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Effects of Streptozotocin-Induced Diabetes on Markers of Skeletal Muscle Metabolism and Monocarboxylate Transporter 1 to Monocarboxylate Transporter 4 Transporters

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Diabetes is known to alter both oxidative and glycolytic pathways in a fiber type-dependent manner. In various skeletal muscles of normal rats, monocarboxylate transporter 1 (MCT1) has been found to be highly correlated to lactate uptake, as well as to oxidative capacity, whereas the distribution and characteristics of MCT4 make it a good candidate for the extrusion of lactic acid from glycolytic muscle cells. Since a previous study found decreased sarcolemmal lactate uptake in streptozotocin (STZ)-diabetic rats, we investigated the presence of MCT1 in relation to enzymatic markers of both oxidative and glycolytic pathways, as well as MCT4 content, in STZ-diabetic rats. Soleus (SOL), red tibialis anterior (RTA), extensor digitorus longus (EDL), heart, and preparations of purified sarcolemmal vesicles (SV) from control and STZ-diabetic rats were harvested for MCT1 and MCT4 content, citrate synthase activity (CS), and lactate dehydrogenase (LDH) isozymes. Basal blood lactate concentration was increased by 38% in the diabetic rats (close to 1.91 mmol/L). However, no change was found in either MCT1 or MCT4 content in these rats. The diabetic rats presented fiber type-specific decrease in CS activity. We noted a redistribution in LDH isozymes in diabetic muscles with a general increase in type H-LDH. Regression analyses indicated (1) a strong relationship between LDH-4 and LDH-5 and (2) MCT1 was still correlated with CS activity in diabetic muscles. These results suggest that diabetes-induced hyperlactatemia is not associated with changes in MCT1 or MCT4 expression, but with alterations of oxidative and glycolytic enzymes.
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THE TRANSPORT OF lactate and other monocarboxylic acids, such as pyruvate, acetoacetate and β-hydroxybutyrate is of major physiologic importance in all mammalian cells. It is now widely accepted that the transport of lactate and pyruvate is mediated by a family of H^+/monocarboxylate transporters, MCTs, named for their characteristic substrate specificity for short chain monocarboxylates (for recent reviews, see Halestrep and Price[1] and Bonen[2]). Accumulating evidence has led to the notion that each isoform of the transporter may have different kinetic and pharmacologic properties related to the unique metabolic requirements of the tissue where it is localized.[1,2] Because both the K_m value of MCT1 was near 5 mmol/L[3] and because McCullagh et al[4] found a strong relationship between MCT1 content and muscle lactate uptake, it was concluded that the role of MCT1 was to take up lactate from circulating blood. On the other hand, Dimmer et al[5] showed a very low affinity/high capacity for MCT4, suggesting a major role in lactic acid efflux from highly glycolytic muscles. The regulation of the muscular MCT isoforms, MCT1 and MCT4, has been widely studied in relation to muscle activity,[6,7] but little is known about regulation in other situations where metabolic fluxes are disturbed, such as in diabetes. Indeed, along with impaired glucose oxidation, several studies have found an increased lactate production by perfused rat hind limbs from ketotic animals, a metabolic state frequently found in such a model.[8,9] Increased lactate production has also been found in adipocytes from streptozotocin (STZ)-diabetic rats[10,11] and may result from the decreased active form of pyruvate dehydrogenase.[11] We previously reported a decrease in total lactate transport activity in STZ-induced diabetes in rat sarcolemmal vesicles (SV),[12] in which the Michaelis-Menten curve fit revealed differences in the number of transporters involved in lactate uptake. Whether this decreased transport activity results from decreased expression of MCT1 or MCT4

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or both, and whether it contributes to the impaired lactate metabolism in STZ-diabetic rats, has not been evaluated.

To more accurately characterize the functional role of each MCT, some investigators have tried to link MCT1 content to the enzymatic markers of the oxidative and glycolytic metabolisms. In normal rats, McCullagh et al[4] found that MCT1 content was correlated with both citrate synthase (CS) activity and heart forms of lactate dehydrogenase (LDH-H). Because both the oxidative and glycolytic metabolisms are known to be altered in a muscle-dependent manner in STZ-diabetic rats, the rat appears to be a good model for investigating whether MCT1 and these enzymatic markers are regulated in a dependent manner. We thus studied the MCT1 content in heart and skeletal muscles in relation to CS activity and LDH distribution in control and STZ-diabetic rats.

MATERIALS AND METHODS

Reagents

Reagents were purchased with the highest quality available from Sigma Chemical (St Quentin Fallavier, France). Antirabbit immunoglobulin G (IgG) was provided by Amersham Pharmacia Biotech (Amersham, Saclay, France).

Animals

Animal experiments were performed according to the Helsinki convention for animal care and use. Male Wistar rats were provided by IFFA CREDO (Paris, France) and assigned to 1 of 2 groups: a control group (n = 7) and a STZ-induced diabetes group (n = 8). They were kept on a reverse 12-hour light/dark cycle at 22°C and housed in individual cages. Standard rat chow and water were provided ad libitum. Type 1 diabetes was induced at the age of 13 to 14 weeks by a single intraperitoneal injection of a freshly prepared solution of (65 mg/kg body weight) in 100 mmol/L citrate buffer (pH 4.5), and it was defined as a nonfasting tail-blood glucose concentration greater than 400 mg/dL on 2 separate occasions (Glucometer Medisense, Schiltigheim, France). Fifteen days after diabetes induction, blood samples for determination of plasma glucose, lactate, ketone bodies, and insulin were collected in basal conditions from rats of each group before death. Rats were then killed by cervical dislocation for tissue preparation.

Tissue Preparation

Muscles from 1 rat of each group were processed on the same day of the experiment. After cervical dislocation, hind limb muscles were rapidly removed. Portions of soleus (SOL), red tibialis anterior (RTA), extensor digitorus longus (EDL), and heart were quickly frozen in liquid nitrogen and stored at −80°C until biochemical assays. These skeletal muscles were selected because they represent primarily slow oxidative (SO), fast oxidative (FOG), and fast glycolytic (FG) fiber types, respectively.[13] Because we refer to previous experiments on SV showing decreased lactate transport,[12] we also prepared purified SV from control and diabetic rats, as described.[12]

Sample Preparation for Western Blotting

Proteins were isolated from muscles for Western blotting using the method of McCullagh et al[4] and previously described.[14] 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.

Western Blotting of MCT1 and MCT4

Affinity-purified polyclonal antibodies directed against the carboxy terminus of rat MCT1 and MCT4 were produced by immunization of New Zealand white rabbits with the synthetic peptide PLQNSSGDPAEEESPV for MCT1 and LREVEHFLKAEPEKNG for MCT4.[15] Polyclonal antibodies yielded a single band on a Western blot that corresponded to 43 to 45 kd, consistent with the molecular mass of each isoform reported earlier.[4,16,17] Antibody specificities were confirmed in preliminary experiments in which the peptides blocked the detection of MCT1 and MCT4 (Fig 1). Samples of muscle, heart homogenates, or purified SV (20 μg protein) and prestained molecular mass markers (Bio-Rad, Ivry-sur-Seine, France) were separated on either 12% sodium dodecyl sulfate (SDS)-polyacrylamide gels for MCT1 (150 V for approximately 90 minutes) or 10% Bis-Tris NuPage gels (Invitrogen, Breda, The Netherlands) for MCT4. Proteins were then transferred from the gels to polyvinylidene difluoride (PVDF) membranes (100 V, 90 minutes). Membranes were incubated overnight at 4°C in blocking buffer (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) and then incubated with either anti-MCT1 or anti-MCT4 antibodies (1:3,000) for 1.5 hours in blocking buffer. After a 15-minute wash followed by two 5-minute washes in 150 mmol/L NaCl, 0.1% Tween 20, and 50 mmol/L Tris, pH 7.5 (TTBS), membranes were incubated for 45 minutes with goat antirabbit immunoglobulin G horseradish peroxidase-conjugated secondary antibody (1:3,000; BI 2407, BioSys, Compiègne, France) in TTBS. Membranes were washed as previously described, and MCT1 or MCT4 expression was detected by enhanced chemiluminescence (ECL) detection (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 (AGFA Duo Scan T1200, New York, NY) using Scion Image software (Scion Corp, Frederick, MD). For MCT1, the signal of control heart preparation was used as a positive control and as a means to fix an arbitrary unit for comparison between experiments (100% equals the MCT1 signal of 20 μg of control heart homogenate).

LDH Isozyme Distribution

LDH isozymes present in muscle homogenates were separated by adding 1 μg of protein to agarose (1%) gels and electrophoreting at 90 V for 30 minutes using a Bio-Rad Sub-Cell system (Ivry sur Seine,

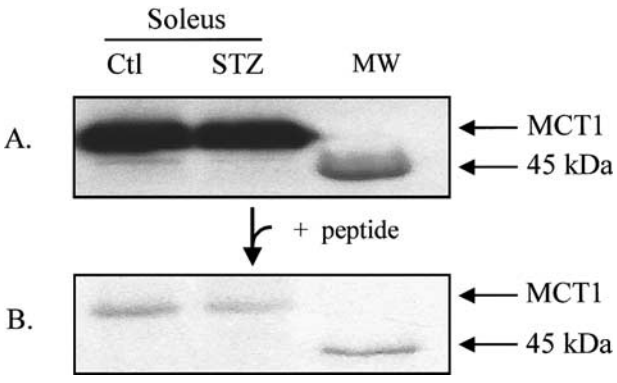

Fig 1. MCT1 antibody specificity. (A) Western blot performed on plasma membrane of soleus muscle from control and diabetic rats. Antibodies were used at a dilution of 1/3,000. (B) Western blot performed with the same plasma membrane preparations as in (A), in which the synthetic peptide used to synthesize MCT1 antibodies was added to a final concentration of 0.4 μg/mL.

Table 1. Body Weight, Nonfasting Blood Glucose, Lactate and Insulin Concentrations in Control and STZ-Diabetic Rats

Group	Body Weight (g)	Blood Glucose (mmol/L)	Blood Insulin (pmol/L)	Plasma Lactate (mmol/L)	Plasma Acac (mmol/L)	Plasma β-OHB (mmol/L)
Control	371 ± 6	7.55 ± 0.25	2.03 ± 0.07	1.38 ± 0.16	0.28 ± 0.05	0.18 ± 0.03
STZ-diabetic	313 ± 8*	35.38 ± 0.94*	0.08 ± 0.02*	1.91 ± 0.27	0.71 ± 0.12*	1.12 ± 0.19*

NOTE. Blood glucose levels were determined using the glucose oxidase assay, and insulin concentrations were determined using a radioimmunoassay kit. Plasma lactate was determined using the method of Gutmann and Wahlefeld[19] and ketone bodies according to Chen et al.[21] Results are the mean ± SE.

*$P < .01$ STZ-diabetic *v* control.

France). An electrophoretic marker (LDH Isotrol, Sigma, L'Isle d'Abeau Chesnes, France) containing LDH isozymes 1 to 5 was used as an aid in identification of isozymes. LDH isozyme activities were visualized by nitroblue tetrazolium reduction to formazan (Sigma Procedure 705). The gels were fixed in 5% acetic acid, and the bands were scanned and quantified spectrophotometrically (AGFA Duo Scan T1200 scanner) using Scion Image software. LDH isozyme (1 to 5) repartition was calculated by dividing the area × mean optic density product for each isozyme by the sum of area × mean optic density of the 5 isozymes (the sum of each area × mean optic density product for each isozyme = 100%). Results are expressed in percentage of all LDH isozymes.

Biochemical Assays

CS (in μmol · min^{-1} · mg^{-1} protein) activity was measured in muscle homogenate spectrophotometrically (Beckman DU-640 spectrophotometer, Paris, France), according to Srere.[18] Plasma lactate concentration was determined using the method of Gutmann and Wahlefeld.[19] Insulin concentrations were measured by the method of Herbert et al.[20] Ketone bodies (acetoacetate, Acac and β-hydroxybutyrate [β-OHB]) were measured according to Chen and Ianuzzo[21] and expressed in millimolars. Proteins were estimated by the Coomassie brilliant blue method using bovine γ-globulin as a standard (Bio-Rad protein assay). Results were expressed in μmol · min^{-1} · g protein^{-1}. Blood glucose levels were determined by the glucose oxidase method.

Statistical Analysis

Results are expressed as means ± SE. The data were analyzed using regression analyses, *t* tests, and analyses of variance when appropriate. The differences were considered significant at $P < .05$.

RESULTS

Body Weight, Nonfasting Blood Glucose, Lactate, Ketone Bodies, and Insulin Concentrations

Body weight, nonfasting blood glucose, lactate, ketones, and insulin concentration values are presented in Table 1. Diabetes induced a decrease in body weight of about 17% ($P < .05$), with an increase in blood glucose concentration to near 35 mmol/L. Insulin fell to near zero concentration, with most diabetic rats at the limit of detection. Blood lactate concentration increased by nearly 38% (up to 1.91 mmol/L, P .05). Fifteen days of diabetes significantly ($P < .01$) increased plasma β-OHB and Acac concentrations in the diabetic rats (Table 1).

MCT1 and MCT4 Expression

MCT1 was detected in heart and the 3 skeletal muscles studied in control and STZ-diabetic rats (Fig 2A). Densitometry analysis did not reveal any difference in MCT1 concentration between the 2 groups in the muscles studied. Equally, the same MCT1 concentration profile was found in the 2 groups: heart contained the greatest amount of MCT1 followed by SOL, RTA, and EDL muscles (Fig 2B). Skeletal muscle MCT1 concentration averaged 34.4% ± 7.2% and 30.9% ± 6.6% for SOL, 12.7% ± 3% and 10.8% ± 4.8% for RTA, 6.3% ± 1.6% and 7.8% ± 3.7% for EDL, and 75.3% ± 2.1% and 77.3% ± 3% for heart in control and STZ-diabetic groups, respectively. The MCT4 signal was absent in heart homogenates in both groups. Like MCT1, MCT4 was detected in SOL, RTA, and EDL muscles (Fig 3A), but no significant difference emerged

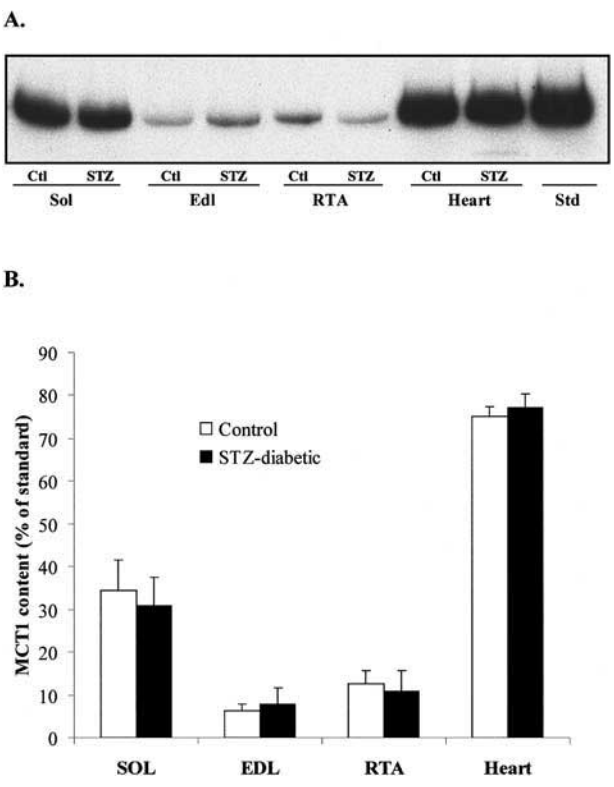

Fig 2. Effect of diabetes on skeletal muscle and heart MCT1 expression. (A) Representative Western blots showing the MCT1 content in 20 μg of SOL, RTA, EDL muscle, and heart preparation of control and STZ-diabetic rats. (B) Comparison of the MCT1 expression in the cited muscles. Values are means ± SE of 7 preparations. Results are expressed in percentage of standard consisting of 30 μg of right ventricle of control rat.

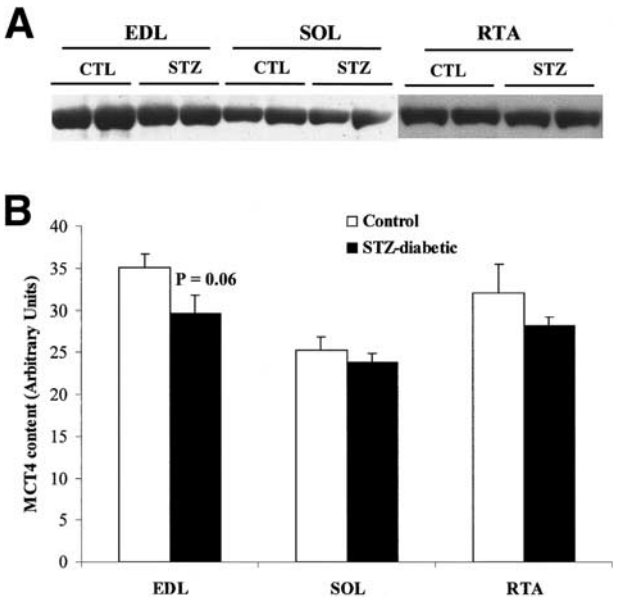

Fig 3. Effect of diabetes on skeletal muscle MCT4 expression. (A) Representative Western blots showing the MCT4 content in 20 μg of SOL, RTA, and EDL muscle preparations of control and STZ-diabetic rats. (B) Comparison of the MCT4 expression in the cited muscles. Values are means ± SE of 6 preparations.

for MCT4 content in the 3 skeletal muscles studied, although we noted a trend ($P = .06$) toward decreased expression in homogenate from EDL muscle of diabetic rats (Fig 3B). Western blot on purified SV confirmed the results in muscle homogenates, in which no significant difference was noted in either MCT1 or MCT4 content (Fig 4).

LDH Isozyme Distribution

LDH isozyme distribution in heart and skeletal muscles is represented in Fig 5. All 5 isozymes of LDH (LDH-1 to LDH-5) could be detected in all muscles. The profile of the relative percentage of each isoform in SOL resembled that of heart, with LDH-1 and LDH-2 being predominant, whereas EDL and RTA were similar with LDH-5 present in higher amounts than the other isoforms.

Diabetes induced changes in the relative percentage of isoform distribution. In SOL muscle, diabetes induced a 31% ($P < .05$) increase in LDH-1, whereas a 41% ($P < .05$) decrease was noted for LDH-3 when compared with control. In EDL muscle, diabetes induced a 142% ($P < .05$) and 58% ($P = .06$) increase in LDH-1 and LDH-2, respectively. We also noted a 15% ($P < .05$) decrease in the LDH-5 isoform, but a 43% increase in LDH-4 ($P < .05$). There were quite similar changes in diabetic RTA muscle: LDH-1 and LDH-2 increased by 63% ($P < .05$) and 46% (not significant [NS]) and LDH-5 decreased by 21% ($P < .05$), whereas LDH-4 increased by 31% ($P = .06$). A similar, but less pronounced, increase in LDH-1 and LDH-2 isoform occurred in heart (21% and 10%, $P < .05$, respectively) with a decrease in LDH-4 and LDH-5 (-52%; $P < .05$ and -20%; $P = .06$).

When regression analysis was used, there was no relationship between MCT1 and any of the 5 LDH isoforms in either group. However, there was a marked difference when isoform distribution was analyzed. In the control group, there was a strong relationship between LDH-1 and LDH-2 ($r = .99$, $P < .05$) and LDH-1 and LDH-3 ($r = .98$, $P < .05$), but not between LDH-2 and LDH-3, as previously reported in normal rats.[4] In addition, a positive relationship was found between LDH-4 and LDH-5 ($r = .94$, $P < .05$) in the STZ-diabetic group, but not in the control group.

CS Activity

Induction of diabetes resulted in a decrease in CS activity, which appears to be fiber type-specific (Fig 6). Indeed, diabetes induced a significant decrease in SO muscle (SOL, -33% ± 5%, $P < .05$) and a trend toward decreased activity in FOG muscle (RTA, -17%, $P = .055$), while no change in FG muscle (EDL) was observed. Moreover, diabetes resulted in a 23% ± 3% decrease in CS activity in heart muscle. To investigate more accurately the role of lactate exchanges in the previous decrease in lactate oxidation,[8] we determined the ratio of MCT1 to CS for each muscle. Although a trend toward an increased ratio was noted in the STZ-diabetic group, significance was noted only for heart muscle compared with the control group (Fig 7).

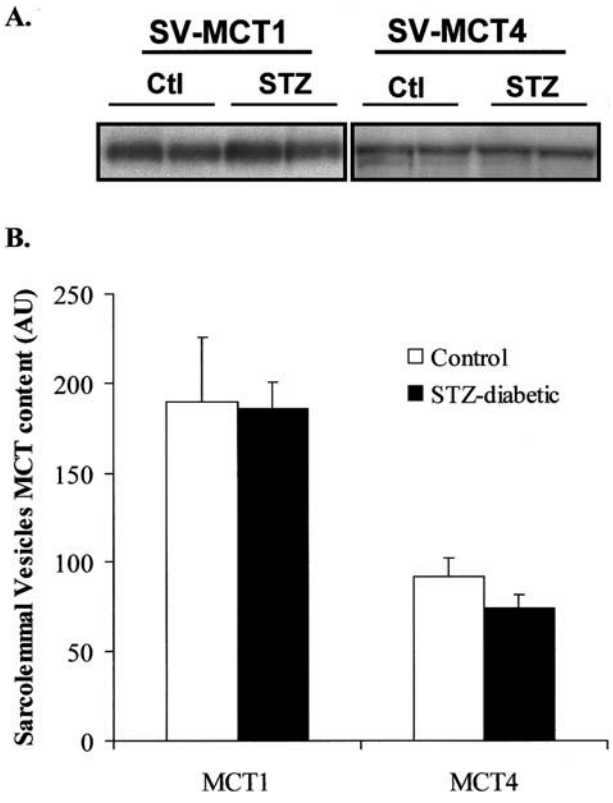

Fig 4. (A) Western blot of MCT1 and MCT4 content present in 20 μg of purified SV preparations of control and STZ-diabetic rats. (B) Comparison of the MCT1 and MCT4 SV content. SV were prepared from whole hind limb muscle mass. Values are means ± SE of 5 preparations.

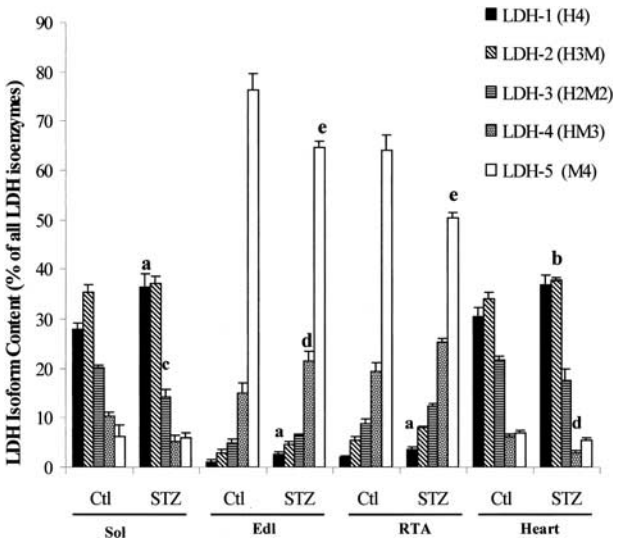

Fig 5. Effect of diabetes on LDH isozyme distribution in heart and skeletal muscles. LDH isozymes are as follows: LDH-1 (H4), LDH-2 (H3M), LDH-3 (H2M2), LDH-4 (HM3), and LDH-5 (M4), in which M is muscle and H is heart. Values are means ± SE of 7 electrophoretic separations. (A) Statistical significance of LDH-1 between control and STZ-diabetic groups, *P* < .05; (B) statistical significance of LDH-2 between control and STZ-diabetic groups, *P* < .05; (C) statistical significance of LDH-3 between control and STZ-diabetic groups, *P* < .05; (D) statistical significance of LDH-4 between control and STZ-diabetic groups, *P* < .05; (E) statistical significance of LDH-5 between control and STZ-diabetic groups, *P* < .05.

DISCUSSION

The results of this study indicate that the altered lactate metabolism in STZ-induced diabetes is not accompanied by changes in MCT1 or MCT4 protein expression in either skeletal muscle or SV preparations. However, diabetes leads to changes in the correlation between the markers of both oxidative and glycolytic pathways and MCT1 content that have been previously described in normal rat muscles.

Here, we showed that metabolic alterations in experimental diabetes are not accompanied by significant changes in MCT1 or MCT4 expression. Since the primary defect in this experimental diabetes is decreased insulin secretion and action, it is tempting to advance the hypothesis that MCT1 and MCT4 expression are not regulated by insulin. Interestingly, data has recently been published on lactate uptake and MCT1 expression in isolated adipocytes from STZ-diabetic rats. Hajduch et al[22] found a 64% decrease in L-(+)-lactate uptake and an 80% decrease in MCT1 expression in isolated adipocytes from diabetic rats compared with controls. Among the possible regulatory mechanisms of MCT1 expression in these STZ-adipocytes, the investigators logically advanced the role of the insulin deficiency.[22] Concerning liver, a previous study of Metcalfe et al[23] found an increased lactate uptake on isolated hepatocytes from starved and diabetic rats, whereas Jackson et al[24] did not, nor did these last investigators find any change in MCT1 or MCT2 content compared with that of normal rats. Such differences or discrepancies regarding MCT regulation between diabetic tissues require further investigation.

As mentioned above, we previously found decreased SV lactate uptake in STZ-diabetic rats compared with controls, which denoted a decreased number of transporters.[12] Our SVs are prepared from pooled hind limb muscles that consist largely of type II fibers containing MCT4.[13] This way, lactate transport experiments in SV are likely to reflect measurements of MCT4 kinetic parameters. Our K_m value supports this hypothesis, as it was closer to that of MCT4 (≈ 30 mmol/L) in both groups.[5]

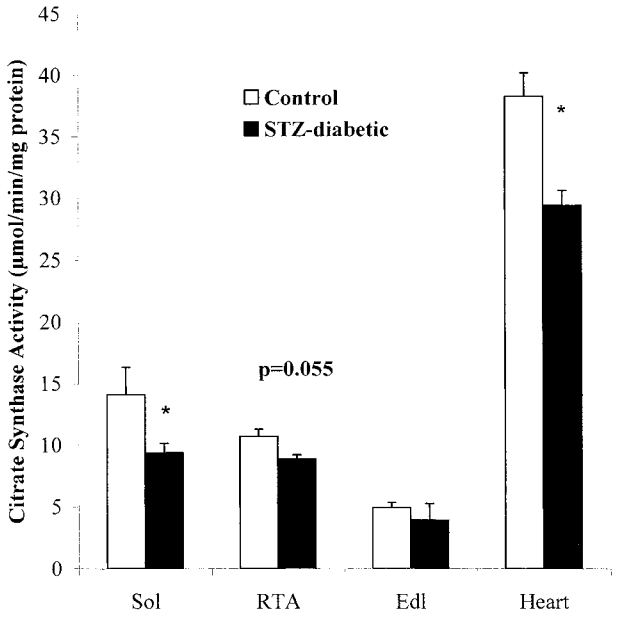

Fig 6. Effect of diabetes on CS activity in SOL, EDL, RTA muscle, and heart. Values are means ± SE (n = 7). Results are expressed in µmol/min/mg protein. *Significantly different from control group, *P* < .05.

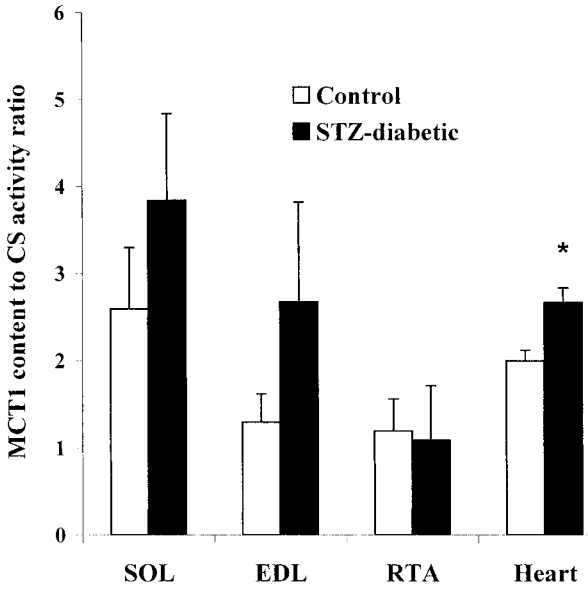

Fig 7. Ratio of MCT1 content to CS activity in SOL, EDL, RTA, and heart muscles. This ratio appears significantly increased only in diabetic heart. *Significantly different from control group, *P* < .05.

Since there was no difference between groups in MCT1 or MCT4 content in the SV preparations, it seems that factors other than simple expression to the sarcolemma are involved in the decreased lactate transport in STZ-diabetic muscle. The possibility that STZ-diabetes leads to posttranslational modifications of the transporters cannot be excluded.[25] In this respect, we[12] and others[26] have reported the presence of increased oxidative stress in skeletal muscle of STZ-diabetic rats. Moreover, in STZ-diabetic rats receiving antioxidant treatment, Khamaisi et al[27] observed normalization of both glucose transport and muscle lactate concentration. It seems unlikely that diabetes led to glycosylation of these transporters since the molecular mass as determined by gel migration was not different between groups ($\approx$ 43 kd on Western blot). Because both MCT1 and MCT4 isoforms interact with the CD147 chaperon protein, the disruption of this interaction by the diabetic process cannot be excluded. In any case, it is clear that additional experiments are needed to more accurately determine the underlying mechanisms of decreased muscle lactate uptake in diabetes.

In the present study, we found decreased CS activity, which appears to be fiber type-dependent and which may contribute to the decreased lactate oxidation and increased lactacidemia in diabetes, as discussed above. This same fiber type-specific decrease in CS activity has already been reported in STZ-diabetic mice and rats.[10,28] To characterize the MCT family, some investigators have tried to link MCT1 content to enzymatic markers of both oxidative and glycolytic metabolism. Thus, McCullagh et al[4] showed a strong correlation between muscle MCT1 content and the oxidative capacity, whether the latter was represented by muscle fiber type composition or CS activity (r = .91 and .82, respectively) in various skeletal muscles. Surprisingly, we were able to find the same positive correlation between MCT1 and CS activity in both groups, while no MCT1 decrease was found, whatever the STZ-muscle studied (data not shown). When the ratio of MCT1 to CS was drawn, only the heart of diabetic rats presented a significant increase compared with controls (Fig 7). A trend towards increase was noted in the SOL and EDL muscles of the diabetic rats. One interpretation of this ratio could be that the lactate clearance in diabetic rats is more related to the decrease in the oxidative pathway than to the distribution of MCT1 or MCT4. It thus seems reasonable to assume that CS, LDH-H, and MCT1 are regulated in separate ways. Similar results concerning the diabetic heart have already been reported by Chatham et al.[25] Indeed, no changes in MCT1 content or LDH isozyme distribution were observed,[25] whereas diabetic hearts presented a decreased lactate oxidation relative to glucose oxidation. More recently, Kitaura et al[29] found that MCT1 decrease in clenbuterol-treated rats was not systematically accompanied by changes in either LDH isozymes or MHC isoforms. In the present study, we noted an increase in LDH-1 proportion in all muscles and hearts in the diabetic group. This increase seems to be inversely related to the oxidative capacity of the muscle with 142%, 63%, 31%, and 21% increases for EDL, RTA, SOL, and heart muscle, respectively. Changes in LDH isozyme distribution have already been explored in diabetes, but conflicting results appear in the literature. Wohlrab and Schmidt[30] noted a decrease in LDH-H subunits in SOL of STZ-diabetic rats compared with controls. LDH-1 (H4) decreased from 30.3% of controls to 18.2%, and the LDH-4 (HM3) increased from 11.1% of controls to 20.1%.[30] On the other hand, the study of Il'in et al[31] also found increased LDH-1 and LDH-2 content in smooth muscle of rabbits with alloxan-diabetes. Our results are in accordance with those of Awaji et al,[32] who found a 21% increase in LDH-H subunit and a reciprocal decrease in LDH-M subunit. These results, together with the previously reported decreased total lactate transport across sarcolemma in the same rat model,[12] may provide insight into the metabolic adaptations in diabetic muscle. We can hypothesize that decreased fluxes of lactate across the sarcolemma have to be matched by an increased rate of lactate into pyruvate transformation. This could be achieved through the redistribution of LDH isozymes, with a shift from M to H isozyme. In support of this hypothesis was the observation that the decrease in CS activity was lessened in relation to higher upregulation of LDH-1 and -2. In addition, the reduced LDH-5 content reported here (primarily in FOG and FG muscles) could be an adaptation to reduced muscle lactate and protons (H^+), which are potent inhibitors of a number of metabolic enzymes. LDH is not the only enzyme, however, that controls lactate oxidation, and this redistribution seems to be insufficient to maintain a normal rate of lactate oxidation in STZ-diabetic muscles. Rather, pyruvate dehydrogenase seems to be the rate-limiting enzyme that controls lactate oxidation. Indeed, Berger et al[8] already noted a 70% decrease in lactate oxidation, while Hagg et al[9] found a 50% decrease in pyruvate dehydrogenase activity in hind limb of STZ-diabetic rats. With an increased ratio of MCT1 to CS activity only in heart muscle, it appears that the previously described decrease in lactate oxidation is more related to factors other than the expression of MCTs. In this respect, it seems that the lactic profile of STZ-diabetic rats is associated more with impaired lactate clearance generated by alterations in enzymatic activities coupled with those of the citric acid cycle. Thus, this rat model shows that the enzymatic activity of CS, LDH distribution, and MCT1 expression are probably associated with one another, but regulated in an independent manner.

We conclude that diabetes leads to changes in the oxidative and glycolytic pathways with a reduction in CS activity and a redistribution of LDH isozymes, both of which appear to be fiber type-dependent and independently regulated with regard to the MCT1 isoform. These cellular modifications are thought to limit the decreased lactate oxidation previously reported in skeletal muscles of this model.[8,25] In addition, the resulting increased plasma and muscular lactate concentrations frequently reported are not associated with changes in MCT1 or MCT4 content of diabetic skeletal muscles. Discrepancies exist in this model in which both heart[33] and skeletal muscles[12] present a decreased lactate uptake without changes in MCT expression.[25] Therefore, additional studies are needed to define the precise MCT regulation in diabetes.

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