# Effect of Food Restriction on Lactate Sarcolemmal Transport

K. Lambert, G. Py, N. Eydoux, S. Matecki, M. Ramonatxo, C. Préfaut, and J. Mercier

**The objective of this study was to investigate the effects of 6 weeks of food restriction (FR) on sarcolemmal lactate transport in rats. The daily food consumption of rats was monitored for 10 days, after which they were assigned to either a control group (CTL, n = 7) that consumed food ad libitum or an FR group (n = 7) that received a daily ration equal to 60% of their predetermined baseline food intake. After the 6-week period, we observed in red gastrocnemius (RG) a fall of 48% in glycogen content ($P < .01$) and a reduction in glutathione peroxidase activity ($P < .05$), confirming that the FR program was well executed. FR resulted in a reduction in muscle lactate ($P < .05$) and liver glycogen contents ($P < .01$). Moreover, hyperlactatemia was noted in the FR group: 1.77 ± 0.24 versus 2.67 ± 0.29 mmol/L ($P < .05$). Lactate transport capacity was significantly increased ($P < .05$) in FR rats, although monocarboxylate transporter isoforms (MCT1 and MCT4) did not change significantly. We conclude that FR alters sarcolemmal lactate transport activity without affecting MCT1 and MCT4 expression.**


DIETARY OR FOOD RESTRICTION (FR), defined as a reduction in food intake without malnutrition, improves insulin sensitivity. It is therefore usually recommended for obesity, insulin resistance, and type 2 diabetes. Such a regimen also appears to extend maximal life span[1] and different explanations have been proposed to explain this finding. Some have suggested that oxidative stress is limited by reducing calorie intake[2,3] or that calorie restriction alters the characteristics of fuel use and oxidative metabolism.[4] Dean et al[5] showed that dietary restriction may both change glucose homeostasis by lowering basal glycemia and insulinemia and increase insulin-stimulated glucose transport by enhancing the steady-state proportion of GLUT-4 residing on the muscle cell surface.

Lactate and glucose metabolism are closely related, since glycolysis produces 2 molecules of lactate for every glucose or glucosyl unit from glycogen consumed and this lactate can serve as substrate for gluconeogenesis. There is strong evidence for a metabolic interaction between lactate and glucose, because hyperlactatemia impairs muscle glucose uptake.[6] Furthermore, lactate is generally recognized to be the major metabolic intermediary in carbohydrate distribution[7] and an important substrate for several tissues (skeletal muscles,[8] heart, brain, and hepatocytes[9]).

Lactate exchange across the cell membrane occurs via a specific family of transporters called monocarboxylate transporters (MCTs). MCT regulation has been investigated during acute[10] and chronic exercise[11] and it has been found to be different from regulation of the glucose transporters, ie, without translocation. Although the findings concerning transport capacity after an exercise training program remain controversial,[12-14] the reduced muscular activity with hypodynamia,[15] or denervation,[16] which causes a decrease in lactate transport, is widely acknowledged.

*From the Département de Physiologie des Interactions, Institut de Biologie, Boulevard Henri IV, Montpellier, France.*

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*Address reprint requests to Dr K. Lambert, Département de Physiologie des Interactions, Institut de Biologie, Boulevard Henri IV, 34060 Montpellier, France.*



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During physical exercise there is an increase in both contractile activity and energetic substrate exchanges. Few studies, however, have specifically investigated lactate exchanges during metabolic alteration. Metcalfe et al[9] studied lactate entry into hepatocytes after 48 hours of food deprivation and found increased entry despite the fact that no modification in MCT1 or MCT2 expression was found by Jackson et al[17] when the same experiments were conducted. Recently, our group showed an impaired lactate sarcolemmal transport in insulin-resistant and diabetic rats[18,19] suggesting MCT regulation by metabolic alteration.

The aim of this study was to investigate the effect of altered metabolism, not associated with pathology, on sarcolemmal transport activity. We used FR to alter metabolism and then compared sarcolemmal lactate transport activity and MCT1 and MCT4 expression in control rats and 6-week FR rats. In addition, we investigated the effect of 6 weeks of FR on muscle enzyme activities and substrate content, as well as blood lactate concentration.

## MATERIALS AND METHODS

### *Treatment of Animals*

All experiments were performed in accordance with the Helsinki specifications for human treatment of laboratory animals. Male Wistar rats (Janvier, Paris, France), weighing 300 ± 5 g, were housed individually in metabolic cages and kept on a 12-hour light-dark cycle with reverse lighting (lights on at 8 PM) and temperature maintained at 22°C. Daily food consumption (Extralabo M20, Pietrement, France) was monitored for 10 days to establish individual baseline food intake. Food consumption was determined by weighing the food provided and correcting for the amount of food not eaten; this was recorded daily. On day 11, animals were assigned to either a control (CTL, n = 7) or a 6-week FR (n = 7) group. FR rats received a daily quantity of food equal to approximately 60% of their predetermined baseline food intake at 10 AM; CTL rats received food ad libitum. Rats from the 2 groups had free access to water and were weighed every 48 hours.

After the 6-week period and before sacrifice, a tail vein blood sample was withdrawn from CTL and FR rats to determine lactate concentration. Animals were subsequently killed by cervical dislocation at 9 AM. Portions of red gastrocnemius (RG) and liver were quickly removed, frozen in liquid nitrogen, and stored at −80°C until biochemical analysis. The remaining hindlimb muscles were used for sarcolemmal isolation.

### *Reagents*

Reagents with the highest quality available were purchased from Sigma Chemicals (Saint Quentin Fallavier, France), unless otherwise stated.

### *Blood Lactate*

Blood lactate content was analyzed enzymatically according to the method of Gutmann and Wahlefeld.[20] Briefly, a 50-$\mu$L aliquot of blood sample was immediately mixed with 200 $\mu$L of ice-cold 7% perchloric acid and centrifuged at 1,500 $\times$ *g* for 10 minutes at 4°C. An aliquot of the resulting supernatant was mixed with NaOH 2N. The reaction beginning with addition of nicotinamide adenine dinucleotide (NAD) and lactate dehydrogenase (LDH) in glycine/hydrazine buffer. The reaction followed the formation of $NADH_2$ at 340 nm.

### *Muscle Lactate*

Muscle lactate content was measured by the method of Gutmann and Wahlefeld.[20] Briefly, 60 to 80 mg of RG was added to 500 $\mu$L of ice-cold 7% perchloric acid, mixed, and centrifuged at 3,000 $\times$ *g* for 10 minutes at 4°C. A 100-$\mu$L aliquot of supernatant was mixed with NAD (40 mmol/L) in glycine/hydrazine buffer and we measured absorbance (A1) against one blank. Then 10 $\mu$L of LDH was added and the absorbance was measured (A2). The final absorbance was A2 $-$ A1.

### *Glutathione Peroxidase*

Glutathione peroxidase (GPX) enzyme activity was determined in RG using the procedure of Flohé and Günzler[21] with t-butyl-hydroperoxide as a substrate. GPX activity determination was based on a coupled reaction where the glutathione reductase enzyme oxidized the $\beta$-NADPH to NADP. GPX activity is directly related to the decrease in NADPH absorbance at 340 nm and expressed as nmol NADPH oxidized per $min^{-1} \cdot$ mg $protein^{-1}$. Muscle protein concentrations were determined in duplicate by Bradford assay with bovine serum albumin (BSA) as standard (Bio-Rad, Ivry-sur-Seine, France).

### *Citrate Synthase, 6-Phosphofructokinase, and LDH*

Homogenates for citrate synthase (CS) and LDH were prepared in buffer A (210 mmol/L sucrose, 2 mmol/L EGTA, 40 mmol/L NaCl, 30 mmol/L HEPES, 5 mmol/L EDTA, and 2 mmol/L phenylmethylsulfonyl fluoride, pH 7.4) and stored at $-$80°C. CS enzyme activity was assayed in RG according to Srere.[22] Changes in absorbance were recorded over 3 minutes at 412 nm, at 25°C. LDH activity was determined using the method of Wroblewski and La Drue (Sigma Diagnostic, Saint Quentin Fallavier, France) and followed the rate of decrease in NADH at 340 nm, at 25°C. Phosphofructokinase (PFK) activity was determined on fresh RG homogenates according to Opie and Newsholme's method[23] and followed the rate of decrease in NADH at 340 nm, at 25°C.

### *Muscle and Liver Glycogen*

Muscle and liver glycogen contents were measured on portions of RG and liver, using the procedure described by Lo et al.[24] Briefly, liver and muscle were boiled in 30% potassium hydroxide (KOH) saturated with $Na_2SO_4$ for 30 min to become soluble, and glycogen was then precipitated from the solution by addition of a 1.2 volume of 95% ethanol. Samples were centrifuged for 30 minutes at 840 $\times$ *g* and pellets were resuspended in $H_2O$. Assays were conducted on aliquots against appropriate blanks at 490 nm. Results were determined from a standard curve generated at the same time and expressed in mg glycogen $\cdot$ g $tissue^{-1}$.

### *Sarcolemmal Isolation and Characterization*

Sarcolemmal vesicles were purified from the remaining hindlimb muscles with a procedure routinely used in our laboratory.[10,12,15] All subsequent steps were carried out at 4°C. After elimination of fatty, nervous, and connective tissues, muscles (usually 15 to 20 g) were homogenized in ice-cold 250 mmol/L sucrose, 1 mmol/L EDTA, and 20 mmol/L HEPES at pH 7.4 with 2 bursts (2 $\times$ 5 seconds) of an Ultra-Turrax T25 (Labo Services, Montpellier, France) at 80% of maximal power. The homogenate was centrifuged twice at 900 $\times$ *g*. Supernatants were filtered and a 1-mL aliquot was partitioned and saved at 4°C for subsequent analysis (termed CH). The remaining crude homogenate was diluted with a volume of KCl medium (3 mol/L KCl, 250 mmol/L sodium pyrophosphate, pH 7.4) equal to 10% of the CH volume and pelleted by ultracentrifugation at 200,000 $\times$ *g* for 45 minutes at 4°C. Pellets were resuspended using Teflon pestle homogenization in 30 mL of sucrose medium. The suspension was centrifuged twice at 280 $\times$ *g* and supernatants were then collected and centrifuged at 200,000 $\times$ *g* for 45 minutes at 4°C. Pellets were homogenized using a glass tissue homogenizer in 7 to 8 ml of 40% (wt/vol) sucrose. A discontinuous density gradient was constructed by addition of 8 mL of each of the following sucrose solutions: 38%, 32%, 27%, and 12% in layers. After an overnight centrifugation at 130,000 $\times$ *g* at 4°C, the 27% sucrose band (corresponding to sarcolemmal vesicles and termed F2) was harvested and diluted with a Krebs-Ringer-HEPES (KRH) buffer (118 mmol/L NaCl, 5 mmol/L KCl, 1.2 mmol/L $MgSO_4$, 50 mmol/L HEPES, pH 7.5) and washed free of sucrose at 200,000 $\times$ *g* for 80 minutes. The vesicles were resuspended in KRH buffer up to 2 mg $\cdot$ $mL^{-1}$ and stored at $-$80°C until used for the transport experiments. Proteins were determined according to the procedure of Bradford using bovine $\gamma$-globulin as a standard.

Sarcolemmal characterization was achieved with $K^+$-stimulated p-nitrophenylphosphatase ($K^+$-pNPPase) assay as described previously.[10,12,15] The 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 (PI) was defined as the ratio of the specific activity from the $F_2$ fraction to the specific activity measured in CH. Skeletal muscle sarcolemmal yield was the ratio of milligrams of sarcolemmal protein obtained in $F_2$ to the muscle weight in grams (wet weight).

### *Lactate Transport Studies*

All measurements were performed in duplicate in zero-*trans* conditions. (U-$^{14}$C)-L-lactate (specific activity, 155 mCi/$mmol^{-1}$; Amersham, Les Ulis, France) 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 $\mu$g of sarcolemmal vesicles in tracer-containing medium and stopped at appropriate time intervals by vacuum filtration on nitrocellulose filters (with an average pore size of 0.45 $\mu$m; Whatman WCN, 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, and dissolved with ethyleneglycolmonomethylether; the radioactivity was then determinated by liquid scintillation counting (2200 Packard, Packard Instruments, Rungis, France). 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 $\cdot$ mg $protein^{-1}$. Measurements of initial lactate uptake were done for 1, 10, 30, and 50 mmol/L external lactate

concentrations at 0 and 10 seconds. Slopes gave initial rates of lactate uptake expressed in nmol of lactate · $min^{-1}$ · mg $protein^{-1}$.

### *Sample Preparation for Western Blotting*

Proteins were isolated from muscles for Western blotting by a method previously described by McCullagh et al[25] and previously used in the laboratory.[12,18] Muscle protein concentrations were determined in duplicate by the bicinchoninic acid assay (Pierce, Interchim, Montluçon, France) with the use of BSA as a standard.

### *Western Blotting of MCT1 and MCT4*

Affinity-purified polyclonal antibodies directed against the carboxy terminus of rat MCT1 were produced by immunization of New Zealand white rabbits with the synthetic peptide PLQNSSGDPAEEESPV for MCT1 and LREVEHFLKAEPEKNG for MCT4[13] (a generous gift from Dr G.A. Brooks, Department of Integrative Biology, University of California, Berkeley, CA). Polyclonal antibodies yielded a single band on a Western blot that corresponded to 43 kd, consistent with the molecular mass reported earlier.[25] Antibody specificities were confirmed in preliminary experiments in which the peptides blocked the detection of MCT1 and MCT4. Samples of muscles homogenates (30 $\mu$g protein) and prestained molecular mass markers (Bio-Rad) were separated on 12% sodium dodecyl sulfate –polyacrylamide gels (200V for ~60 minutes) with the Novex system (Invitrogen, Groningen, The Netherlands). Proteins were then transferred from the gels to polyvinylidene difluoride (PVDF) membranes (30 V, 60 minutes), and the 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 mol/L HCl, adjusted to pH 7.5, 0.1% [vol/vol] Tween 20, and 5% [wt/vol] nonfat dried milk). The membranes were then incubated with diluted carboxy-terminal of either MCT1 antibody (1:2,500) or MCT4 antibody (1: 2,500) in buffer D for 1.5 hours, followed by 3 washes in buffer E (ie, buffer D without dried milk: 3 × 5 minutes washes), and then incubation for 45 minutes with goat anti-rabbit immunoglobulin G horseradish peroxidase–conjugated secondary antibody (1:2,500, BI 2407, BioSys, Compiègne, France) in buffer E. Membranes were washed as previously described and MCT1 or MCT4 expression was detected by ECL (Biomax MR films, Kodak, Reuil-Malmaison, France). Films were developed and fixed using a Hyperprocessor, RNP 1700 (Amersham, Les Ulis, France). MCT1 and MCT4 protein band densities were determined by scanning the blots on a scanner (AGFA Duo Scan T1200, New York, NY) and Scion Image software (Scion Corp, Frederick, MD). Results were expressed in arbitrary OD units as used by others.[18]

### *Statistical Analysis*

Results are expressed as means ± SE. Statistical significance was assessed by the Student's *t* test to determine the effect of FR on the variables studied. To compare initial rates of lactate uptake between the FR and CTL groups at the different external concentrations, the Mann-Whitney test was used. Throughout the study, a probability level of $P < .05$ was used.

## RESULTS

Body weights were significantly different between the CTL and FR groups (Fig 1). The body weight of the FR rats was decreased by 41% after the 6-week period of restricted diet ($P < .05$).

Blood lactate was higher in FR rats than in CTL ($P < .05$). In contrast, RG muscle lactate content was significantly decreased after the regimen ($P < .05$) (Table 1). RG glycogen concentration was 48% lower in FR rats compared with CTL (Table 1). In liver, glycogen content was reduced by 60% in FR group (Table 1).

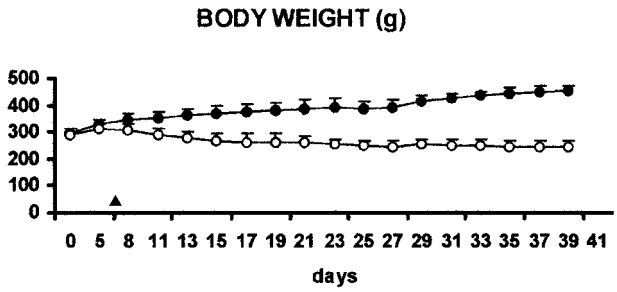


**Fig 1. Effect of 6 weeks of FR (○) or ad libitum (CTL, ●) food intake on total body weight in rats. Results are expressed as means ± SE. (▲) Beginning of food restriction.**

CS activity was lower ($P < 0.01$) in RG muscle from FR rats, whereas 6-PFK activity was unaffected after the 6-week diet (Table 2). Moreover, LDH activity was significantly higher ($P < .05$) in the FR rats (Table 2) in contrast with muscle GPX activity, which was lower ($P < .05$) after the FR program (Table 2). Table 3 shows that vesicles purified from the FR and CTL groups had similar protein yields and purification indices of $K^+$-pNPPase. Initial rates of lactate uptake at 1 mmol/L ($P < .05$) external lactate concentration were significantly higher in the FR group (Fig 2). No significant differences were observed for others external lactate concentrations.

FR did not affect MCT protein, whatever the isoform.

## DISCUSSION

This study shows that a 6-week program of FR decreased muscle lactate and glycogen content and increased blood lactate concentration and lactate sarcolemmal transport capacity, without changes in MCT1 and MCT4 expression.

Several investigators have shown the ability of FR to modulate oxidative stress. To verify our model, we measured GPX activity in skeletal muscle. In accordance with the results of Luhtala et al,[26] we found a reduction in GPX activity in RG, which suggests a lower level of oxidative stress. This result, associated with the decreased glycogen content in RG, indicates a well-executed FR program. Using the classical procedure for purification of sarcolemmal vesicles performed in our laboratory, we obtained similar characteristics in the 2 groups (FR and CTL) in terms of sarcolemmal protein yields and $K^+$-pNPPase purification indices. With this methodology, we previously demonstrated that the isolated vesicles are sensitive to alpha-cyano-4-hydroxycinnamate (specific inhibitor) and exhibit detectable lactate transport. Moreover, although several investigators have hypothesized that an alteration in lactate transport capacity may be due to free radicals,[27] we found that muscle GPX activity was significantly reduced in our FR group. Thus, we concluded that the increase in lactate transport activity observed in this study was due to a specific muscle adaptation to FR and not the consequence of sarcolemmal alteration by free radicals.

Concerning the metabolic adaptations, as expected, we found a decrease in glycogen content in the RG of FR rats. This is

**Table 1. Lactate in Blood and Muscle and Glycogen Content in Muscle and Liver in Rats After 6 Weeks of Consuming Food Ad Libitum (CTL) or 40% Food Restricted (FR)**

| | Lactate | | Glycogen | |
|---|---|---|---|---|
| | Blood (mmol/L) | Muscle ($\mu$mol/g wet muscle) | Muscle (mg/g muscle) | Liver (mg/g liver) |
| FR | 2.67 ± 0.2* | 8.48 ± 0.44* | 1.80 ± 0.18† | 13.8 ± 4.1† |
| CTL | 1.77 ± 0.24 | 10.31 ± 0.18 | 3.45 ± 0.47 | 34.1 ± 5.1 |

NOTE. Values are mean ± SE, n = 7.
*$P < .05$, †$P < .01$ different from CTL.

consistent with the finding of the study of Cartee et al.[28,29] It seems that glycogen reduction may be linked with the duration of FR since other investigators have found that muscle glycogen content was preserved in a trial of 40% caloric restriction over 20 days[5] or longer.[30] This reduced glucose storage could be associated with lower glycemia and insulinemia, which are common features of such a diet[5,29]; however, they were not measured in this study. These results concerning glucose availability point out the need of extrahepatic substrate for gluconeogenesis.

The lower RG lactate content in FR rats may be the consequence of impaired glucose storage or higher utilization, which is consistent with the lower glycogen content. The PFK activity in our study was not affected by FR, but the literature are inconsistent in this regard. For example, Ballor et al[31] reported that PFK activity in rat muscle and heart is differentially affected by dietary restriction. On the basis of CS and LDH activity measurements, it appears that FR may induce a shift from oxidative metabolism towards glycolytic metabolism; our decrease in CS activity is consistent with the study of Feuers et al,[32] who observed a decrease in mitochondrial electron transport system.

Lactate plays a central role in the distribution of potential energy from carbohydrate among tissues[7,33-35] and may be considered as a major respiratory fuel under some conditions. Thus, the increased activity of sarcolemmal lactate transport observed in FR rats may be an adaptive response to ensure the availability of another substrate.

In this context, MCTs have important implications for substrate distribution since several isoforms have been found and they are ubiquitously distributed among many tissues. Nine MCT-related sequences have been identified and seven have been cloned.[36] In skeletal muscle, 2 isoforms, MCT1 and MCT4, coexist and have a fiber type-specific distribution,[37,38] suggesting a physiological significance to either their properties or their regulation.[39] MCT1, which is present in almost all tissues, is correlated with mitochondrial content and may reflect

**Table 2. Effects of 6 Weeks of FR in Activities of GPX, LDH, CS, and PFK in RG From Control and FR rats**

| | GPX ($nmol \cdot min^{-1} \cdot mg\ protein^{-1}$) | LDH ($\mu mol \cdot min^{-1} \cdot mg\ protein^{-1}$) | CS | PFK |
|---|---|---|---|---|
| FR | 21.40 ± 3.41* | 3,771 ± 352* | 4.66 ± 0.39† | 22.5 ± 3.53 |
| CTL | 31.76 ± 3.47 | 2,104 ± 100 | 7.32 ± 0.41 | 26.4 ± 2.01 |

NOTE. Values are mean ± SE, n = 7.
*$P < .05$, †$P < .01$ different from CTL.

**Table 3. Biochemical Characteristics of the Sarcolemmal Vesicle Preparations from Control and FR Muscles in Rats**

| | SL Protein Yield (mg/g tissue) | PI $K^+$ -pNPPase |
|---|---|---|
| FR | 0.072 ± 0.008 | 17.8 ± 1.86 |
| CTL | 0.091 ± 0.015 | 15.9 ± 1.37 |

NOTE. Values are mean ± SE, n = 7. SL yield was the ratio of milligrams of sarcolemmal protein obtained in vesicles fraction after the last ultracentrifugation to the muscle weight in grams of hindlimb rat. PI $K^+$-pNPPase is the purification index of the $K^+$-stimulated *para*-nitrophenylphosphatase defined as the ratio of the specific activity from the vesicle fraction to the specific activity measured in crude homogenate.

the ability to use lactic acid for respiratory fuel in slow oxidative fibers.[25] In contrast, MCT4 seems more important for lactate efflux from muscles that rely more on glycolytic metabolism.[37] Recently, Fishbein et al reported a third isoform of unknown significance in human skeletal muscle: MCT2.[40] MCT2 expression was limited in type I fiber and showed great variability, depending on biopsy site. Our model, using all hindlimb muscles, did not allow us to separate the different isoforms so the measured lactate transport capacity reflected the activity of all MCT isoforms of sarcolemmal membrane. However, the major characteristic of this model is that sarcolemmal vesicles are made from a greater proportion of white glycolytic muscles than red oxidative ones. The greater lactate sarcolemmal transport observed in the FR rats is thus probably related to the MCT4 isoform. Because we did not observed any increase in MCT4 with Western blot, we hypothesize that the change in lactate transport resulted from the increased affinity of lactate transporter for substrate rather than an overexpression of this isoform. The discovery of MCT2 in humans, however, suggests such an isoform in rat muscle and the increase in muscle lactate transport observed in our study may therefore be due to its increased activity and/or expression. Since MCT2 is expressed in type I fibers, this would explain the lack of MCT1 and MCT4 changes in RG, which are composed of a great proportion of type I fibers. We also cannot exclude the possibility that the increase was due to modifications in glycosylation or phosphorylation sites, but this hypothesis is unlikely since the sequences for potential glycosylation sites are in loop

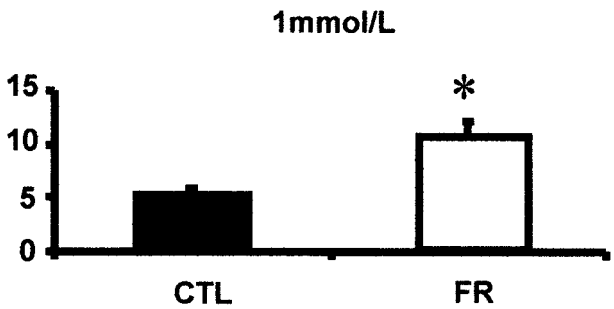


**Fig 2. Lactate uptake in sarcolemmal vesicles purified from hindlimb muscle rat after 6 weeks of consuming food ad libitum (CTL) or 40% FR. Values are means ± SE, n = 4-5 different membrane preparations. *Significantly different from CTL, $P < .05$.**

regions too small for N-linked glycolsylation.[36] Moreover, Carpenter et al demonstrated that glycosylation is not required for MCT1 function.[41] The increase in lactate transport activity probably resulted in greater lactate extrusion from muscle and may have contributed to the higher level of blood lactate. The hyperlactatemia under the FR condition may have come from adipocytes[42] or it may have been caused by impaired removal or clearance by the liver. But our results taken together with those of Dhabbi et al,[4] who demonstrated that a 50% caloric restriction resulted in increased gluconeogenesis enzyme activities in liver, suggest enhanced gluconeogenesis from lactate. However, this needs further investigation since we did not measure enzyme capacities for gluconeogenesis in this study.

In conclusion, 6 weeks of FR alters sarcolemmal lactate transport activity without any change in MCT1 and MCT4 expression. Furthermore, this change is associated with alteration in substrate concentration in blood and muscle as well as muscle enzyme activities, which suggests an enhancement of gluconeogenesis from lactate. Further investigations of lactate uptake by liver in relation to lactate release by muscle during food restriction are needed.

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