Impaired sarcolemmal vesicle lactate uptake and skeletal muscle MCT1 and MCT4 expression in obese Zucker rats

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Py, Guillaume, Karen Lambert, Antonia Perez-Martín, Eric Raynaud, Christian Préfaut, and Jacques Mercier. Impaired sarcolemmal vesicle lactate uptake and skeletal muscle MCT1 and MCT4 expression in obese Zucker rats. Am J Physiol Endocrinol Metab 281: E1308–E1315, 2001.—The present experiments were undertaken to characterize 1) the hindlimb muscle mass lactate uptake and expression of monocarboxylate transporter isozymes in obese and MCT1 and MCT4, as well as lactate dehydrogenase (LDH) isozyme distribution, in various skeletal muscles of Zucker fa/fa rats taken as a model of insulin resistance-related obesity. Initial lactate uptake at six different concentrations was measured in sarcolemmal vesicles (SV) by use of L-(1-[14]C) lactate. Compared with controls, the maximal rate of lactate uptake and affinity were decreased in SV of Zucker rats (−30%) in which MCT4 content was significantly decreased (P < 0.05). MCT4 expression was decreased in soleus, extensor digitorum longus, and red tibialis anterior (RTA; P < 0.05), but not in white tibialis anterior, whereas MCT1 expression was decreased only in RTA of Zucker rats (P < 0.05). Obesity led to a shift toward type M-LDH isozyme in mixed muscles. We conclude that obesity leads to changes in muscular MCT1 and MCT4 expression, which, when associated with LDH isozyme redistribution, may contribute to the hyperlactatemia noted in insulin resistance.

LACTATE PRODUCTION is a metabolic event that is receiving increasing attention, because peripheral conversion of glucose into lactate plays a significant role in the synthesis of liver glycogen via the gluconeogenic pathway after glucose ingestion (27). Lately, it has become evident that adipose tissue is an important source of lactate production in vivo (19) and that this process is enhanced by both insulin and catecholamine stimulation (20). Interestingly, a significant inverse correlation between overnight fasting lactate level and insulin sensitivity has been found in obese subjects (26), and this correlation is stronger than the relationship between lactate levels and body mass index in this population. Moreover, it has been observed that patients with type 2 diabetes have even greater elevations in basal lactate levels than do obese subjects (34). This association between elevated basal lactate levels, insulin resistance, and diabetes is supported by epidemiological studies in healthy subjects in whom an elevated fasting lactate level was found to be a significant and independent risk factor for the development of type 2 diabetes (32).

Quantitatively, skeletal muscle mass appears to be by far the most important insulin-sensitive tissue, indeed, far more important than liver and adipose tissue. On the other hand, this insulin-sensitive skeletal muscle mass can both produce and utilize lactate as a fuel for mitochondrial oxidation relative to fiber type, and it presents a net lactate release in the resting condition in normal subjects (8). In this respect, it is tempting to implicate skeletal muscle mass in the mechanisms leading to basal hyperlactatemia through an increased lactate production/interconversion rate with pyruvate, as previously described (1), impaired fiber type-dependent lactate uptake and oxidation, or both mechanisms.

In the past few years, it has been acknowledged that skeletal muscles and most other tissues have a membrane transport system mediating a coupled lactate and proton translocation (33). Moreover, in muscle, several lactate-proton monocarboxylate transporter isoforms (MCTs) are coexpressed, two of which have already been cloned: MCT1 and MCT4 (33). Whether an impaired lactate exchange across key plasma membrane organs is involved in obesity and the insulin-resistant state and whether this contributes to reduced lactate clearance have not yet been evaluated. Moreover, a potential redistribution in lactate dehydrogenase (LDH) isozymes that could participate in the previously described increased lactate/pyruvate interconversion rate with pyruvate has not yet been investigated. Therefore, this study aimed to 1) characterize the hindlimb muscle mass lactate uptake by use of the model of sarcolemmal vesicles coupled with the expression of

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MCT1 and MCT4 and 2) determine LDH distribution in various skeletal muscles of insulin-resistant obese Zucker fa/fa rats.

**MATERIALS AND METHODS**

**Reagents.** Reagents were purchased with the highest quality available from Sigma Chemical (L’Isle d’Abeau Chesnes, St Quentin Fallavier, France) unless otherwise stated.

**Animals.** Animal experimentation was performed according to the Helsinki convention for animal care and use. Male Zucker fa/fa and Wistar rats, 11 wk old, were purchased from IFFA CREDO (Charles River, L’Abresle, France). They were kept on a reverse 12:12-h light-dark cycle (lights on at 7 PM) at 22°C and housed in individual cages. Standard rat chow and water were provided ad libitum.

Two weeks after their arrival in the laboratory (13 wk old), blood samples for determination of plasma glucose, lactate, and insulin were collected in the nonfasting state from rats of each group before euthanasia. One Zucker rat and one control rat were then killed on the same day by cervical dislocation for tissue preparation.

**Tissue preparation.** After cervical dislocation, hindlimb muscles were rapidly removed. Portions of red and white tibialis anterior (RTA and WTA, respectively), extensor digitorum longus (EDL), and soleus (SOL) were quickly frozen in liquid nitrogen and stored at −80°C until biochemical assays were performed. The remaining hindlimb muscles were used for sarcolemmal isolation.

**Sarcolemmal vesicle isolation and characterization.** Sarcolemmal vesicles (SV) were purified from hindlimb muscles with a procedure already described (10, 11, 14). Proteins were determined according to the procedure of Bradford with bovine γ-globulin as a standard. SV characterization was achieved with K+-stimulated p-nitrophosphophatase (K+-pNPPase) assay, as previously described (10, 11). The purification index (PI) was defined as the ratio of the specific activity from the sarcolemmal fraction (SF) to the specific activity measured in the crude homogenate (CH). Skeletal muscle SV yield was the ratio of sarcolemmal proteins (mg) obtained in SF to the muscle weight in grams after trimming (wet weight).

**Lactate transport studies.** All measurements were performed in zero-trans conditions and in duplicate. L-[1-14C]lactate (sodium salt) was purchased from Amersham (specific activity 155 mCi/mmol) and was diluted in 280 mM L-lactate concentrations (150, 200, 250, 300, 350 mM in 50 mM Tris, pH 7.4). Intravesicular medium consisted of 250 mM sucrose and 50 mM Tris, pH 7.4. Equilibrium L-lactate uptake was determined after 3-min incubations, and results were expressed in nanomoles per milligram protein per 3 min.

**Sample preparation for Western blotting.** Proteins were isolated from muscles for Western blotting, as previously described by McCullagh et al. (29) and previously used in this laboratory (13, 14). Muscle protein concentrations were determined in triplicate by the bicinchoninic acid assay (Pierce, Interchim, Montluçon, France) with bovine serum albumin 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 PLOQNSSGD-PAEESPVP for MCT1 and LREVHFLKAEPEKNG for MCT4 (9). Polyclonal antibodies yielded a single band on a Western blot that corresponded to 43 kDa, consistent with the molecular mass reported earlier (23, 29, 37). Antibody specificities were confirmed in preliminary experiments in which the peptides blocked the detection of MCT1 and MCT4. Protein (20 µg) of muscle homogenates and prestained molecular mass markers (Bio-Rad, Ivry-sur-Seine, France) were separated on 12% SDS-polyacrylamide gels (140 V for ~90 min). Proteins were then transferred from the gels to polyvinylidene difluoride membranes (100 V, 90 min). Membranes were incubated at a shaker overnight at room temperature in buffer D (20 mM Tris base, 137 mM NaCl, 0.1 M HCl, adjusted to pH 7.5, 0.1% (vol/vol) Tween 20, and 5% (wt/vol) nonfat dried milk). Membranes were then incubated with diluted carboxy terminus of either MCT1 antibody (13,000) or MCT4 antibody (13,000) in buffer B for 1 h 30 min, followed by three washes in buffer E (i.e., buffer D without dried milk: 3 × 5-min washes), followed by incubation for 45 min with goat anti-rabbit IgG horseradish peroxidase-conjugated secondary antibody (1:3,000; BI 2407, BioSys, Compiègne, France) in buffer E. Membranes were washed as previously described, and MCT1 or MCT4 expression was detected by enhanced chemiluminescence (Biomax MR films, Kodak). 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) and with Scion Image software. For MCT1, the signal of control heart was determined in triplicate by the bicinchoninic acid assay (Pierce, Interchim, Montluçon, France) and used as a positive control and to fix an arbitrary unit to allow comparison between experiments (100% equals the MCT1 signal of 20 µg of control heart homogenate).
RESULTS

Basal blood glucose, lactate, and insulin concentrations. Basal blood glucose, lactate, and insulin concentration values are presented in Table 1. Obese Zucker rats were characterized by increased basal plasma insulin and lactate concentrations, whereas plasma glucose remained unchanged. Plasma lactate concentration increased by 48%, i.e., up to 2.22 mmol/l (P < 0.05). Moreover, insulin concentration was increased by 10.22 ± 0.33 ng/dl (P < 0.05).

Characterization of SV. Using gradient density fractionation, we obtained SV preparations with similar biochemical characteristics in both groups. A slight but significant increase in the SF enrichment in the Zucker group was observed (0.16 ± 0.02 vs. 0.11 ± 0.01 mg/g protein, P < 0.05). However, there was no significant difference in the purification indexes of K+-pNPPase between the control and Zucker groups (Table 2).

Lactate transport kinetics. Figure 1 shows the lactate uptake kinetics in vesicles from the control and Zucker fa/fa groups plotted as a function of external lactate concentration. SV from obese rats showed a general decrease in total lactate influx rates compared with control rats. Initial rates displayed saturation kinetics with Vmax values of 340 and 259 nmol·min⁻¹·mg protein⁻¹ and Km values of 30 and 41 mM for control and Zucker groups, respectively. Thus the maximal rate of lactate influx into SV from the Zucker rats was decreased by nearly 34%, whereas the affinity for the substrate decreased by 37%.

Effect of osmotic forces. Figure 2 shows the effect of osmolarity on 1 mM L-(−)-lactate uptake at equilibrium in SV from the control and Zucker groups. A two-way ANOVA revealed that decreasing the intravesicular space by higher external sucrose concentration resulted in less L-(−)-lactate accumulation in vesicles from the two groups at pH 7.4 (P < 0.05). Moreover, lactate uptake at equilibrium was significantly higher in the control than in the Zucker group (P < 0.05). Thus, whereas osmotic pressure exerted the same ef-

Table 2. Characterization of sarcolemmal vesicles

<table>
<thead>
<tr>
<th>Group</th>
<th>CH Proteins, mg/g</th>
<th>SF Proteins, mg/g</th>
<th>PI K+-pNPPase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>99.36 ± 3.75</td>
<td>0.11 ± 0.01</td>
<td>23.1 ± 1.4</td>
</tr>
<tr>
<td>Zucker</td>
<td>104.7 ± 7.91</td>
<td>0.16 ± 0.02a</td>
<td>27.3 ± 2.3</td>
</tr>
</tbody>
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Values are means ± SE. CH, crude homogenate; SF, sarcolemmal fraction; PI K+-pNPPase, purification index of sarcolemmal enzyme K+-p-nitrophenylphosphatase. *Significantly different from control values, P < 0.05.

Table 1. Basal plasma glucose, insulin, and lactate concentrations in control and obese Zucker fa/fa rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma Glucose, mmol/l</th>
<th>Plasma Insulin, ng/dl</th>
<th>Plasma Lactate, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.04 ± 0.27</td>
<td>2.06 ± 0.07</td>
<td>1.5 ± 0.09</td>
</tr>
<tr>
<td>Zucker fa/fa</td>
<td>7.282 ± 0.14</td>
<td>10.48 ± 1.08*</td>
<td>2.22 ± 0.08*</td>
</tr>
</tbody>
</table>

Plasma glucose levels were determined using the glucose oxidase assay, and insulin concentrations were determined using a radioimmunoassay kit. Plasma lactate levels were determined using the method of Gutmann and Wahlefeld (18). Results are means ± SE. *P < 0.01, Zucker fa/fa vs. control.

Fig. 1. Kinetics of initial lactate uptake rate (lactate uptake10s) into vesicles from control and Zucker groups. Initial rate of lactate influx from experiments with external lactate only (zero-trans condition) is depicted in curves that are the best Michaelis-Menten fit to the data. Values are means ± SE; n = 7 different membrane preparations. All assays were performed in duplicate at pH 7.4.
ffect on the two groups, a more pronounced decrease in lactate transport in SV from Zucker rats was observed when compared with SV from control rats.

**MCT1 and MCT4 expression in skeletal muscles.** In control and Zucker rats, MCT1 was detected in the four skeletal muscles studied (Fig. 3A). Densitometry revealed the same MCT1 concentration profile in both groups: SOL contained the highest level of MCT1, followed by RTA, EDL, and WTA muscles (Fig. 3B). Relative to control heart as the positive standard, there was a trend toward a reduced MCT1 expression in SOL, EDL, and WTA muscles of the Zucker group (45.4, 11.6, and 3.9% for SOL, EDL, and WTA, respectively, in the control group and 35.5, 10.7, and 2.3%, respectively, in the Zucker group). However, a significant decrease was observed only in RTA of the Zucker rats (17 vs. 32.1%, P < 0.05; Fig. 3B).

In the control group, densitometry of MCT4 protein revealed a different expression profile than that of MCT1. MCT4 expression level was quite similar for all muscles except SOL, which showed the lowest expression level. The MCT4 band was absent in heart preparations (data not shown). Compared with the control group, the expression level of MCT4 was decreased in SOL (218%), RTA (237%), and EDL (230%), but not in WTA (Fig. 4) of Zucker rats.

**MCT1 and MCT4 expression in SV.** When possible, we performed immunoblots of SV preparations from both groups. Figure 5 shows that the number of MCT4 transporters present in the SV of Zucker rats was decreased compared with controls, whereas only a trend toward the decreased MCT1 transporter was noted.

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**Fig. 2.** Effect of medium osmolarity on L(1+)-lactate uptake into vesicles from control (■) and Zucker (●) rats. Assays were performed in duplicate at pH 7.4 with external sucrose concentrations adjusted to give the indicated osmolarities. Values are means ± SE on 4 different membrane preparations.

**Fig. 3.** Monocarboxylate transporter 1 (MCT1) expression in soleus (SOL), extensor digitorum longus (EDL), red tibialis anterior (RTA), and white tibialis anterior (WTA) expressed as a percentage of MCT1 in control heart (set to 100% in each Western blot). A: representative Western blot showing expression of MCT1 in total muscle homogenates in SOL, EDL, RTA, and WTA of control (Ctl) and Zucker (ZK) rats. *P < 0.05, significantly different from control group. Values are means ± SE; n = 7 rats in each group. Results are expressed as a percentage of MCT1 content in heart control (100% = heart MCT1 signal in each Western blot).

**Fig. 4.** MCT4 expression in SOL, EDL, RTA, and WTA. A: representative Western blot showing the expression of MCT4 in total muscle homogenates in SOL, EDL, RTA, and WTA of control and Zucker rats. *P < 0.05, significantly different from control group. Values are means ± SE; n = 7 rats in each group. Results are expressed in arbitrary units.
LDH isozyme distribution. The LDH isozyme distribution in skeletal muscles is represented in Table 3. All LDH isozymes (LDH-1 to LDH-5) were detected in SOL, EDL, and RTA muscles, whereas only LDH-3, -4, and -5 were detected in WTA of the control and Zucker rats. The profile of the relative percentage of each isoform in SOL, with LDH-1 and LDH-2 predominant, differed from that of the EDL, RTA, and WTA muscles, in which LDH-5 was present in the highest amount. Obesity led to changes in the relative percentage of isozyme distribution in the Zucker rats, with differences between muscles relative to their oxidative and glycolytic capacities. A 14% decrease was noted in the LDH-2 isozyme in SOL, although this was not significant. No change was found in the more glycolytic WTA muscle. For EDL and RTA muscles we observed similar changes, with the LDH-1 decrease more pronounced in RTA (70%, \( P < 0.05 \)) than in EDL (53%, \( P < 0.05 \)). LDH-5 was also decreased by 18 and 15% (\( P < 0.05 \)) in RTA and EDL, respectively. Moreover, there was a 61% increase in the intermediate isoforms LDH-3 and LDH-4 for both muscles and a 35 and 51% increase for LDH-4 in RTA and EDL, respectively.

Relationship between MCT1 and LDH isozyme distribution. Using Pearson product-moment correlations, a positive relationship in the two groups appeared between their MCT1 and LDH-3 isozyme content (\( r = 0.95 \) and 0.98 for control and Zucker groups, respectively, \( P < 0.05 \); Fig. 6) and between MCT1 and the LDH-2 isozyme (\( r = 0.96, P < 0.05 \)) in the Zucker group. Surprisingly, we were not able to find the same positive correlations between MCT1 content and heart-type isozymes (LDH-1 and -2) as previously described in the control rats (29). Nevertheless, a strong correlation appeared between LDH-1 and -2 in both groups (\( r = 0.99 \) and 0.98 for the control and Zucker groups, respectively, \( P < 0.05 \); Fig. 6). Moreover, comparison between MCT1 and the LDH-5 isozyme resulted in a strong negative relationship only in the Zucker group (\( r = -0.99, P < 0.05 \); Fig. 6).

**DISCUSSION**

We attempted to characterize hindlimb muscle mass lactate uptake and MCT1-MCT4 expression, as well as LDH isozyme distribution, in various skeletal muscles of Zucker rats to specify the possible contribution of skeletal muscles in the basal hyperlactatemia present in this model of insulin resistance. The main results we observed in Zucker rats compared with controls are 1) a decrease in the maximal rate of lactate uptake in SV, 2) a fiber type-dependent decrease in MCT1 and MCT4 expression, and 3) an effect of obesity on the redistribution of LDH isoforms with a shift toward more type M-LDH.

Blood lactate concentration increases in many physiological and pathological conditions, such as physical exercise, fasting, type 2 diabetes, obesity, and hypertension (6). When released in the circulation, lactate may be taken up by different organs, but skeletal muscle, heart, and liver appear to be major sites of lactate removal. In resting conditions, muscle lactate utilization appears to be largely dependent on oxidation (17). Insulin resistance-related obesity in Zucker rats, like that in humans, seems to be characterized by
increased resting blood lactate concentration that may be the result of both impaired lactate metabolism and impaired exchanges in skeletal muscle.

We investigated lactate transport into SV from control and Zucker rats and found a decreased total influx rate at all external lactate concentrations in the Zucker group. The decreased maximal rates of lactate uptake (34%) found in the SV of these rats may represent a decreased number of transporters present in the SF. The properties of lactate transport in our SV were similar to the standard results in isolated membranes (24, 31, 35). We previously reported that our system exhibits detectable lactate transport properties, saturation with increasing external lactate concentration, sensitivity to pH and α-cyano-4-hydroxycinnamate (a monocarboxylate transport inhibitor), and some transstimulatory properties at high external L-(+)-lactate concentration (10, 14). Despite low sarcolemmal recovery from skeletal muscle, isolated SV constitute a nonmetabolic system that allows detailed studies of membrane properties. In the present study, the SV of the two groups appeared to be sealed, because they showed identical sensitivity to changes in osmotic conditions (Fig. 2). In equilibrium conditions, SV from the Zucker group also showed reduced lactate accumulation. This result could indicate that SV from the Zucker group were smaller than those of the control group. It has been reported that when measurements are made in initial rate conditions, the uptake is dependent only on the transporter activity. This is unlike equilibrium conditions, where the amount of accumulated substrate in SV is dependent on both transporter activity and vesicle volume (22). These findings suggest that obesity and/or insulin resistance could either influence the sarcolemmal isolation procedure or induce membrane alterations that might perturb lactate transport properties, resulting in decreased lactate accumulation. Because the protein yields, although slightly increased in the Zucker group, and the purification indexes were quite similar in both groups, it is unlikely that obesity and/or insulin resistance could have affected the vesicle volume and purification of SV.

Recently, it was established that skeletal muscles and most other tissues possess a membrane transport system mediating a coupled lactate and proton translocation (33). MCT1 was the first rat and human skeletal muscle isoform to be cloned, by Garcia et al. (15). More recently, Wilson et al. (37) cloned MCT4 (formerly MCT3), which showed a strong expression in rat and human skeletal muscle. MCT4 content appears to be similar in the various skeletal muscles except for a markedly lower content in SOL, suggesting that only the fast oxidative glycolytic (FOG) fibers in SOL possess this isoform (37). In the present study, the Zucker rats had a more pronounced decrease in the expression of MCT4 than of the MCT1 isoform, relative to the fiber type composition of muscle. MCT1 expression was decreased in RTA muscle, which is composed primarily of type IIA (FOG) fibers, although only a trend toward decreased expression was noted in both slow-twitch and fast-twitch muscles; MCT4 expression was decreased in all muscles except the fast-twitch WTA muscle. These results may explain why the MCT4, but not the MCT1, content in SV prepared from the Zucker rat hindlimb muscles appeared to be decreased (Fig. 5). Furthermore, when SV are prepared, the rat hindlimb muscles that are pooled consist largely of type II fibers that contain predominantly MCT4 (5). Thus MCT4 was expected to be quantitatively the more represented isoform in the SV of both groups. Therefore, with both decreased MCT1 expression in FOG fibers and essentially decreased MCT4 expression in a wider range of muscle fiber types, the SV of Zucker rats were expected...
to have a decreased content in MCTs appreciated by the decreased $V_{\text{max}}$ in this group. Moreover, another clue that might provide information about the percentage of each transporter present in SV appears to be the transport affinity determined in SV. The determined $K_m$ of both groups (30–40 mM) was closely related to the recently determined $K_m$ of MCT4 when expressed in *Xenopus laevis* oocytes (34 mM) with radiotracer (7), whereas with the same model, the determined $K_m$ of MCT1 is ~5 mM (4). However, with a decreased lactate uptake at 1 and 10 mM in SV of Zucker rats, it seems that the MCT1 content of our vesicles was sufficient to detect visible changes in lactate transport at physiological concentrations.

From the study of McCullagh et al. (29), it appears that muscle MCT1 content is highly correlated with muscle lactate uptake as well as oxidative capacity. On the other hand, because MCT4 expression was found to be highly correlated with type II fiber type, it has been suggested that this expression may reflect the muscle requirement for lactate efflux (37). This situation is quite different from what occurs in vitro, with predominant MCT4 content in SV and the two isoforms showing identical influx or efflux kinetics (25). Even if basal lactatemia is increased in both rat (present study) and human insulin resistance-related obesity (6), the values do not exceed the value of MCT1 $K_m$, suggesting that, in vivo, MCT1 is still the major isoform for lactate uptake. Interestingly, mixed muscle such as RTA or red gastrocnemius (RG) has a greater lactate uptake than more oxidative muscle (such as SOL) in normal rats (29). Thus the hyperlactatemia in obesity could be more related to a decreased muscle lactate uptake, primarily in mixed muscle where MCT1 content is significantly decreased. We may therefore hypothesize that the decreased MCT1 content in mixed muscles would be sufficient to significantly decrease lactate uptake in vivo at the physiological concentrations found in obesity.

From the recent literature, we know that both MCT1 expression and MCT4 expression are regulated by contractile activity. Endurance training programs and chronic electrical stimulation upregulate MCT1 but not MCT4 (2). On the other hand, denervation (37) and hypokinesia (hindlimb suspension) (11) decrease SV lactate transport as well as MCT1 and MCT4 expression (37). Interestingly, a series of data shows the decreased spontaneous activity in obese compared with lean Zucker rats (12, 16, 36). Indeed, when fed ad libitum, obese Zucker rats appear to be less active than lean ones (16). We can hypothesize that the long-term adaptation in Zucker rats, i.e., chronic hypokinesia, might mimic what occurs in hindlimb suspension for the expression of both MCT isoforms and sarcolemmal lactate transport. Hypokinesia is generally accompanied by reduced skeletal muscle oxidative capacity (11, 38) and increased glycolytic enzymes (28). Here, what might corroborate the published data indicating a decreased oxidative capacity of skeletal muscles from obese Zucker rats is the redistribution in LDH isozymes toward more type M-LDH. LDH-1 was decreased in RTA and EDL (by 70 and 53%, respectively), where oxidative profile is representative of the majority of hindlimb muscles, but it remained unchanged in highly oxidative or highly glycolytic muscles (SOL and WTA). This shift toward type M-LDH could contribute to the decreased lactate oxidation by skeletal muscles, along with reduced oxidative capacity, in obese rats. From the study of McCullagh et al. (29), we know that MCT1 is strongly correlated with lactate uptake, oxidative capacity, and type H-LDH isozymes. Because the Zucker rats had fiber type-dependent alterations in MCT1 expression and LDH isozymes, we reevaluated the validity of the correlations between MCT1 and LDH isozymes in this model. MCT1 content in control rats was correlated with LDH-3 ($r = 0.95$) but not with the LDH-2 isozyme, whereas in Zucker rats it was correlated with both the LDH-2 and LDH-3 isozymes ($r = 0.96$ and 0.98). A correlation between MCT1 content and the LDH-5 isozyme was more surprising, because MCT1 content appeared to decrease in the RTA and EDL muscles of Zucker rats only. However, neurons in which LDH-1 is the predominant isozyme do not express MCT1 but rather the MCT2 isoform, whereas glial cells that contain LDH-5 express only the MCT1 isoform (3). As a result, the hypothesis advanced by McCullagh et al. (30) of coordinated regulation of MCT1 and H-LDH subunit genes is not verified, and more complex mechanisms in their expression may be involved.

In summary, we found a decreased total lactate influx in the skeletal muscles of obese Zucker rats by use of the SV model. This decreased uptake of lactate was accompanied by reduction in the skeletal muscle expression of the MCT1 and MCT4 isoforms, both of which appeared fiber type dependent, with a more pronounced decrease in MCT4 expression. As a consequence of the model, MCT4 content was also reduced in SV of the Zucker rats, which could explain the decreased maximal rate of lactate uptake. We hypothesized that the reduced spontaneous activity of the strain would account for the decreased expression of both MCT isoforms. Consistent with this hypothesis is the redistribution in LDH isozymes in the skeletal muscle of the Zucker rats, which also appeared fiber type specific. A strong relationship was found between LDH-5 and MCT1 only in the obese rats. Although the SV model may be not strictly representative of what occurs in vivo, our evidence suggests that the decreased lactate uptake, along with a shift toward type M-LDH, could reduce skeletal muscle lactate oxidation in obese Zucker rats and thus contribute to basal hyperlactatemia. Further investigations are needed, however, to evaluate the relative part of the impaired skeletal muscle metabolism and exchanges in obesity-related hyperlactatemia. As we pointed out in the introductory paragraphs, adipose mass appears to be a significant site of lactate production. Whether impaired lactate transport and metabolism of other organs, including adipose tissue, participate in obesity-impaired lactate clearance remains to be established.
MUSCLE LACTATE EXCHANGES AND OBESITY

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REFERENCES


