Changes in MCT 1, MCT 4, and LDH expression are tissue specific in rats after long-term hypobaric hypoxia

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McClelland, Grant B., and George A. Brooks. Changes in MCT 1, MCT 4, and LDH expression are tissue specific in rats after long-term hypobaric hypoxia. *J Appl Physiol* 92: 1573–1584, 2002. First published January 18, 2002; 10.1152/japplphysiol.01069.2001.—Little is known about the effect of chronic hypobaric hypoxia on the enzymes and transporters involved in lactate metabolism. We looked at the protein expression of monocarboxylate transporters MCT 1, MCT 2, and MCT 4, along with total lactate dehydrogenase (LDH) and LDH isozymes in skeletal muscle, cardiac muscle, and liver. Expression of these components of the lactate shuttle affects the ability to transport and oxidize lactate. We hypothesized that the expression of MCTs and LDH would increase after acclimation to high altitude (HA). The response to acclimation to HA was, however, tissue specific. In addition, the response was different in whole muscle (Mu) and mitochondria-enriched (Mi) fractions. Heart, soleus, and plantaris muscles showed the greatest response to HA. Acclimation resulted in a 34% increase in MCT 4 in heart and a decrease in MCT 1 (–47%) and MCT 4 (–47%) in plantaris Mu. In Mi fractions, the heart had an increase (+40%) and soleus a decrease (–40%) in LDH. HA also had a significant effect on the LDH isozyme composition of both the Mu and Mi fractions. Mitochondrial density was decreased in both the soleus (–17%) and plantaris (–44%) as a result of chronic hypoxia. We conclude that chronic hypoxia had a tissue-specific effect on MCTs and LDH (that form the lactate shuttle) but did not produce a consistent increase in these components in all tissues.

monocarboxylate transporters; lactate dehydrogenase isozymes; lactate shuttle; lactate metabolism; high-altitude acclimation; cytochrome oxidase

THE CHANGES THAT OCCUR in gene and protein expression with long-term exposure to hypobaric hypoxia associated with high-altitude (HA) acclimation have received little attention. The effects of acute hypoxia exposure have been more extensively studied (23). For example, the machinery involved in carbohydrate metabolism, including membrane transporters and many glycolytic enzymes, is upregulated in skeletal muscle at the level of mRNA and proteins on acute exposure to low O₂ (21, 43). Whether the same is true after acclimation is unknown. Two recent studies suggest that changes in

gene and protein expression may be attenuated in chronic hypoxia in skeletal muscle and brain (16, 41). Lactate is a metabolic intermediate with high turnover and oxidation rates that exceed those of glucose during exercise in rats (11, 18) and humans (37). Moreover, its metabolism is affected by altitude exposure (9). Little is known with regard to the effects of chronic hypoxia on the expression of enzymes and transporters of lactate metabolism, but the response(s) may be tissue specific. For instance, red and white muscles have very different patterns of gene expression, even in normoxia (13), and respond to perturbations in energy balance in different ways.

Monocarboxylate transporters (MCTs) are transmembrane proteins that facilitate the transport of lactate in and out of cells (30). Two isoforms most commonly found in locomotory and cardiac muscle are MCT 1 and MCT 4. Also, the apparent $V_{\max}$ of lactate uptake reflects the density of MCTs, and MCT 1 expression is known to increase with contractile activity associated with exercise training or chronic electrical stimulation (e.g., Refs. 19, 40). This increase in MCT 1 expression is correlated with changes in mRNA, is highest in oxidative muscle fibers, and facilitates lactate uptake at the sarcolemmal and mitochondrial membranes (3, 5, 8). MCT 4 expression does not change in concert with mRNA (5), MCT 4 is found predominantly in glycolytic fibers, and its putative role is lactate extrusion (17). The extent to which these transporters are affected by exposure to chronic hypoxia has never been documented. It is not known, for instance, whether they respond through a well-characterized induction pathway like other components of the glycolytic pathway (21, 43). MCT 2, another isoform known to transport lactate, is found primarily in liver and brain (42). Little is known about the regulation of this isoform. An increase in its density, however, could enhance lactate transport for use as a gluconeogenic precursor in liver.

MCTs and lactate dehydrogenase (LDH) are involved in the shuttling of lactate between cells via the proposed cell-cell lactate shuttle (6). A proposed intracellular lactate shuttle directs endogenous lactate

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toward oxidation by mitochondria (10, 46). Consequently, changes in expression of shuttle machinery may affect the cellular transport and oxidation of lactate. For example, with endurance training in humans, there is a correlation between lower lactate release with higher MCT 1 content in mitochondria or sarcolemmal membranes (19). Therefore, changes in MCT expression with chronic hypoxia may shed some light on the well-documented alteration of lactate metabolism (reduced peak and submaximal exercise blood lactate concentrations) with the transition from acute to chronic hypoxia (7, 22). Because blood lactate concentrations ($[\text{lactate}]_b$) represent a balance between uptake and release by tissues, it is interesting that human skeletal muscles after acclimation become net lactate consumers during exercise (7). Whether this is due to changes in sarcolemmal or mitochondrial MCT 1 and/or LDH is not yet known. Lactate oxidation can also be enhanced by increasing mitochondrial membrane density and, therefore, associated transporters. Past findings in this area are varied, with hypoxia acclimation either decreasing (14), increasing (33), or not affecting (28, 35) mitochondrial density. Moreover, it is unknown whether hypoxia affects expression of mitochondrial components of the lactate shuttle, namely mitochondrial MCT 1 and LDH (mLDH). Also, the cellular distribution and composition of LDH isozymes may affect lactate kinetics at altitude. Measurement of these components will illuminate the role of the lactate shuttle before and after altitude acclimation.

In this study, we used HA-acclimated rats to test the hypothesis that chronic hypoxia will increase the expression of MCT 1 and LDH in oxidative muscle fibers. To look at the tissue specificity of this response, we examined these changes in four locomotory muscles, in cardiac muscle, and in liver.

METHODS

Animals. As part of a previous study (39), female Wistar rats (Charles River, St. Hyacinthe, QC, Canada) were randomly assigned to two groups, one kept under normoxic sea level (SL) conditions and the other under hypobaric hypoxia equivalent to 4,300 m HA. Each group had free access to food and water as well as a water supplement (Transgel, Charles River). The HA rats were acclimated by progressively decreasing the chamber pressure over a 10-day period to a final pressure of 450 mmHg, as described before (38). The acclimation period was 8 wk. Average weights for the two groups used in this study were 230 ± 4 g for SL controls and 236 ± 5 g for acclimated (HA) rats.

Tissue sampling. As part of the previous study (39), rats were exercised twice, 48 h apart, for 60 min at 60% maximal O_2 uptake (12.3 ± 0.1 ml/min) under normoxia. At the end of the second exercise bout, they were quickly euthanized (20 mg/100 g pentobarbital sodium) via a previously implanted arterial catheter. Right and left hindlimb muscles (soleus, plantaris, red and white gastrocnemius), liver, and heart were removed and quickly frozen by use of liquid N_2 -cooled aluminum tongs and then submerged in liquid N_2 before storage at -80°C until analysis. Visual inspection did not reveal any obvious differences in daily activity between HA and SL rats. Both groups performed the same acute exercise

in normoxia, and this may have some impact on the results but is unlikely to affect HA-to-SL comparisons.

Sample preparation. Homogenates were prepared as previously described (19) with some modification. Briefly, tissues were homogenized by using a motorized glass homogenizer at 4°C in buffer containing 210 mM sucrose, 2 mM EGTA, 40 mM NaCl, and 30 mM HEPES at pH 7.4 (*buffer A*), supplemented with 0.15% protease inhibitor cocktail (Sigma Chemical). Total muscle homogenate was prepared by centrifugation at 600 g for 10 min. One milliliter was removed, and, after dilution ($0.75 \times$ total volume) with buffer containing 1.167 M KCl and 58.3 mM $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10 \text{H}_2\text{O}$, pH 7 (*buffer B*), the homogenates were centrifuged at 230,000 g for 120 min. The pellet was resuspended in buffer consisting of 1 mM EDTA and 10 mM Tris at pH 7.4 (*buffer C*). One-third volume of 16% SDS was added to the resulting suspension and centrifuged at room temperature for 25 min at 1,000 g, resulting in a supernatant containing whole muscle membrane preparations (Mu). To separate mitochondrial fractions, the remaining original homogenate (after 600 g spin) was centrifuged at 10,000 g for 20 min at 4°C . This pellet was resuspended in *buffer C* as above, and aliquots of the supernatant, before the addition of SDS, were saved for LDH isozyme measurements. The remaining solution was mixed with one-third volume of 16% SDS and then treated like Mu homogenates and represents mitochondria-rich fractions (Mi). Purity of mitochondrial fractions was confirmed in preliminary experiments by the absence of GLUT1 reactivity in immunoblots. The supernatant was diluted with 0.75 volume of *buffer B* and spun at 230,000 g. Aliquots of the resulting supernatant were collected as pure cytosol (Cy), lacked SDS, and were used for LDH isozyme measurements. All of the resulting homogenates were aliquoted and frozen at -80°C .

RBC membrane "ghosts." Red blood cell (RBC) membrane "ghosts" were used as a positive control for MCT 1 immunoblotting. Whole blood from a donor rat (Sprague-Dawley, UCB) was anticoagulated by mixing with seven volumes of acid-citrate-dextrose buffer containing 75 mM sodium citrate, 38 mM citric acid, and 138 mM D-glucose. At 4°C , blood was mixed with sedimenting buffer (1:4 vol/vol, pH 7.5) consisting of 150 mM NaCl and 5 mM sodium phosphate. The RBCs were allowed to settle out for 1–2 h. After being washed three times with physiological saline, they were centrifuged at 2,000 g for 5 min. Cells were lysed by rapid addition of 10 vol of a pH 7.5 buffer consisting of 7.5 mM sodium phosphate and 1 mM EDTA containing protease inhibitors (antipain at 2 $\mu\text{g}/\text{ml}$). The lysed cells were centrifuged at 37,000 g for 10 min at 4°C , and the resulting pellet was washed with lysing buffer until white in appearance. The "ghosts" were divided into aliquots and stored at -80°C for future use.

Immunoblotting. Custom polyclonal antibodies to MCT 1 and MCT 4 from rabbit were used; their specificities have been confirmed elsewhere (8). Rat RBC membrane ghosts were used as a positive control for MCT 1 with an arbitrary unit of MCT set at 0.25 μg of RBC protein. Chicken anti-MCT 2 antibody (Chemicon International) was used to probe liver homogenates. Mitochondrial density was estimated by using a monoclonal mouse anti-cytochrome oxidase against the IV subunit of cytochrome oxidase (COX; Molecular Probes). LDH antibodies were polyclonal specific to the whole enzyme (not subunit specific) in rabbit (Rockland). Secondary antibodies were donkey anti-rabbit (MCT 1 and 4) and sheep anti-mouse (COX, Amersham), anti-goat (LDH, Santa Cruz), and anti-chicken (MCT 2, Chemicon International) horseradish peroxidase-linked monoclonal antibodies. Protein content of each sample was determined by the bicinchoninic acid

assay (Pierce), using BSA as a standard. Equal amounts of protein were separated on 10% SDS-PAGE and transferred by electroblotting onto polyvinylidene difluoride membranes (Invitrogen). Membranes were incubated for 1 h at room temperature or overnight at 4°C in 10% blocking buffer [150 mM NaCl, 0.1% Tween 20, and 50 mM Tris, pH 7.5 (TTBS), 10% nonfat dried milk] before incubation with antibodies for 2 h at room temperature or overnight at 4°C in 5% blocking buffer (in Tris-buffered saline). Membranes were washed once for 15 min and then twice for 5 min in TTBS and then incubated for 90 min at room temperature with secondary antibody in Tris-buffered saline. Membranes were washed as above, and protein expression was then detected by enhanced chemiluminescence by standard methods (Renaissance; NEN). Autoradiographic films (Kodak X-Omat Blue XB-1) were exposed to membranes and developed (Konica QX-130A Plus). Band densities were determined with a Bio-Rad GS-700 densitometer and quantified by using Molecular Analyst software (Bio-Rad Laboratories). Molecular weight standards were used to identify appropriate antibody binding (Rainbow Marker, Amersham Life Sciences). The membranes were stripped of antibody signal by a Western blot recycling kit (Alpha Diagnostic International) and washed with TTBS before being reprobed. Complete stripping of signal was verified in preliminary experiments by the absence of signal after membranes were treated with enhanced chemiluminescence and processed as above.

LDH isozymes. The cellular composition of LDH isozymes (LDH 1–5) was evaluated in Cy and Mi fractions. Protein (1.5 µg Cy or 10 µg Mi) was loaded onto 1% agarose gels (Reliant precast gels; FMC) and separated for 60 min at 100 V by using 2 µl of isotrol (Sigma Chemical) as an internal positive control. Bands representing the different isozymes were revealed colorimetrically (Sigma Chemical procedure 705), and the gels were fixed with 5% acetic acid. The resulting bands were quantified as described above for Western blots. When a band was not detected, it was given a value of 0% but may have been below the detection limits of the assay.

Statistics. Results were analyzed by using a *t*-test, and linear regressions were tested by using an ANOVA ($P < 0.05$). All percentages were arcsine-square-root transformed. When tests for normality failed, a Mann-Whitney rank-sum test was used. All the values presented are means $\pm$ SE.

RESULTS

MCT 1, MCT 4, and LDH expression. Protein expression was determined by Western blot techniques; representative blots are shown in Fig. 1. The expression of MCT 1 was not affected by chronic hypoxia in heart, soleus, or red gastrocnemius Mu or Mi fractions ($P > 0.05$, Figs. 2, A and E; 3, A and E; and 4, A and E). MCT 4 expression, in contrast, was increased by 34% in heart Mu preparations but not in other oxidative tissues. Moreover, total LDH expression was affected by chronic hypoxia differently in heart (increased, +40%) and soleus (decreased, -40%) mitochondria (Figs. 2F and 3F). Also, there was a positive and significant correlation between MCT 1 and LDH in heart Mu ($r^2 = 0.90$, $P < 0.001$) as well as a positive correlation between MCT 1 and COX in soleus ($r^2 = 0.35$, $P < 0.03$). Acclimation had no significant effect on the expression of these variables in the red gastrocnemius muscle (Fig. 4). Chronic hypoxia had its most pronounced effect on the mixed muscle plantaris. In this muscle

Fig. 1. Representative Western blots showing amounts of monocarboxylate transporters (MCT) 1 and 4, lactate dehydrogenase (LDH), and cytochrome oxidase (COX) in whole muscle (Mu; A) and mitochondrial (Mi; B) fractions in heart, soleus, red gastrocnemius (Gastroc.), plantaris, and white gastrocnemius muscle (liver not shown) in sea level (SL) control (–) and high-altitude (HA) acclimated (+) female Wistar rats.

there was a large decrease in the expression of MCT 1 (–47%) and MCT 4 (–47%) in Mu preparations (Fig. 5, A and B, $P < 0.05$). LDH showed a nonsignificant increase with altitude acclimation, and this led to a significantly negative correlation between MCT 1 and LDH in this muscle ($r^2 = 0.42$, $P = 0.01$). The more glycolytic white gastrocnemius, on the other hand, showed no response to chronic hypoxia acclimation. Also, there was no significant correlation between LDH and MCT 4 content in the muscles studied (data not presented).

Changes in aerobic capacity. Expression of COX, used as an index of tissue aerobic ability and mitochondrial density, was decreased after altitude acclimation in both the soleus (–17%) and plantaris (–44%) muscles. Other tissue showed no change in COX expression in the Mu fraction (Figs. 2G–7G). There was also no change in the COX content per gram of mitochondrial protein as demonstrated in the Mi fractions (Figs. 2G–7G).

LDH isozymes. The distribution of the five different LDH isozymes consisting of combinations of the two subunits LDH A (M type) and LDH B (H type) were measured by gel electrophoresis. The five isozymes are

Fig. 2. Expression of MCT 1 (A and E), MCT 4 (B), LDH (C and F), and COX (D and G) in heart Mu and Mi cell fractions in rats before (SL) and after (HA) long-term acclimation to hypobaric hypoxia; $n = 8$ for HA and $n = 6$ for SL. *Significantly different from SL controls ($P < 0.05$).

LDH5 (A_4), LDH4 (A_3B_1), LDH3 (A_2B_2), LDH2 (A_1B_3), and LDH1 (B_4). The isozyme distribution for LDH in the Cy and Mi fractions of the tissue studied appears in Table 1. In all of the tissues studied from SL and HA individuals, there was a significant difference in LDH isozyme composition between the cytosol and mitochondria. There were also significant differences in the responses of these two cellular fractions to altitude acclimation. Heart and red gastrocnemius Mi fractions showed a decrease in LDH4. The heart Mi also showed an increase in LDH1 with acclimation to altitude ($P < 0.05$) (Table 1). This was reflected in a significant increase in the percentage of LDH B subunits in heart

Mi fractions from HA rats (Table 2). The Mi fractions were higher than Cy in LDH A subunits in all tissues except soleus (Table 2).

DISCUSSION

This is the first study to examine the effects of acclimation to chronic hypoxia on the expression of MCTs. It is also the first attempt to study the effects of hypoxia on the cellular components of the lactate shuttle. Acclimation to chronic hypobaric hypoxia elicits its effects in a tissue-specific manner. The heart (Fig. 2) and plantaris (Fig. 5) show profound plasticity to ac-

Fig. 3. Expression of MCT 1 (A and E), MCT 4 (B), LDH (C and F), and COX (D and G) in soleus Mu and Mi cell fractions in SL and HA; $n = 8$ for HA and $n = 6$ for SL. *Significantly different from SL controls ($P < 0.05$).

climation, whereas liver (Fig. 7) along with red (Fig. 4) and white (Fig. 6) gastrocnemius muscles show no significant response to this treatment. Moreover, hypoxia can elicit changes independently in the cytosol and mitochondria. The expression of MCT 1 and MCT 4 is regulated in different ways by hypoxia depending on the tissue. MCT 4 protein was increased in the heart (Fig. 2B), but acclimation resulted in a large decrease in the plantaris (Fig. 5B). The tissue specificity of this response to chronic hypoxia rules out a coordinated response by the machinery involved in lactate metabolism. Although some tissues may be able to increase their lactate uptake and oxidation, others show a decrease in transporters, enzymes, and mitochondrial

density. These results do not provide direct evidence for increased ability for uptake and oxidation of lactate as an explanation for the previously reported (32) perturbation caused by chronic hypoxia on lactate kinetics.

Chronic hypoxia and protein expression. Chronic hypoxia and its effects on the expression of genes and gene products involved in energy metabolism are underexplored. Here we find that acclimation to a simulated altitude (4,300 m) had a tissue-specific effect on protein expression. This is perhaps not surprising considering that, in the absence of experimental perturbations, red and white muscles show distinctly different gene expression patterns (13). Moreover, many pro-

Fig. 4. Expression of MCT 1 (A and E), MCT 4 (B), LDH (C and F), and COX (D and G) in red gastrocnemius Mu and Mi cell fractions in SL and HA rats; $n = 8$ for HA and $n = 6$ for SL.

teins, including LDH A (21, 26, 43), show isozyme- and isoform-specific regulation of expression with acute hypoxia. These genes are regulated through the well-characterized hypoxia-inducible factor-1 acting on a hypoxia response element of their respective genes (44). There is no present evidence that the genes encoding MCT 1, 2, or 4 contain sequences binding hypoxia-inducible factor or any other factor. However, differences in MCT's response to hypoxia may reflect differences in some other promoters. Interestingly, the response for one of these isoforms (MCT 4) is in opposing directions depending on the tissue examined.

Changes in LDH isozyme composition (Table 1) demonstrate that the two genes encoding subunits A (M type) and B (H type) respond differently to hypoxia.

Transcription of LDH A is known to be upregulated by acute hypoxia (26), whereas LDH B generally shows no response to hypoxia (21, 25). We confirm here that chronic hypoxia affects the differential expression of LDH isozymes (25). The ultimate consequences of these differences after acclimation are uncertain because the metabolic significance of LDH isozyme changes in relation to reaction kinetics have recently been questioned (24).

For the first time, the effects of hypoxia on isozymic composition of mLDH were measured (Table 1). Acclimation had a significant effect on mLDH, shifting the pattern of isozymes in heart and red gastrocnemius Mi fractions. Interestingly, in heart mitochondria there was a shift toward a greater percentage of LDH B

Fig. 5. Expression of MCT 1 (A and E), MCT 4 (B), LDH (C and F), and COX (D and G) in plantaris Mu and Mi cell fractions in SL and HA rats; $n = 8$ for HA and $n = 6$ for SL. *Significantly different from SL controls ($P < 0.05$).

subunits (Table 2), normally found to be insensitive to hypoxia (21, 25). This occurred in the absence of MCT 1 changes in mitochondrial membranes (see MCTs below).

Expression of COX was reduced in some of the tissues examined (soleus and plantaris, Figs. 3D and 5D). This is consistent with cell line work in which mRNA of COX subunit III and other mitochondria transcripts were reduced after 16 and 48 h of hypoxia (21). Other HA acclimation studies sampled mixed muscle or looked at single specific muscles and found a variety of results (14, 28, 33, 34). Any changes seen in this study are probably not the result of changes in myosin isoforms that are fairly insensitive to acclimation in rats (1).

Changes in mRNA expression of MCTs have been measured neither after acute nor after chronic hypoxia. Differences in the regulation of MCT 1 and MCT 4 have been noted in exercise training studies (5). The differential response of these two isoforms, even in the same muscles, of acclimated rats (e.g., Fig. 2) suggests that they are regulated through different mechanisms by hypoxia as well. Also, the possibility that MCTs undergo posttranslational modification has not been explored.

MCTs. The expression of MCTs in muscle is malleable, as shown by several studies on electrical stimulation, exercise training, limb unweighting, and addition of a β_2 -receptor agonist (e.g., Refs. 5, 19, 20, 36). This first study to examine the expression of MCTs with

Table 1. *LDH isozyme composition (% total) in the Cy and Mi fractions of heart, liver, and skeletal muscle of rats before and after acclimation to high-altitude hypoxia*

Tissue	LDH5		LDH4		LDH3		LDH2		LDH1	
	SL	HA	SL	HA	SL	HA	SL	HA	SL	HA
Heart										
Cy	0.4 ± 0.1	0.5 ± 0.1	5.0 ± 0.4	5.6 ± 0.4	18.9 ± 0.7	17.9 ± 1.1	42.6 ± 1.0	39.6 ± 0.7	33.2 ± 1.3	36.4 ± 1.8
Mi	2.2 ± 0.4†	3.5 ± 0.9†	9.5 ± 0.8†	4.9 ± 0.6*	21.1 ± 0.9	17.3 ± 1.0*	37.0 ± 1.9†	36.7 ± 1.1	30.2 ± 3.0	37.6 ± 1.6*
Soleus										
Cy	1.9 ± 0.9	5.9 ± 2.6	3.4 ± 1.0	3.0 ± 0.8	12.2 ± 1.1	11.2 ± 0.7	35.3 ± 0.6	34.5 ± 1.3	47.3 ± 2.4	45.4 ± 2.7
Mi	4.1 ± 2.8	6.1 ± 2.5	6.4 ± 1.6	7.0 ± 0.9†	15.1 ± 0.6	14.5 ± 0.7†	33.4 ± 2.5	33.9 ± 2.3	41.9 ± 3.7	38.6 ± 3.6
RG										
Cy	61.6 ± 3.1	64.8 ± 2.5	9.1 ± 1.2	5.6 ± 0.8*	6.7 ± 0.3	5.2 ± 0.5	8.5 ± 0.9	10.2 ± 0.6	14.1 ± 1.8	14.3 ± 1.2
Mi	78.3 ± 3.8†	79.2 ± 3.6†	2.0 ± 0.5†	0.8 ± 0.6*†	4.2 ± 0.9	4.1 ± 0.7	7.4 ± 1.3	7.2 ± 1.4	8.1 ± 1.8	8.8 ± 1.5†
Plantaris										
Cy	73.9 ± 3.2	64.4 ± 7.5	10.4 ± 2.6	13.9 ± 1.5	6.0 ± 0.6	7.6 ± 0.6	5.2 ± 1.5	7.7 ± 0.8	4.5 ± 1.9	6.3 ± 1.6
Mi	74.2 ± 8.6	84.0 ± 3.8	9.8 ± 4.4	7.4 ± 2.2	7.2 ± 1.3	4.2 ± 0.6†	6.7 ± 2.7	3.0 ± 0.7†	2.1 ± 1.7	1.5 ± 0.5
WG										
Cy	96.9 ± 0.4	100	1.8 ± 0.3		0.8 ± 0.3		0.5 ± 0.1		0.02 ± 0.01	
Mi	100	98.1 ± 1.9		1.1 ± 1.1		0.4 ± 0.4		0.3 ± 0.3		0.1 ± 0.1
Liver										
Cy	100	100								
Mi	96.5 ± 1.1	97.5 ± 0.7			1.0 ± 0.5	0.03 ± 0.03	2.4 ± 0.9	2.0 ± 0.3	0.06 ± 0.05	0.5 ± 0.2

Values are means ± SE. Cy and Mi, cytosolic and mitochondrial fractions, respectively; LDH, lactate dehydrogenase. SL and HA, before ($n = 6$) and after [$n = 8$ except for plantaris ($n = 3$)] acclimation to high-altitude hypoxia, respectively. LDH5 = A₄, LDH4 = A₃B₁, LDH3 = A₂B₂, LDH2 = A₁B₃, and LDH1 = B₄; RG, red gastrocnemius; WG, white gastrocnemius. *Significantly different from SL; †significantly different from Cy fraction.

acclimation to simulated altitude reveals its ability to affect expression in some but not all tissues examined. Also, past studies have failed to detect MCT 4 in the adult rat heart (31), and it was thought to be absent from this tissue (4). However, we report MCT 4 expression in both SL and HA heart muscle and that this isoform shows significant response to acclimation (Fig. 2B). The specificity of our antibody has been confirmed (8), but the presence of MCT 4 is perhaps not surprising because cardiac muscles simultaneously take up and release lactate (15). MCT 2 levels were the same in liver before and after acclimation (Fig. 7B). In fact, overall, the liver shows little response to chronic hyp-

oxia (35). This suggests that the ability of this tissue to transport lactate as a gluconeogenic precursor is not adjusted with this experimental protocol. To date, there have been no studies on the regulatory effects of MCT expression on lactate metabolism. Although past correlative data are intriguing, more detailed measurements of these relationships are warranted.

MCT 1 is highly related to muscle oxidative capacity, and so the decrease in MCT 1 seen in plantaris at first suggests a transition to more fast-twitch myosin isoforms. This hypothesis is not supported by MCT 4, which is also decreased, and LDH, which is unaffected by acclimation.

The lactate shuttle at high altitude. Elements of the cell-cell and intracellular lactate shuttles have been implicated to explain the effects of exercise and hypoxia on lactate metabolism (7). The mechanisms and degree of plasticity of these shuttles, however, are not fully understood. According to the lactate shuttle hypothesis, changes in kinetics should be a product of changes in the components of the shuttle. Here we demonstrate that the components of the shuttle show changes in expression with chronic hypoxia. The effect is tissue specific, and even in the same tissue hypoxia does not result in a coordinated change in all components. Some tissues showed a more synchronous regulation of shuttle components, there being a strong correlation between Mu MCT 1 and Cy LDH expression and a weaker but significant relationship between Mi MCT 1 and mLDH in heart (HA and SL data pooled). In this tissue, which is most affected by hypoxia, coordination between two important components of the shuttle may be necessary to preserve oxidative function. There was a negative correlation between Mu MCT 1 and Cy LDH in plantaris (Fig. 5). This mixed

Table 2. *Occurrence of A and B LDH subunits (% total) in Cy and Mi fractions of heart and skeletal muscle of rats before and after acclimation to high-altitude hypoxia*

Tissue	A		B	
	SL	HA	SL	HA
Heart				
Cy	24.2 ± 0.5	23.5 ± 0.9	75.8 ± 0.5	76.5 ± 0.9
Mi	29.1 ± 1.2†	25.0 ± 1.4*	70.9 ± 1.2†	75.0 ± 1.4*
Soleus				
Cy	14.2 ± 1.9	20.2 ± 2.2	85.8 ± 1.9	79.8 ± 2.2
Mi	24.8 ± 3.6	27.0 ± 2.9	75.3 ± 3.6	73.0 ± 2.9
RG				
Cy	73.9 ± 2.4	74.1 ± 1.9	26.1 ± 2.4	25.9 ± 1.9
Mi	83.8 ± 3.0†	83.6 ± 2.8†	16.2 ± 3.0†	16.4 ± 2.8†
Plantaris				
Cy	86.0 ± 2.9	80.6 ± 2.9	14.0 ± 2.9	19.4 ± 2.9
Mi	86.9 ± 4.6	92.4 ± 1.8†	13.1 ± 4.6	7.6 ± 1.8†

Values are means ± SE. SL, $n = 6$; HA, $n = 8$ except for plantaris ($n = 3$). *Significantly different from SL; †significantly different from Cy fraction.

Fig. 6. Expression of MCT 1 (A and E), MCT 4 (B), LDH (C and F), and COX (D and G) in white gastrocnemius Mu and Mi cell fractions in SL and HA rats; $n = 8$ for HA and $n = 6$ for SL.

muscle, high in type II fibers (1), shows a downregulation of MCT 1 and 4 in addition to a decrease in mitochondria density (indexed by COX expression). Surprisingly, there was no decrease of LDH in Cy or Mi fractions (Fig. 5). This most likely reflects the fast-twitch nature of this muscle geared toward glycolysis and net lactate release.

As mentioned above, chronic hypoxia changed the pattern of protein expression in skeletal muscle and heart mitochondria. This novel finding points out that the effects of a systemic environmental stressor can have important subcellular consequences. Here we confirm the presence of LDH in the mitochondria (10, 19) and show that the most profound effect of chronic hypoxia was seen in the expression of mLDH (see Figs. 2F and 3F). Whether there is a resulting change in

mitochondrial lactate oxidation rates is unknown. The isolation and respiration of mitochondria from acutely hypoxic and acclimated rats may illuminate putative limiting intracellular steps in lactate oxidation. The differences in LDH isozyme composition between Cy and Mi in most tissues examined rule out contamination of the Mi fraction by cytosolic LDH. The Mi fraction routinely had a greater percentage of LDH A subunits than the Cy fraction. This resulted in higher proportions of LDH 5 and LDH 4 in the Mi of most tissues (Tables 1 and 2).

Lactate metabolism and altitude acclimation. Many hypotheses have been put forward to explain the paradoxical decrease in blood lactate during exercise in the transition from acute to chronic hypoxia (e.g., Refs. 27, 32). We propose a mechanism by which increased

Fig. 7. Expression of MCT 1 (*A* and *E*), MCT 4 (*B*), LDH (*C* and *F*), and COX (*D* and *G*) in liver whole tissue (Mu) and Mi cell fractions in SL and HA rats; $n = 8$ for HA and $n = 6$ for SL.

transport and oxidation of lactate in skeletal muscle and heart decreases $[\text{lactate}]_b$. This hypothesis was born out of the fact that net lactate release is lower and lactate uptake predominates (9) after acclimation compared with acute hypoxia, independent of O_2 delivery to muscle (27). Also, after acclimation, muscle lactate concentration during exercise is lower than acute hypoxia values (29), providing further evidence of lower lactate production (27). Similarly, endurance training decreases lactate concentration (12), and this correlates with increases in MCT 1 content in sarcolemmal and mitochondrial membranes (19) and is thought to involve increased utilization by muscles (18). Changes in the components of the cell-cell and intracellular

lactate shuttles do not adequately explain all of the observed responses of lactate metabolism to chronic hypoxia. Although arterial lactate concentration theoretically could be reduced by an increase in oxidation in situ, acclimation did not result in a concerted increase in shuttle components. Except in the heart (Fig. 2), most tissues showed no change or even a decrease in the expression in the components of the lactate shuttle. The downregulation of MCT 4 would result in lower $[\text{lactate}]_b$ as well. Decreases in MCT 4 seen in plantaris muscles of HA (Fig. 4*B*) may contribute to lower lactate concentration, but clearly other mechanisms must also come into play (see Ref. 27 for review). It should be noted that decreased rate of appearance of lactate is

not completely attributed to active muscle. Decreased [lactate]_b can be partially explained by decreases in epinephrine with acclimation (27) and lower systemic lactate release. Future experiments integrating changes in lactate transport and oxidation ability with measurements of lactate kinetics during exercise in acutely hypoxic and HA-acclimated rats are clearly warranted.

Conclusions and implications. The underlying mechanisms of the tissue-specific response to chronic hypoxia observed here are not known. The tissue specificity of protein expression in skeletal muscle, in heart, and in liver may result from differences in regional oxygenation. Changes in systemic hypoxia or exercise can lead to regional areas of hypoxia in muscle (45). The patterns of blood flow at rest and during exercise (2) may result in different levels of hypoxia and may be the driving force for changes in hypoxia-induced gene expression, depending on the muscle or other tissues. To date, there has been no study looking at potential promoter regions on the genes encoding MCT 1, 2, or 4. Also, there are no known promoters or induction factors known to affect the transcription of these genes. Discovery of these factors; how they change with exercise training, hypoxia, or other perturbations; and their tissue specificity are potent areas for future research.

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