muscle with different fiber type composition has not revealed any differences in K_m, which was found to be 15 mM in both oxidative and glycolytic fibers (Juel and Pilegaard 1998). Therefore, if the MCT isoforms have different roles in different muscle fiber types, these would be based only on different distributions and not on different transport kinetics. However, the existence of two MCT isoforms could be an advantage if they are regulated differently and could adapt to different demands.

Regulation

Studies in the rat have demonstrated that endurance training and high-intensity training, as well as chronic stimulation, can result in a 30%–100% increase in the lactate/H^+ transport capacity measured in sarcolemmal vesicles (McCullagh and Bonen 1995; McCullagh et al. 1996a; McDermott and Bonen 1993; Pilegaard et al. 1993). These experiments have clearly shown that V_{max} of the lactate/H^+ transporter can be changed, whereas alterations in K_m have not been clearly demonstrated. Using specific antibodies, it has been shown in rats that training can induce an increase in the MCT1 content (present mainly in red muscle). Furthermore, the increase in MCT1 protein density was paralleled by an augmented rate of lactate uptake in isolated muscle (Baker et al. 1998). In another study, also using vesicles, it was found that the main part of the training-induced improvement in lactate transport capacity took place in the white fibers (Juel and Pilegaard 1998). Thus, lactate membrane transport in rat skeletal muscle can undergo adaptive changes.

The training-induced improvement in lactate/H^+ cotransport observed in rat muscle is large (up to more than 100%) and must be evaluated against the background that control rats are extremely untrained. It was

therefore of interest to investigate whether the lactate transport capacity in human subjects with normal physical activity could be improved by training.

A cross-sectional human study using vesicles made from needle biopsy samples from muscle revealed large interindividual differences and showed that athletes can have an improved lactate transport capacity (Pilegaard et al. 1994). The questions posed were whether training can increase the membrane transport capacity and whether the improved membrane transport capacity can have any functional significance.

A large number of studies have demonstrated that training can change lactate kinetics in the human body, but most of those changes can be ascribed to metabolic alterations and not to improved membrane transport. It could be speculated, however, that there might be a relationship between the content of membrane MCT protein and the release of H^+ and lactate from human muscle during intense exercise. At least three studies have addressed this question. One study, where subjects underwent 7 days of bicycle endurance training, reported that the MCT1 content was increased (18%) after training and that the lactate concentration in the femoral vein during exercise was higher for a given amount of muscle lactate (Bonen et al. 1998). In a similar study, where subjects performed intermittent high-intensity training with one leg, it was found that the MCT1 and MCT4 content was increased by 76% and 32%, respectively, compared to the untrained leg. The increase in MCT protein content was associated with a 12% improvement in sarcolemmal lactate transport, which was measured in giant vesicles made from needle biopsy samples of muscle. In addition, the release of lactate and H^+ was similar in the trained and untrained leg, although the cellular-to-interstitial concentration gradients of lactate and H^+ were lower after training than before training (Pilegaard et al. 1999a). Recently, Dubouchaud et al. (2000) reported that levels of sarcolemmal MCT1 and MCT4 can be increased by training, and that MCT1 content is positively correlated to the leg lactate release. Based on these studies, it can be concluded that the lactate/H^+ transport capacity can be improved by training in humans, and that the two MCT isoforms can adapt differently, which may suggest different regulatory mechanisms. Furthermore, it appears that the elevated density of membrane transporter proteins resulting from training is of functional importance for the translocation of H^+ and lactate from muscle to blood during high-intensity exercise. In line with these findings, it has been reported that extremely low muscle activity, as in patients with spinal cord injuries (Pilegaard et al. 1998), or in denervated rat muscle (McCullagh and Bonen 1995; Pilegaard and Juel 1995) reduces the capacity for lactate/H^+ transport.

Thus, there is good evidence for the long-term regulation of lactate/H^+ cotransporters associated with training; however, the mechanism for regulation has not been established. In contrast, there is yet no evidence for any short-term (hormonal) regulation of these cotransporters.

Lactate/H^+ transport and pH regulation

Lactate and H^+ accumulate in muscle during activity. Intense exercise with a limited muscle mass may result in a cellular lactate concentration of more than 40 mmol/l of cell water (Bangsbo et al. 1993; Juel et al. 1990). At the same time, pH decreases by approximately 0.5 pH units, which may impair muscle function (Fitts 1994). The activity of the lactate/H^+ cotransporter counteracts the accumulation of lactate and H^+ during muscle activity. Although the ratio between the produced and released lactate varies in different experiments, all studies have reported a considerable release of lactate and H^+ during and after muscle activity. Even during short-lasting activity, approximately one-third of the produced lactate and H^+ is released in the exercising period, and another fraction is released in the recovery period after exercise (Bangsbo et al. 1993; Juel et al. 1990). Thus, the existence of the cotransporter both reduces the accumulation of lactate and H^+ during activity and shortens the recovery phase. Furthermore, the presence of the lactate/H^+ transporter facilitates uptake in other fibers and reduces acidification of the muscle interstitium and the blood.

No matter whether lactate crosses the sarcolemmal membrane via the lactate/H^+ cotransporter or via simple diffusion of undissociated lactic acid, the movement is always coupled to the movement of H^+ in a 1:1 ratio. In contrast, H^+ transport can be mediated by other transport systems independent of lactate. Consequently, any change in lactate flux and lactate concentration will be associated with a change in pH. It could therefore be expected that lactate/H^+ transport is important for muscle pH regulation. In general, muscle pH homeostasis is a balance between H^+ accumulation (H^+ influx, metabolic production of acid) and H^+ removal mediated by transport systems located in the sarcolemma (Juel 1995, 1998). The importance of the lactate/H^+ transporters for pH regulation in skeletal muscle is supported by the finding in a rat model system, where the cotransporter possesses the highest capacity for mediating H^+ efflux when compared with the systems for Na^+/H^+ exchange and bicarbonate-dependent transport (Juel 1995). In human exercise experiments it is possible to quantify the lactate release from the femoral venous–arterial concentration difference and blood flow; similarly, H^+ release can be calculated from the femoral venous–arterial difference of base excess and blood flow. It is therefore possible to compare the total release of lactate and H^+ from muscle to blood, and thereby to evaluate the importance of the cotransporter for pH regulation. During, and in the first phase of recovery from high-intensity knee-extensor exercise, lactate release was approximately two-thirds of the total H^+ release (Bangsbo et al. 1993; Juel et al. 1990). In another study with intense knee-extensor exercise, lactate-coupled H^+ release was approximately 50% of the total H^+ release (Pilegaard et al. 1999a), and this fraction was reduced during sub-maximal exercise (Bangsbo

et al. 1997). Such experiments imply that lactate/H^+ cotransport is the single transport system mediating the highest H^+ removal from human muscle during intense exercise, and confirm the prime importance of the lactate/H^+ cotransporter for pH regulation. However, it must be noted that the activity of the cotransporter is driven mainly by the lactate gradient, is only slightly sensitive to internal pH, and is not activated by hormones. In contrast, the classical pH regulating transport system, the Na^+/H^+ exchanger, is activated strongly when internal pH is reduced, thereby functioning as a safety system against any major changes in internal pH. Bicarbonate-dependent exchange or cotransport systems present in the sarcolemmal membrane may possess similar properties, but these transport systems have not been investigated in detail (Juel 1995). Thus, the transport systems not involving lactate are well suited for fine adjustments of pH and for pH regulation at rest, when the lactate gradient is small or absent, whereas during intense exercise, when lactate production is large, the lactate/H^+ cotransporter will handle most of the H^+ produced.

The involvement of lactate/H^+ cotransport in pH regulation may also be important for the interstitial pH homeostasis, which may have important regulatory consequences, since the interstitial H^+ concentration may influence sensory nerve endings involved in the reflex regulation of blood flow and ventilation (muscle metabo-reflexes).

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