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Metabolism Clinical and Experimental 54 (2005) 634–644

Metabolism
Clinical and Experimental

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Role of hypoxia-induced anorexia and right ventricular hypertrophy on lactate transport and MCT expression in rat muscle

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Received 2 July 2004; accepted 17 December 2004

Abstract

To dissect the independent effects of altitude-induced hypoxemia and anorexia on the capacity for cardiac lactate metabolism, we examined the effects of 21 days of chronic hypobaric hypoxia (CHH) and its associated decrease in food intake and right ventricle (RV) hypertrophy on the monocarboxylate transporter 1 and 4 (MCT) expression, the rate of lactate uptake into sarcolemmal vesicles, and the activity of lactate dehydrogenase isoforms in rat muscles. In comparison with control rats (C), 1 mmol/L lactate transport measured on skeletal muscle sarcolemmal vesicles increased by 33% and 58% in hypoxic (CHH, barometric pressure = 495 hPa) and rats pair-fed an equivalent quantity of food to that consumed by hypoxic animals, respectively. The increased lactate transport was higher in PF than in CHH animals ($P < .05$). No associated change in the expression of MCT1 protein was observed in skeletal muscles, whereas MCT1 mRNA decreased in CHH rats, in comparison with C animals (42%, $P < .05$), partly related to caloric restriction (30%, $P < .05$). MCT4 mRNA and protein increased during acclimatization to hypoxia only in slow-oxidative muscles (68%, 72%, $P < .05$, respectively). The MCT4 protein content did not change in the plantaris muscle despite a decrease in transcript levels, related to hypoxia and caloric restriction. In both the left and right ventricles, the MCT1 protein content was unaffected by ambient hypoxia or restricted food consumption. These results suggest that *MCT1* and *MCT4* gene expression in fast-glycolytic muscles is mainly regulated by posttranscriptional mechanisms. Moreover, the results emphasize the role played by caloric restriction on the control of gene expression in response to chronic hypoxia and suggest that hypoxia-induced right ventricle hypertrophy failed to alter MCT proteins.

1. Introduction

The rapid transport of lactate across the plasma membrane is of crucial importance for the maintenance of cell homeostasis, mainly if high rates of glycolysis are to be maintained [1]. Cell homeostasis in fast-twitch skeletal muscle is related to lactate output and appearance in the blood, whereas lactate uptake by oxidative muscles such as heart and soleus (Sol) permits the use of lactate as a respiratory fuel. There is now considerable evidence that transport of lactate across the plasma membrane is catalyzed by protein-linked monocarboxylate transporters (MCTs) [2]. A large family of MCTs has been cloned over the past few years, and different MCT isoforms can be coexpressed in the same tissue, suggesting that MCT isoforms play different physiological roles [3]. MCT1 and MCT4 are the major lactate transporters found in skeletal muscle and in the heart. The abundance of MCT1 is highly correlated with the oxidative capacity of muscle and is found at higher levels in the heart than in slow-oxidative skeletal muscles [4,5]. MCT1 expression is responsive to exercise training, and, taken together, these findings suggest that MCT1 expression is correlated with lactate uptake from the circulation, facilitating its oxidation by oxidative myofibers [6,7]. In contrast, MCT4 is mainly expressed in fast-glycolytic fibers, and this supports the notion that this isoform is important for lactic acid efflux from muscles that rely on glycolytic

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doi:10.1016/j.metabol.2004.12.007

metabolism. Whether MCT4 expression is altered by changes in functional load is not clear, but several studies suggest that the expression of this isoform is induced by increased glycolytic activity [8]. Lactate dehydrogenase (LDH) is an important family of metabolic enzymes, which exists as multiple molecular isoforms as an association of 2 isoforms of monomer subunits, known as M and H. Several previous studies showed a good relationship between MCT1 expression, H-LDH activity, and oxidative capacity in rat muscles, suggesting that oxidative muscles are strongly involved in lactate uptake and oxidation in the mitochondria [5,8]. These results support the notion that MCT and LDH isoforms are important components of the shuttling of lactate as implemented by the "lactate shuttle" hypothesis [9].

Chronic exposure to hypobaric hypoxia has been shown to induce marked adaptive changes that result in the improvement of oxygen transport from the environment to the blood and then to tissues. Acclimatization to chronic hypobaric hypoxia (CHH) leads to biochemical adaptation affecting the energy metabolism of both the skeletal muscle and the heart, and especially lactate metabolism [10,11]. Lower lactate production, release, and higher levels of lactate uptake were shown in muscles after acclimatization, in comparison with acute hypoxia [12,13]. Moreover, hypoxia leads to an increased reliance on glucose as energy substrate [13]. Because lactate traverses the sarcolemmal membrane via MCTs, whether CHH elicits changes in the expression of these specific transport proteins in several tissues needs to be examined. Because MCTs and LDH isoforms are involved in the shuttling of lactate between cells [9], the responses of these components of the lactate shuttle have been examined after acclimation to high altitude [14]. A decrease in MCT1 and MCT4 proteins occurred in a mixed skeletal muscle, whereas MCT4 expression increased in the heart. However, long-term exposure of rats to CHH is associated with cachexia and depressed growth rate, mainly attributable to a decrease in food consumption in comparison with normoxic rats [15-17]. Caloric restriction has been reported to alter glucose metabolism and compromise glycolysis, especially in slow-oxidative muscles [18,19]. Moreover, caloric restriction alone increases the lactate transport capacity in skeletal muscle, without significant change in MCT expression [20]. It is thus clear that the specific effects of the decreased food consumption need to be taken into account to examine the expression of MCT and LDH isoforms in response to prolonged exposure to hypoxia.

A marked increase in resistances in pulmonary circulation occurs during exposure to hypobaric hypoxia, resulting in increased right ventricular workload [21]. This, in turn, involves a true hypertrophy of the right ventricle (RV), mainly related to a major increase in the mean myofiber size, which likely affects energy metabolism [22]. Earlier studies reported that mechanically induced myocardial hypertrophy provoked a decrease in the H/M ratio of the LDH isozymes and a shift in metabolism toward the glycolytic pathway [23]. These responses are consistent with the adaptive changes of LDH in the heart after acclimatization to prolonged hypoxia [24-26]. However, the extent to which MCT expression is differentially affected by exposure to CHH in RV and left ventricle (LV) has never been documented.

Clearly, the responses to ambient hypoxia of MCT and LDH isoforms, involved in the lactate shuttling, need to be considered together with the specific impact of reduced dietary energy intake and the compensatory RV hypertrophy in the heart. Therefore, the purpose of this work was to evaluate the impact of prolonged exposure to ambient hypoxia, and its associated effects on food consumption, hypoxemia, pulmonary hypertension, and RV hypertrophy, on the expression of MCT and LDH isoforms. In the present study, we assessed the tissue-specific changes in MCT expression and the alterations in LDH activity and isozyme expression in skeletal muscle, RV, and LV, after 3 weeks of exposure to ambient hypoxia. Changes in MCT1 and MCT4 were examined at both the protein and mRNA levels. In addition, to examine the functional effects of changes in MCT1 and MCT4 proteins in skeletal muscles, we assessed the rate of lactate transport in sarcolemmal vesicles. To differentiate the effects of hypoxia per se from those related to a CHH-induced decrease in food intake, we used a group of rats exposed to normoxia and pair-fed (PF) quantities of food equivalent to those consumed by animals subjected to ambient hypoxia.

2. Materials and methods

2.1. Animals

Thirty male Wistar rats (IFFA CREDO, France), weighing 300 ± 20 g (10 weeks old), were divided into 3 groups of ten animals. These groups were assigned to either a normobaric control (C) group, a CHH group, or a PF group. Pair-fed rats were maintained in normobaric conditions, received the same amount of food as the hypoxic ones, and were destined to study the specific effects of decreased food intake. Chronic hypobaric hypoxia rats were housed in individual cages with a 12:12-hour dark-light cycle (lights on at 7 PM) in an experimental hypobaric chamber. They were exposed to conditions equivalent to those found at 5500 m (FiO_2 = 0.1) for 21 consecutive days. The pressure in the hypobaric room was raised daily to normobaric level for 1 hour for cleaning, food, and rat weighing. Each animal, weighed daily to monitor their growth, received a standard diet and water ad libitum throughout the experimental period, except the PF group which received a measured amount of food equal to what was eaten and drunk by the CHH rats. The temperature was 22 ± 1°C under both environmental conditions. All experiments were performed

in accordance with the Helsinki accords for humane treatment of laboratory animals.

2.2. Tissue and sample preparations

After cervical dislocation, a blood sample was withdrawn from the abdominal aorta for hematocrit analysis, and hindlimb muscles and the heart were rapidly removed. Right ventricle and LV, left Sol, and plantaris (Pla) muscles were excised, weighed, and quickly frozen in liquid N_2 and stored at −80°C until used for biochemical analysis and Western blotting. All muscles of the right hindlimbs were used for sarcolemmal isolation. Reagents of the highest quality available were purchased from Sigma Chemicals (Lyon, France) unless otherwise stated.

Soleus, Pla, RV, and LV muscle homogenates (1:40) were prepared at 4°C in 2 mL of *buffer A* [in mmol/L: 210 sucrose, 2 ethylene glycol-bis(*β*-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid, 40 NaCl, 30 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, 5 EDTA, and 2 phenylmethylsulfonyl fluoride, pH 7.4] for ten passes in a glass tissue homogenizer. Two milliliters more of *buffer A*, used to rinse the glass homogenizer, was added to these tubes. Then, homogenates were subjected to a low-speed centrifugation (1000*g*) for 10 minutes at 4°C before the supernatants were divided into aliquots (one of 3.8 mL for immunoblotting and two others with the remaining volume for total LDH activity and isozymes separation) and stored at −80°C.

2.3. Western blotting of MCT1 and MCT4

On the day of immunoblotting and according to the slightly modified procedure of McCullagh et al [5], 2.85 mL of *buffer B* (1.167 M KCl, 58.3 mmol/L tetrasodium pyrophosphate) was added to the 3.8-mL aliquot of Sol, Pla, RV, and LV muscle homogenate supernatant, mixed briefly, and then set on ice for 15 minutes. After centrifugation at 230 000*g* for 75 minutes at 4°C, the supernatant fluid was discarded and the pellet was washed thoroughly with 1 to 2 mL of *buffer C* [10 mmol/L tris(hydroxymethyl)aminomethane (Tris) base, 1 mmol/L EDTA, pH 7.4]. The pellet was resuspended in 570 μL of *buffer C* and homogenized for 2 interrupted 10-second bursts with an Ultra-Turrax T25 (Labo Moderne, Paris, France) set at 50% of maximal power. Then 3.33 μL of 16% sodium dodecyl sulfate (SDS) was added per milligram of muscle, and the samples were removed from ice, vortex mixed, and centrifuged at 1100*g* for 20 minutes at room temperature. The supernatant was divided into aliquots for protein assays and immunoblot detection of MCT1 and MCT4. Muscle protein concentrations were determined in triplicate by the bicinchoninic acid assay (Pierce, Interchim, Montluçon, France) with the use of bovine serum albumin as a standard.

Affinity-purified polyclonal antibodies (a gift from Professor GA Brooks, Department of Integrative Biology, University of California, Berkeley, Calif) directed against rat MCT1 and human MCT4 were produced by immunizing New Zealand White rabbits with the synthetic peptide PLQNSSGDPAEEESPV for MCT1 [27] and LREVEHFLKAEPEKNG for MCT4 [28]. Antibody specificities were confirmed in preliminary experiments in which the peptides blocked the detection of MCT1 and MCT4. The secondary antibody used was a goat antirabbit IgG horseradish peroxidase–linked monoclonal antibody (BioSys, Compiègne, France).

Protein (20 μg) samples of the muscles and prestained molecular mass markers (Bio-Rad SA, Marnes la Coquette, France) were separated on 12% sodium dodecyl sulfate–polyacrylamide gels (Novex system, Invitrogen, Groningen, The Netherlands) for 60 minutes at 200 V. Proteins were then transferred from the gels to polyvinylidene difluoride membranes (30 V, 60 minutes). Membranes were incubated on a shaker for 1 hour at room temperature in *buffer D* (20 mmol/L Tris base, 137 mmol/L NaCl, 0.1 M HCl, adjusted to pH 7.5, 0.1% [v/v] Tween 20, and 5% [w/v] nonfat dried milk). Membranes were then incubated with either anti-MCT1 antibody (1:2500) or anti-MCT4 antibody (1:2500) in *buffer D* for 2 hours at room temperature. After a 15-minute wash and two 5-minute washes in *buffer E* (ie, *buffer D* without dried milk), membranes were incubated for 1 hour with goat antirabbit immunoglobulin G horseradish peroxidase–conjugated secondary antibody (1:2500; BI 2407, BioSys) in *buffer E*. Membranes were then washed as before in *buffer E*, and MCT1 or MCT4 expression was detected with the use of an enhanced chemiluminescence detection according to the manufacturer's instructions (Biomax MR, Kodak). Films were developed and fixed using a Hyperprocessor, RNP 1700 (Amersham, Les Ulis, France). Films were scanned with an AGFA T1200 Duo Scan densitometer, and MCT1 and MCT4 band densities were quantified by Scion Image software (Scion Corporation, Frederick, Md). MCT1 and MCT4 protein content values were normalized to those measured in Sol muscles from the C group.

2.4. MCT mRNA determination

2.4.1. Total RNA isolation

Total RNA was extracted from 15 mg of frozen muscle samples by using the RNeasy mini kit procedure (Qiagen SA, Courtaboeuf, France) following the company protocol. The RNA concentration was determined by optical density (OD) at 260 nm (by using an OD_{260} unit equivalent to 40 μg/mL). This technique provides well-preserved RNA, free of DNA and proteins, which is confirmed by an OD_{260}/OD_{280} ratio of ~1.90. Samples were stored at −80°C until subsequent analyses.

2.4.2. Oligonucleotide primers

All primers used in this study were synthesized by Eurogentec (Saraing, Belgium) and designed with the

MacVector software (Accelrys). Selected forward (F) and reverse (R) primers for MCT1, MCT4, and cyclophylin-A (CycA) were as follows:

MCT1	F: 5′-GCCTCTACCTCTTCATTGGTATGG-3′
	R: 5′-CTCATCAACATCAGTGCTGGTCTC-3′
	Generating a 116-bp DNA fragment
MCT4	F: 5′-TTCAATGGCTTCACTGACCTGACAG-3′
	R: 5′-CCATACGAGATCCCAAAGAAGATGC-3′
	Generating a 89-bp DNA fragment
CycA	F: 5′-AGCATGTGGTCTTTGGGAAGGTG-3′,
	R: 5′-CTTCTTGCTGGTCTTGCCATTCC-3′
	Generating a 92-bp DNA fragment

2.4.3. Reverse transcriptase reaction

One microgram of total RNA was reverse transcribed for each muscle sample by using the following composition of the reverse transcriptase (RT) mixture (Roche Applied Science, Meylan, France): 2 μL 10× first strand buffer, 4 μL $MgCl_2$ (25 mmol/L), 2 μL deoxy nucleic acid triphosphate (dNTP) mix (10 mmol/L each), 2 μL oligo-p $[dT: deoxythimidine]_{15}$ primer (0.8 μg/μL), 1 μL RNase inhibitor (50 U/μL), and 0.8 μL AMV RT. Depending on the volume of total RNA corresponding to 1 μg, the volume of the reaction mixture was adjusted to 20 μL by adding RNase-free water. The complete mix was incubated for 10 minutes at +25°C, then 60 minutes at +42°C, followed by 5 minutes at +99°C and then cooling to +4°C for 5 minutes.

2.4.4. Real-time polymerase chain reaction

The amplification reaction mixture contained 2 μL of cDNA in a final volume of 20 μL, and the final concentrations were, respectively, 4 mmol/L for $MgCl_2$ and 0.4 μmol/L for primers. The reaction was carried out with the LightCycler (Roche Diagnostics, Meylan, France) for 45 cycles with each cycle consisting of 15 seconds at 94°C, 5 seconds at 60°C (MCT1), 54°C (MCT4), or 65°C (CycA), then 8 seconds at 72°C.

2.4.5. Quantification and normalization

The accuracy of mRNA quantification depends on the linearity and efficiency of polymerase chain reaction (PCR) amplification, which were determined from the comparative threshold cycle (C_T) approach. This method represents a fast and simple way for quantification, which is based on very close amplification efficiencies between target and reference genes [29]. A reliable quantitative RT-PCR method requires corrections for experimental variability among different samples (ie, cDNA amount and minor differences in PCR efficiencies). Using real-time PCR, quantification is based on C_T values, which are determined rapidly in the exponential phase of the reaction, and thus the intersample variability in amplification efficiencies does not confound the results [30]. Normalization to a housekeeping gene is currently the most acceptable method to avoid these discrepancies [30], but the choice of an appropriate gene is particularly relevant for reliability of the results. No significant change was detected in the expression of CycA gene in the present study, and this gene has been used as a housekeeping gene (data not shown).

2.5. Lactate uptake by sarcolemmal vesicles

2.5.1. Sarcolemmal isolation and characterization

Sarcolemmal vesicles were purified from hindlimb muscles of the 2 legs, except the left Sol and Pla muscles, with a procedure routinely used in our laboratory [6,31,32]. Muscle samples were homogenized in ice-cold 250 mmol/L sucrose, 1 mmol/L EDTA, and 20 mmol/L HEPES at pH 7.4. The homogenate was centrifuged twice at 900*g*, supernatants were then filtered, and a 1-mL aliquot was partitioned and saved at 4°C for subsequent analysis. The remaining crude homogenate was diluted with a volume of KCl medium equal to 10% of its volume and pelleted by ultracentrifugation (200 000*g* for 45 minutes at 4°C). Pellets were resuspended using Teflon pestle homogenization, then centrifuged twice at 280*g* and supernatants were collected and centrifuged (200 000*g*; 45 minutes at 4°C). Pellets were homogenized, and a discontinuous density gradient was constructed by addition of several sucrose solutions in layers. After an overnight centrifugation, the 27% sucrose band was harvested, diluted with a Krebs-Ringer-HEPES buffer, and washed free of sucrose. The vesicles were resuspended and stored at −80°C until used for the transport experiments. Proteins were determined according to the procedure of Bradford [33] using bovine γ globulin as a standard.

Sarcolemmal characterization was achieved with K^+-stimulated *p*-nitrophenyl-phosphatase (K^+-pNPPase) assay as described previously [31,32]. Its total activity was measured in 40 mmol/L HEPES, 0.8 mmol/L EGTA, 4 mmol/L $MgCl_2$, 20 mmol/L KCl, and 5 mmol/L *p*-nitrophenylphosphate, pH 7.4. The absorbance of the *p*-nitrophenol formed was read at 410 nm. Nonspecific K^+-pNPPase activity was determined in a KCl-free medium which, when subtracted from the total activity, gave the specific K^+-pNPPase activity expressed in $\mu mol \cdot h^{-1} \cdot mg^{-1}$. The purification index was defined as the ratio of the specific activity from the F_2 fraction to the specific activity measured in the crude homogenate. Skeletal muscle sarcolemmal yield was the ratio of milligrams of sarcolemmal protein obtained in F_2 to the muscle weight in grams (wet weight). The vesicle "sideness" was estimated in a set of experiments, by measurement of the K^+-pNPPase activity with and without a previous 2 mmol/L deoxycholate incubation [31]. As previously reported, right-side-out vesicles represented 65% to 70% of the total vesicle population.

2.5.2. Lactate transport studies

All measurements were performed in zero-*trans* conditions in duplicate. [^{14}C]-L-Lactate (ul) (specific activity 155 mCi·mmol^{-1}; Amersham) was diluted in 280 mmol/L sucrose and 50 mmol/L HEPES, pH 7.4, and different unlabeled L(+)-lactate concentrations. Reciprocal decreases in sucrose were used to maintain the same total isosmotic buffer strength. Reactions were initiated by delivering 50 μg of sarcolemmal vesicles in tracer-containing medium and stopped at appropriate time intervals by vacuum filtration on nitrocellulose filters (Whatman WCN, average pore size of 0.45 μm; Bio-Rad, Ivry-sur-Seine, France). Filters were then rinsed 3 times with an ice-cold isosmotic medium consisting of KRH buffer with 3 mmol/L $HgCl_2$, pH 7.4, dissolved with ethyleneglycolmonomethylether, and the radioactivity was counted in a scintillation analyzer (Packard 2200, Calif, USA). Nonspecific transport activities were determined by preincubation of vesicles in tracer-containing medium with KRH buffer containing 3 mmol/L $HgCl_2$ which was used to fix the time 0 points. Results were expressed in nmol mg protein^{-1}. Measurements of initial lactate uptake were done for 1, 10, 30, and 100 mmol/L external lactate concentrations at 0 and 10 seconds, where uptake rates are assumed to be constant and zero-*trans* conditions respected [34]. Slopes gave initial rates of lactate uptake expressed in nanomoles of lactate min^{-1} mg protein^{-1}.

2.6. Lactate dehydrogenase isozyme distribution and specific activities

Lactate dehydrogenase isozymes present in Sol, Pla, LV, and RV muscle homogenate supernatants, thawed at room temperature, were separated by electrophoresis. Typically, 1 μg protein was loaded on 1% agarose gels and separated for 90 minutes at 200 V. Individual isozymes, that is, the 5 homo- (H_4 and M_4) and hetero- (H_3M, H_2M_2, and HM_3) tetramers of LDH, were resolved with a commercial revelation system (Sigma LDH reagent kit). Lactate dehydrogenase Isotrol marker (Sigma) containing LDH isozymes 1 to 5 was used as an aid in isoforms identification. Gels were fixed in 5% acetic acid and scanned with an AGFA Duo Scan 1200 densitometer, and the different bands were quantified with Scion Image software. The relative content of the LDH-H subunit was calculated as follows: H-LDH = H_4 + 3/4 H_3M + 1/2 H_2M_2 + 1/4 HM_3, whereas the relative content of the LDH-M subunit was similarly calculated. The specific activities of the H and M isomers were obtained by multiplying each percentage by total activity, as determined spectrophotometrically [24]. Results were expressed as UI/g wet wt.

2.7. Statistical analysis

All results are expressed as means ± SE. One-way analysis of variance was used to determine the effects of experimental conditioning on variables studied, and a Student-Newman-Keuls post hoc test was used to determine the significance of difference between means. Throughout the study, statistical significance was accepted at $P < .05$.

3. Results

3.1. Body and muscle weights, and hematocrit values

Mean body, heart, and muscle weight values from C, PF, and CHH animals are provided in Table 1. Final body weight values were affected by the environment or treatment and were reduced by 11% and 13% in CHH and PF rats, respectively, compared with C rats ($P < .001$). Absolute weight gain of C animals averaged 23%, whereas CHH and PF body-weight gains represented only 25% and 21% of that of C rats, respectively. This was associated with a 32% decrease in daily food intake in the CHH group, in comparison with C animals (Fig. 1), despite the progressive age-related increase in daily food intake.

Relative heart, RV, and LV weights were significantly altered under hypoxia and food restriction. A slight increase in relative heart and LV weights was observed in PF rats (7%, $P < .05$). As expected, the relative heart weight was markedly increased in CHH rats, in comparison with that of C animals (49%, $P < .001$) and PF rats (39%, $P < .001$). This resulted from an increase in both the relative LV weight (24%, $P < .001$) and the relative RV weight, especially in comparison with C rats (167%, $P < .001$) (Table 1). No significant differences were observed in relative skeletal muscle weights between the 3 groups. As expected, chronic hypoxia induced hematological adaptations that increased hematocrit by an average of 40% in CHH rats compared with the C group (Table 1).

3.2. Monocarboxylate transporter expression

A representative blot showing MCT1 protein content in hindlimb skeletal muscles (Sol and Pla muscles), LV, and

Table 1
Body weight, muscle-to-body weight ratio, and hematocrit values

	C (n = 10)	PF (n = 10)	CHH (n = 10)
Initial body weight (g)	297.4 ± 3.5	304.4 ± 6.4	308.5 ± 5.7
Final body weight (g)	366.1 ± 4.3	319.1 ± 3.2**	326.4 ± 4.5**
Percent gain (%)	23.1	4.8	5.8
Heart–body weight ratio ([mg/g] × 100)	219.1 ± 1.8	233.8 ± 3.6*	326.2 ± 5.1**,***
LV–body weight ratio ([mg/g] × 100)	178.9 ± 3.0	191.0 ± 3.1*	222.6 ± 3.9**,***
RV–body weight ratio ([mg/g] × 100)	39.3 ± 2.0	42.7 ± 2.4	103.6 ± 4.6**,***
Sol–body weight ratio ([mg/g] × 100)	34.9 ± 2.3	38.4 ± 1.7	40.4 ± 1.2
Pla–body weight ratio ([mg/g] × 100)	91.2 ± 1.8	97.6 ± 1.9	93.6 ± 2.0
Hematocrit (%)	46.5 ± 0.6	44.8 ± 0.8	65.1 ± 0.6**,***

Values are expressed as means ± SE.
* Significantly different from C group, $P < .05$.
** Significantly different from C group, $P < .001$.
*** Significantly different from PF group, $P < .001$.

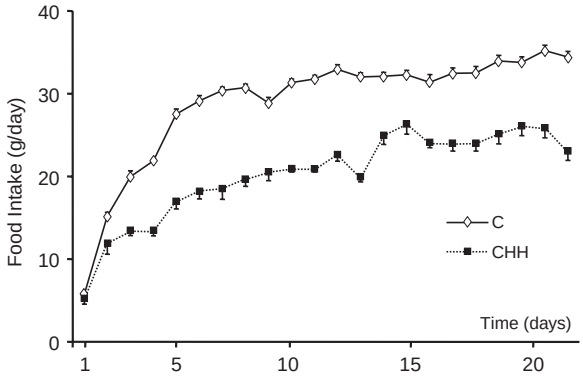

Fig. 1. Mean daily food intake in normoxic (C) and CHH rats. Values are means ± SE; $n = 10$ in each group. Absolute values of food intake in the PF group are strictly those of CHH rats.

RV from C, PF, and CHH animals, is shown in Fig. 2. MCT1 protein was barely detected in the fast-glycolytic Pla muscle, with no difference between groups. In contrast, the largest concentrations of MCT1 were present in LV and RV. The MCT1 protein content in skeletal muscles and in the heart was not affected by either hypoxia exposure or caloric restriction (Fig. 2B). Despite a lack of detectable change in MCT1 protein, a decrease in MCT1 mRNA abundance was observed in the Pla muscle of CHH rats in comparison with C animals (42%, $P < .05$), partly related to the caloric restriction (30%, $P < .05$) (Fig. 2C). A decrease in MCT1 mRNA was also detected in the LV of CHH rats, in comparison with both C and PF animals ($P < .05$).

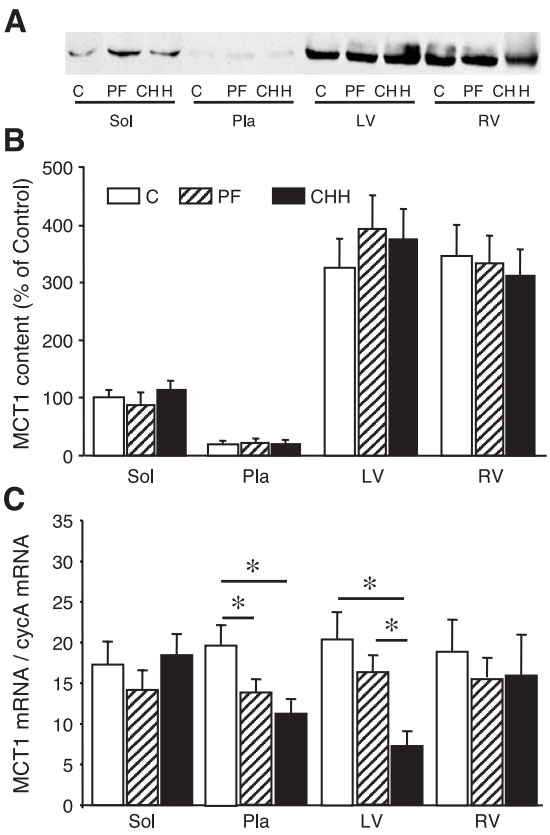

Fig. 2. Representative Western blots (A) and changes in MCT1 protein content (B) and mRNA (C) in Sol, Pla muscles, LVs, and RVs from C (open bars), PF (hatched bars), and CHH (black bars) rats. Pair-fed and CHH muscular MCT1 contents are expressed in OD arbitrary units and as a percentage of control group, where MCT1 content was set at 100%. Quantitative analysis of MCT1 gene expression has been performed by real-time RT-PCR, using CycA as an internal standard. MCT1 content values and mRNA values are expressed as means ± SE ; $n = 10$ in each group. Significant difference with C group, $^*P < .05$.

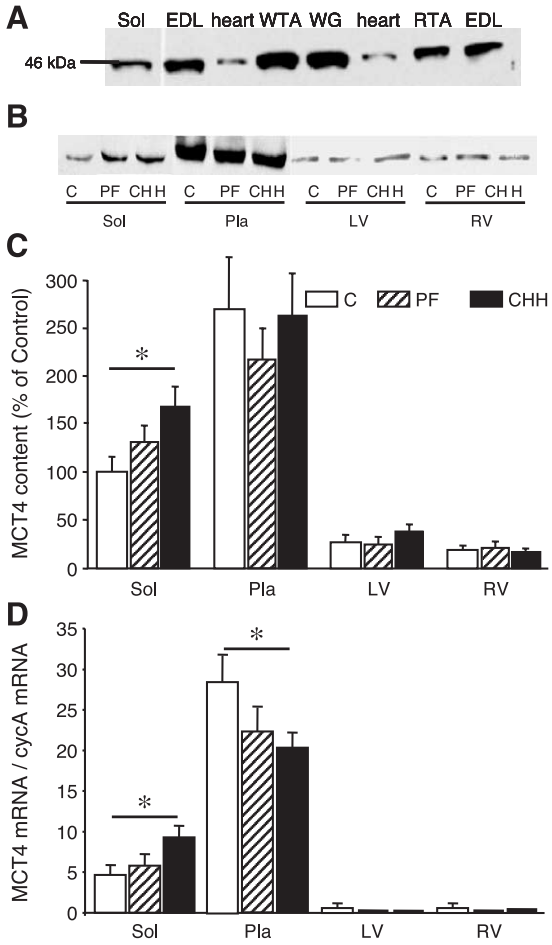

Fig. 3. Representative Western blots of MCT4 protein in heart and rat skeletal muscles. (A) EDL indicates extensor digitorum longus; WTA, white tibialis anterior; WG, white gastrocnemius; RTA, red tibialis anterior muscles from control normoxic rats. For protein determination, 20 and 35 μg protein were loaded into each well for skeletal muscles and heart, respectively. Representative Western blots (B) and changes in MCT4 protein content (C) and mRNA (D) in Sol, Pla muscles, LVs, and RVs from C (open bars), PF (hatched bars), and CHH (black bars) rats. Pair-fed and CHH muscular MCT4 contents are expressed in OD arbitrary units, and as a percentage of control group, where MCT4 content was set at 100%. Note that heart and skeletal muscle data are not directly comparable because 60 μg per lane for heart and 20 μg per lane for skeletal muscles were loaded into each well. Quantitative analysis of MCT4 gene expression has been performed by real-time RT-PCR, using CycA as an internal standard. MCT4 content values and mRNA values are expressed as means ± SE; $n = 10$ in each group. Significant difference with C group, $^*P < .05$, $^{**}P < .01$.

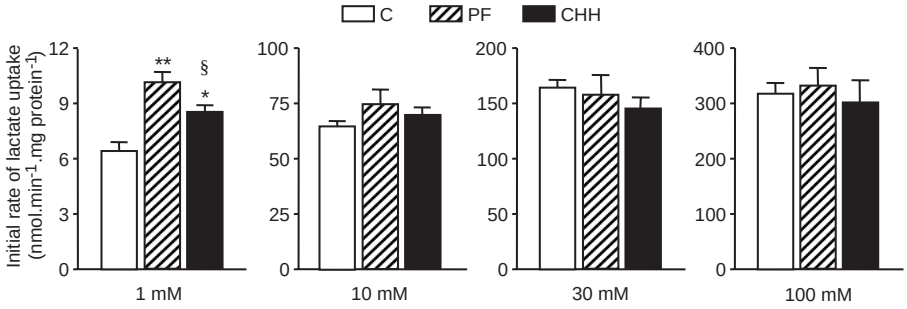

Fig. 4. Effect of chronic hypoxia and decreased food consumption on initial rates of lactate uptake in skeletal muscle sarcolemmal vesicles from C (open bars), PF (hatched bars), and CHH (black bars) rats, at 1, 10, 30, and 100 mmol/L external lactate concentrations. Values are means ± SE of four experiments with different membrane preparations. All assays were performed in duplicate at pH = 7.4. Significantly different from control rats, *$P < .01$, **$P < .001$. Significantly different from PF animals, §$P < .05$.

A representative MCT4 Western blot obtained from fast-, slow skeletal muscles, and the heart of C, PF, and CHH rats is shown in Fig. 3A. When a constant protein amount was loaded into each well, MCT4 protein was only barely detected in the heart. Moreover, the MCT4 protein content of fast-glycolytic muscles was largely higher than that of slow muscles such as Sol and heart. The MCT4 protein content in the Sol muscles of CHH rats was higher than that of C rats (68%, $P < .05$) (Fig. 3B,C). This increase was only partly related to a specific effect of caloric restriction. An increase in MCT4 mRNA was shown in the Sol muscles of CHH rats in comparison with C animals (72%, $P < .05$) (Fig. 3D). No significant change was detected in MCT4 in the Pla muscle, although a decrease in MCT4 mRNA occurred in hypoxic rats, mainly related to caloric restriction (Fig. 3D). The Western blot analysis detected MCT4 protein in heart muscle after higher protein/lane amounts than for skeletal muscles were loaded in each well (Fig. 3B). Hypoxia exposure or caloric restriction did not affect MCT4 protein in the heart, despite a 46% increase in the LV of CHH rats, in comparison with both C and PF animals ($P = 0.08$). Because MCT4 mRNA was only detected at very low levels in the heart, no significant change was detected with hypoxia exposure or caloric restriction.

3.3. Lactate uptake

Initial rates of lactate uptake in skeletal muscle sarcolemmal vesicles were plotted as a function of 4 different external concentrations (ie, 1, 10, 30, and 100 mmol/L, in the C, PF, and CHH groups) (Fig. 4). The rate of 1 mmol/L lactate uptake was increased in the vesicle preparations from CHH (33%, $P < .01$) and PF rat skeletal muscles (58%, $P < .001$), compared with C animals (Fig. 4). Moreover, the 1 mmol/L lactate uptake rate was significantly higher in the PF group than the rate measured in the CHH group ($P < .05$). For higher external lactate concentrations, the lactate transport activity of vesicle preparations did not differ between groups.

3.4. LDH isozyme profile

The total LDH activity was 38% higher in the Sol of CHH rats than in C or PF animals (38% and 20%, $P < .01$ and $P < .05$, respectively) (Table 2). An increase in the total LDH activity occurred in the LV of CHH rats, in comparison with control animals (21%, $P < .05$), mainly related to caloric restriction (32%, $P < .01$). Except in the Pla muscles, an increase in the percentage of the M-LDH subunits occurred with hypoxia acclimatization, partly related to caloric restriction (Table 2). The percentage of M-LDH subunits in the heart of CHH rats was higher than that of PF animals (14% and 19%, in LV and RV, respectively, $P < .05$), and this result highlights a specific effect of hypoxia per se. For each tissue, the specific activity of the 2 subunits M-LDH and H-LDH was differently altered (Fig. 5). Compared with C rats, the specific activity of M-LDH was increased in the Sol muscles, LV, and RV of both CHH and PF animals. This increase was more marked in the CHH than in the PF group

Table 2
Total LDH activity and percentage of M-LDH subunits

	C (n = 10)	PF (n = 10)	CHH (n = 10)
Total LDH activity	667.8 ± 15.1	721.5 ± 15.8	725.0 ± 94.2
Sol	146.1 ± 12.9	166.7 ± 9.8	201.8 ± 11.8**,***
Pla	667.8 ± 15.1	721.5 ± 15.8	725.0 ± 94.2
LV	430.1 ± 31.8	567.0 ± 20.7**	518.7 ± 23.3*
RV	422.0 ± 39.0	453.1 ± 15.5	486.8 ± 22.2
Percentage of M-LDH subunits			
Sol	31.5 ± 1.5	38.9 ± 1.1*	40.6 ± 1.4**
Pla	75.7 ± 1.4	71.4 ± 0.8	71.9 ± 4.2
LV	33.3 ± 0.8	38.3 ± 0.7*	43.7 ± 0.4**,***
RV	30.2 ± 0.9	37.5 ± 0.7*	44.6 ± 0.6**,***

Values are expressed as means ± SE. The total LDH activity was expressed as μmol of substrate transformed by minute and per gram wet mass of tissue at 25°C. Significantly different from PF group.

* Significantly different from C group, $P < .05$.

** Significantly different from C group, $P < .01$.*

*** Significantly different from PF group, $P < .05$.**

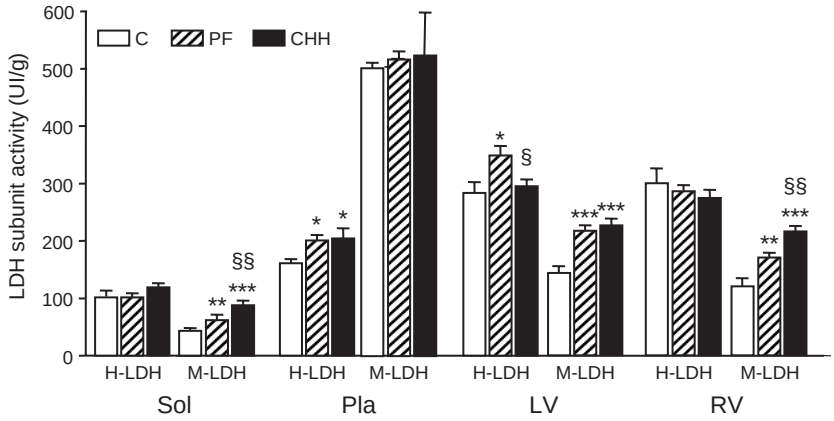

Fig. 5. H- and M-LDH subunit-specific activities in Sol, Pla muscles, LV, and RV muscles from C (open bars), PF (hatched bars), and CHH (black bars) rats. Values are means ± SE. Enzyme activity is expressed as units of activity per gram of tissue wet weight (UI/g). Significantly different from control rats, $*P < .05$, $**P < .01$, $***P < .001$. Significantly different from PF rats, §$P < .05$, §§$P < .01$.

for Sol muscles and RV ($P < .01$) (Fig. 5). The specific activity of H-LDH was also increased in the Pla muscle of CHH and PF rats ($P < .05$), and by 25% in the LV of PF animals, compared with C rats ($P < .05$).

4. Discussion

The main purpose of this study was to examine the effects of prolonged exposure to ambient hypoxia, and its associated decreased food intake and RV hypertrophy, on MCT expression in heart and skeletal muscles and lactate transport activity in hindlimb muscles. The expected and well-known general responses to CHH were confirmed. The decreased growth rate, mainly during the first days of exposure, the increased hematocrit, and RV hypertrophy contribute to verify the efficiency of hypoxia exposure. In skeletal muscles, we showed (1) only an increase in MCT4 protein and mRNA levels in slow-oxidative muscles, as a result of hypoxia, not MCT1 protein; (2) an enhanced total LDH activity and M-LDH–specific activity in CHH rat Sol muscle, due to both hypoxia per se and caloric restriction; (3) an increased rate of 1 mmol/L lactate uptake in skeletal muscle vesicle preparations from both CHH and PF rats, compared with the C group ($P < .01$, $P < .001$, respectively); (4) hypoxia per se slightly decreased the lactate transport at low lactate concentration. In the heart, we observed (5) no significant change in MCT1 or MCT4 protein, despite a marked decrease in MCT1 mRNA in the LV, (6) an increase in total LDH activity, and M-LDH–specific activity in the LV of CHH rats, mainly related to a decrease in energy intake.

The present data confirm that the food consumption of rats exposed to CHH was 22% to 25% lower than that of normoxic animals [15-17]. One hypothesis was that the hypoxia-induced decrease in caloric intake could influence MCT expression. A decrease in both MCT1 and MCT4 mRNA levels occurred in the Pla muscle of CHH rats, mainly attributable to the decreased food consumption of hypoxic rats, but not paralleled by alterations in protein expression. Small changes in MCT1 mRNA, whereas MCT1 protein concentrations were largely increased, have been reported in chronically stimulated muscles [35]. Consistent with the present study, a decrease in MCT1 mRNA without change in the MCT1 protein content was observed in developing rat brain [36]. Whether chronic hypoxia increased polysomes, ribosomal mRNA and/or affected the phosphorylation state of translational initiation factors in fast muscles, and thus increased translational efficiency, needs to be examined in the future. Moreover, these results are fully consistent with previous observations that MCT1 and MCT4 protein expression are regulated by both transcriptional and posttranscriptional mechanisms in fast muscles [35,37,38]. We failed to show significant changes in MCT1 or MCT4 proteins in fast-glycolytic muscle, although a decrease in these MCT isoforms has been previously reported in rat Pla muscle after altitude acclimatization [14]. Differences between these results and those reported here remain unexplained, but could be related to a sex effect or to differences in duration of altitude exposure. In contrast, the changes in the relative levels of MCT4 mRNA in the Sol muscle paralleled the pattern observed for MCT4 protein. The mechanisms of the transcriptional control of *MCT4* gene during hypoxia exposure predominantly observed in slow-oxidative muscles require additional experiments.

The changes in MCT expression were studied in parallel with those of total LDH and isoform activities. The increase in the H-LDH activity shown in the Pla muscles of hypoxic rats, related to the decrease in energy intake, was not paralleled by significant changes in MCT protein expression. In contrast, the increased M-LDH activity observed in the Sol muscles of hypoxic rats, related to a marked increase in the percentage of M-LDH subunits, was paralleled by an increase in the MCT4 content, suggesting that chronic hypoxia enhanced the glycolytic metabolism, the production of lactate from pyruvate, and the capacity of lactate

extrusion from muscle fibers. However, this adaptive response is tissue-specific, mainly shown in slow-oxidative muscle, and related to the hypoxia-induced decrease in caloric intake.

One additional objective of this study was to examine the functional impact of alterations in MCT expression on the skeletal muscle lactate transport capacity. It has been previously suggested that MCT1, mainly found in oxidative fibers, fulfils the role of a high-affinity/low-capacity transporter in muscle that can be faced with low lactate concentrations, whereas MCT4, mainly found in glycolytic fibers, has been viewed as a low-affinity/high-capacity transporter, effective when lactate concentrations are high [39]. Because no change was shown in the MCT1 protein content of both slow-oxidative and fast-glycolytic muscles, we did not expect major changes in the lactate transport at low external lactate concentration. However, an increased lactate transport was observed in CHH in comparison with C rat skeletal muscles, entirely attributed to the hypoxia-induced decrease in energy intake. These specific effects of caloric restriction are consistent with the results of a recent study [20]. Only an increase in MCT4 protein was shown in the Sol muscle mainly composed of oxidative fibers; because oxidative fibers account only for ~25% of all fibers in total hindlimb muscles, and MCT4 is only slightly expressed in those muscles, it is thus unlikely that the changes in MCT4 protein content in Sol could explain the change in the increased rate of lactate uptake observed in hypoxic animals in comparison with the control ones.

Furthermore, hypoxia per se, as estimated by comparing experimental data between the CHH and PF groups, slightly decreased the lactate transport at a low lactate concentration. We failed to detect any specific effect of hypoxia per se on the decrease in MCT1 and/or MCT4 protein content of either slow-oxidative or fast-glycolytic muscles. Therefore, all these findings on changes in the lactate transport across the sarcolemma could be explained by (*a*) alterations in the content of another MCT isoform in muscle, such as MCT2 [40]; (*b*) changes in the affinity and activity of MCT isoforms for their substrate, as recently shown under conditions of increased effective buffer capacity [41]; and/or (*c*) changes in the subcellular distribution of MCT isoforms with a greater localization in plasma membrane. These hypotheses have not been tested in the present study and need to be examined in further experiments.

The MCT1 protein expression was positively correlated with muscular oxidative capacities, and rat hearts mainly express this MCT isoform [37,38]. No change in the MCT1 content in heart of rats acclimatized to hypoxia has been reported to date [14]. However, RV and LV were not considered separately, and the present study extends this previous report to an examination of the tissue-specific changes in MCT1 protein and its mRNA in LV and RV. Despite a decrease in MCT1 mRNA abundance in the LV of CHH rats, the MCT1 protein content was not altered by hypoxia exposure or caloric restriction, neither in the LV nor in the RV. This decrease in MCT1 mRNA without change in protein levels suggests that, as for skeletal muscles, MCT1 expression in the heart is also predominantly regulated by posttranscriptional mechanisms.

Previous studies failed to detect MCT4 protein in rat hearts [35,38,42]. In accordance with a recent study, we detected MCT4 protein in the heart as a single band of very low density at approximately 43 kDa (Fig. 3A,B) [14]. When higher protein amounts were loaded, we were able to quantify the MCT4 protein content. Using real-time PCR, which is the most sensitive technique for detecting even slight variations in mRNA levels, we were also able to detect MCT4 mRNA in the heart. In comparison with transcript levels in skeletal levels, only a faint MCT4 mRNA signal was detected in both the LV and the RV. MCT4 protein and mRNA levels were not altered after chronic hypoxia and/or caloric restriction, neither in the LV nor in the RV. This lack of change in MCT4 protein or gene expression clearly suggests that the hypoxia-induced pressure overload did not affect MCT4 expression. Because MCT4 relies mainly on glycolytic metabolism, this finding contrasts with the switch in substrate preference from fatty acids to glucose, observed in the heart in response to increased load such as in hypoxia-induced hypertrophied RV [43].

An increase in total LDH and M subunit activity occurred in the LV, whereas an increase in only the LDH-M subunit was detected in the RV. These results are in agreement with previous observations [24-26] and are related to several origins. The hypoxia-induced decrease in energy intake at least partly account for the increased M-LDH activity in the heart; but the increased percentage of the M-LDH subunits in both LV and RV in CHH rats, in comparison with PF animals, suggests also a role of hypoxia per se, consistent with the well-known effects of the oxygen tension in the regulation of the *M-LDH* gene [44]. However, the changes in the isozyme distribution for LDH were similarly observed in the LV and RV, without the specific effect of the hypoxia-induced RV hypertrophy.

In summary, we have shown that prolonged exposure to hypoxia affected the *MCT1* and *MCT4* gene expression in a tissue-specific manner. One of the main results of this study was that the effects of hypobaric hypoxia on the *MCT* gene expression were mainly related to decreased caloric intake. However, no change in MCT proteins was detected in fast-glycolytic muscle, and the decreased lactate transport at low external lactate concentration observed in rats acclimatized to hypoxia in comparison with control animals was not directly related to alterations in MCT1 or MCT4 muscle protein content. Together with the responses of the M-LDH–specific activity, this finding contributes to emphasize the role played by caloric restriction on the control of gene expression in response to chronic hypoxia [16]. The mechanisms involved in the regulation of *MCT* genes are

largely unknown [1], but our results suggest that *MCT1* and *MCT4* gene expression in fast-glycolytic muscles is mainly regulated by posttranscriptional mechanisms. Moreover, the lack of specific changes in the MCT protein content and M-LDH activity in the heart suggests that, in contrast with other components of energy metabolism, the hypoxia-induced RV hypertrophy failed to alter MCT proteins and M-LDH activity.

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