

Streptozotocin-Induced Diabetes Decreases Rat Sarcolemmal Lactate Transport

Guillaume Py, Nicolas Eydoux, Antonia Perez-Martin, Eric Raynaud, Jean-Frédéric Brun, Christian Préfaut, and Jacques Mercier

Impaired lactate metabolism is a metabolic disorder, which is not fully understood in the diabetic state including streptozotocin (STZ)-induced diabetes. We investigated whether STZ-induced diabetes results in altered lactate exchanges using the rat muscle sarcolemmal vesicles (SV) model. Fifteen days after diabetes onset (1 STZ-injection, 65 mg/kg, intraperitoneal [IP]), rats had higher blood and muscle lactate concentrations compared with normal rats (1.50 ± 0.09 v 1.95 ± 0.21 mmol/L (not significant [NS]) and 21.02 ± 1.26 v 25.53 ± 0.98 mmol/kg wet weight (ww); $P < .05$). The initial rate of lactate uptake was measured at various external lactate concentrations using SV of both group in zero-*trans* conditions. STZ-induced diabetes decreased the initial rate of total lactate influx at external lactate concentrations from 1 to 100 mmol/L ($P < .05$). This decrease in lactate transport was found in addition to an increased free radical production, as indicated by a significant increase in malonedialdehyde (MDA) concentration (64.3 ± 8.7 v 100.3 ± 13.5 nmol \cdot g $^{-1}$ ww, $P < .05$), coupled with a higher glutathione peroxidase (Gpx) activity (48.03 ± 3.13 v 84.7 ± 15.01 μ mol \cdot min $^{-1}$ \cdot mg $^{-1}$ protein, $P < .05$) in red gastrocnemius. We concluded that STZ-induced diabetes decreases total lactate transport activity in rat SV and is associated with increased muscular oxidative stress.

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IN NORMAL CONDITIONS, skeletal muscle is the main source of circulating lactate, showing a net lactate release,¹ in contrast to heart, which shows a net lactate uptake.² However, in pathologic conditions such as diabetes and obesity, lactate metabolism is altered.^{3,4} Indeed, an earlier study noted increased lactate production in the hind limbs of diabetic rats.³ Although there are several studies on the alterations in lactate metabolism and its role in impaired glucose homeostasis of diabetes,⁵ only 1 study has focused on how lactate is exchanged. In their study, Metcalfe et al⁶ found an enhanced carrier-mediated lactate entry into isolated hepatocytes in diabetic rats. Skeletal muscles and most other tissues possess a membrane transport system mediating a coupled lactate and proton translocation.⁷ Moreover, muscle possesses several lactate-proton transporter isoforms, 2 of which have already been cloned.⁸ Because different metabolic pathways and different forms of monocarboxylate transporter (MCT) exist in liver and muscle,⁸ it seems highly likely that any alterations in lactate transport would also be different. There are currently no data on the specific properties of lactate transport in diabetic rat muscles, which present higher lactate concentrations.⁹ The lactate-proton transporter has some plasticity, which raises the question of its regulation. The most thoroughly studied regulation concerns exercise training programs. These studies have shown that training increases lactate uptake,¹⁰ as well as MCT1 isoform content and lactate release by muscle.¹¹ In contrast, denervation¹² and unweighting¹³ downregulate the lactate transport capacity. The possibility that short-term effects on the

lactate proton transport capacity mediated by hormones exist has not been systematically investigated.

In the present study, we hypothesized that impaired lactate exchanges across the sarcolemmal membrane may play a role in the impaired lactate metabolism of experimental type I diabetes.

On the other hand, oxygen free radicals have been involved in the pathophysiology of various disease states including diabetes mellitus.¹⁴ Free radicals exert their cytotoxic effect by peroxidation of membrane phospholipids leading to changes in permeability and loss of membrane integrity.¹⁵ Thus, we also hypothesized that oxidative stress is present in skeletal muscle and may interact with lactate transport-mediated protein mechanisms.

The aim of this study was to investigate whether blood and muscle lactate levels in diabetes were linked to an up or downregulated transport activity. We investigated the lactate transport in streptozotocin (STZ)-induced diabetes in rats using a subcellular system: isolated sarcolemmal vesicles (SV) from rat muscle. In addition, we measured the antioxidant glutathione peroxidase (Gpx) enzyme activity and the malonedialdehyde (MDA) concentration as a marker of lipid peroxidation present at the muscular level.

MATERIALS AND METHODS

Reagents

Reagents were purchased with the highest quality available from Sigma Chemical (St Quentin Fallavier, France) unless otherwise stated. Antirabbit immunoglobulin G (IgG) was provided by Amersham Life Science (Amersham, UK).

Animals

Animal experimentation was 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 and a STZ-induced diabetes group. They were kept on a reverse 12-hour light/dark cycle (lights on at 7:00 PM) 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 (IP) injection of a freshly prepared solution of STZ (65 mg/kg body weight)⁹ in 100 mmol/L citrate buffer (pH 4.5).

From the Département de Physiologie, Institut de Biologie, Montpellier; and the Département de Biochimie Métabolique et Clinique, Faculté de Pharmacie, Montpellier cedex, France.

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Address reprint requests to Guillaume Py, PhD, Département de Physiologie, Institut de Biologie, Boulevard Henri IV, 34060 Montpellier, France.

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Diabetes was defined as a nonfasting tail-blood glucose concentration greater than 400 mg/dL on 2 separate occasions (Glucometer Medisense, Schiltigheim, France).⁹ Fifteen days after diabetes induction, blood samples for determination of plasma glucose, lactate, and insulin were collected in the fed state from rats of each group before death. Rats were then killed by cervical dislocation for tissue preparation.

Tissue Preparation

The 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 red and white gastrocnemius (RG and WG, respectively), tibialis anterior (TA), soleus (Sol), and heart (H) were quickly frozen in liquid nitrogen and stored at -80°C until biochemical assays. The remaining hind limb muscles were used for sarcolemmal isolation.

SV Isolation and Characterization

SV were purified from hind limb muscles with a procedure used in our laboratory.^{13,16,17} All subsequent steps were performed 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 × 5 s) of an Ultra-Turrax T25 at 80% of maximal power. The homogenate was centrifuged twice at 900g. Supernatants, termed crude homogenate (CH), were filtered and a 1-mL aliquot was partitioned and saved at 4°C for subsequent analysis. CH was diluted with a volume of KCl medium (3 mol/L KCl) equal to 10% of the CH volume and pelleted by ultracentrifugation at 200,000g for 45 minutes at 4°C (60Ti rotor, Beckman Instruments, Gagny, France). Pellets were resuspended using Teflon pestle homogenization in 30 mL of sucrose medium. The suspension was centrifuged twice at 280g, and supernatants were then collected and centrifuged (200,000g; 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: 36%, 32%, 27% (4 mL), and 12% in layers. After an overnight centrifugation at 130,000g at 4°C in a SW27 rotor, the 27% sucrose band (corresponding to SV) was harvested and diluted with a Krebs-Ringer-HEPES (KRH) buffer (118 mmol/L NaCl, 5 mmol/L KCl, 1.2 mmol/L MgSO₄, 50 mmol/L HEPES, pH 7.5) and washed free of sucrose in a 60Ti rotor (200,000g for 80 minutes). The vesicles were resuspended in KRH buffer (F fraction) up to 4 mg·mL⁻¹ and stored at -80°C until used for the transport experiments. Proteins were determined according to the procedure of Bradford using bovine γ globulin as a standard.

SV characterization was achieved with K⁺-stimulated p-nitrophenylphosphatase (K⁺-pNPPase) assay as previously described.^{13,16} Its total activity was measured in 40 mmol/L HEPES, 0.8 mmol/L EGTA, 4 mmol/L MgCl₂, 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 as $\mu\text{mol} \cdot \text{h}^{-1} \cdot \text{mg}^{-1}$. The purification index (PI) was defined as the ratio of the specific activity from the F fraction to the specific activity measured in the CH. Skeletal muscle SV yield was the ratio of milligrams of sarcolemmal proteins obtained in F to the muscle weight in grams after trimming (wet weight).

Lactate Transport Studies

All measurements were performed in zero-trans conditions in duplicate. [U-¹⁴C]-L-lactate (sodium salt) was purchased from Amersham (specific activity, 155 mCi · mmol⁻¹) 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.^{13,16} Reactions were initiated by delivering 50 μg of protein in tracer-containing medium and then stopped at appropriate time intervals by vacuum filtration on nitrocellulose filters (Whatman WCN, Fisher Scientific, Elancourt, France; average pore size of 0.45 μm). Filters were then rinsed 3 times with an ice-cold isosmotic medium consisting of KRH buffer with 3 mmol/L HgCl₂, pH 7.4, dissolved with ethyleneglycolmonomethyl-ether, and the radioactivity was counted in a scintillation analyzer (Packard 2200 CA, Packard Instruments, Rungis, France). Nonspecific transport activities were determined by preincubation of vesicles in tracer-containing medium with KRH buffer containing 3 mmol/L HgCl₂, which was used to fix the time 0 points. Results were expressed as nmol · mg protein⁻¹. Rates of initial lactate uptake were measured for 1, 10, 30, 50, and 100 mmol/L external lactate concentrations at 0 and 10 seconds in which uptake rates are assumed to be constant for this period of time¹⁸ and during which zero-trans conditions are respected. Slopes gave initial rates of lactate uptake (lactate uptake_{10s}) expressed in nmol of lactate · min⁻¹ · mg protein⁻¹. We also measured the time course of zero-trans lactate uptake with an external lactate concentration of 1 mmol/L.

From the best Michaelis-Menten curve fit to the data depicted, we estimated a maximal velocity (V_{max}) value and a $K_{1/2}$ value corresponding to the substrate concentration at 50% V_{max} .

Vesicle Sensitivity to Osmotic Forces

The effect of medium osmolarity on L(+)-lactate uptake at equilibrium in SV was determined using different external media containing 1 mmol/L L(+)-lactate plus tracer and various sucrose concentrations: 150, 200, 250, 300, and 350 mmol/L in 50 mmol/L Tris, pH 7.4. Intravesicular medium consisted of 250 mmol/L sucrose, 50 mmol/L Tris, pH 7.4. Equilibrium L(+)-lactate uptake was determined after 3-minute incubations, and results were expressed as nmol · mg protein⁻¹ · 3 min⁻¹.

Biochemical Analysis

MDA was measured using the thiobarbituric acid method.¹⁹ Briefly, muscles (RG) were homogenized (10% wt/vol) in 8.1% sodium dodecyl sulfate (SDS), acetate buffer (20% acetic acid solution adjusted to pH 3.5 with NaOH), and 0.8% aqueous solution of thiobarbituric acid. After heating at 95°C for 60 minutes, the red pigment produced was extracted with n-butanol-pyridine mixture and estimated by the absorbance at 532 nm. Tetramethoxypropane was used as an external standard, and results were expressed as nmol · mg⁻¹ protein in tissue homogenate.

Gpx activity was determined in RG by a spectrophotometric method.²⁰ This method is based on the coupling of Gpx activity to the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) by glutathione reductase (GR). Oxidized glutathione (GSSG) formed during the Gpx reaction is instantly and continuously reduced by an excess of GR activity providing for a constant level of glutathione (GSH). The concomitant oxidation of NADPH is monitored photometrically at 340 nm.

Other Assays

Protein content was measured using the Bio-Rad protein assay (Sigma).²¹ Plasma lactate concentration was determined using the method of Gutmann and Wahlefeld.²² Insulin concentrations were measured by the method of Herbert et al²³ using an antibody supplied by Miles Laboratories (Paris, France). ¹²⁵I-labeled insulin was obtained from International CIS (Gif-sur-Yvette, France); the standard used was rat insulin (Novo, Copenhagen, Denmark), whose biologic activity was 22.3 $\mu\text{U}/\text{ng}$. The intra and interassay coefficients of variation were 9%

and 13.5%, respectively. The sensitivity was 0.1 ng/mL. Blood glucose levels were determined either by Exactech glucometer (Medisense) or by the glucose oxidase method.

Statistical Analysis

Results are expressed as means \pm SE. We used a Student's *t* test and, when normality failed, we also used a Mann-Whitney test to examine the effects of induced diabetes on the variables studied. A 2-way analysis of variance (ANOVA) was used to compare initial rates of lactate uptake between the control and STZ-diabetic groups at the different external concentrations or lactate uptake at different osmotic pressure. The differences were considered significant at $P < .05$.

RESULTS

Body Weight, Nonfasting Blood Glucose, Lactate, Insulin Concentrations

Body weight, nonfasting blood glucose, lactate, and insulin concentration values are presented in Table 1. Diabetes induced a decrease in body weight of about 15% ($P < .005$) with an increase in blood glucose concentration to near 35 mmol/L. Insulin fell to near 0 concentration, with most diabetic rats at the limit of detection. Diabetic rats were also ketotic (data not shown). Blood lactate concentration increased by 30% (up to 1.95 mmol \cdot L⁻¹), but this increase was not statistically different from the control group.

Characterization of SV

Using gradient density fractionation, we obtained SV preparations with similar biochemical characteristics in the 2 groups. Protein yields were 97 mg of protein \cdot g⁻¹ (wet weight [ww]) for both groups, and the purification indices of K⁺-pNPPase were 21.7 and 17.8 ($P > .05$) for the control and STZ-diabetic groups, respectively (Table 2).

Lactate Transport Kinetics

Figure 1 shows the time course of 1 mmol/L L-(+)-lactate uptake at pH 7.4 into vesicles from the control and STZ-diabetic groups. Diabetes induced a decrease of the lactate uptake at 1 mmol/L relative to the control group ($P < .05$), whereas the equilibrium level was attained at the same time in both groups.

Figure 2 shows the lactate uptake_{10s} in vesicles from the control and STZ-diabetic groups plotted as a function of external lactate concentration. The shape of the curves confirms the saturable process of the lactate transport.

Kinetic constants for transport in control and STZ-diabetic groups (Table 3) were estimated from the data in Fig 2. V_{\max}

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)
Control	371 \pm 6	7.55 \pm 0.25	2.10 \pm 0.07	1.50 \pm 0.09
STZ-diabetic	313 \pm 8*	35.38 \pm 0.94*	0.06 \pm 0.01*	1.95 \pm 0.21

NOTE. Blood glucose levels were determined using the glucose oxidase assay, and insulin concentrations were determined using a radioimmunoassay kit. Results are the mean \pm SE.

* $P < .001$ STZ-diabetic v control.

Table 2. Characterization of SV

Group	Proteins in CH (mg \cdot g ⁻¹)	Proteins in SF (mg \cdot g ⁻¹)	PI K ⁺ -pNPPase
Control (12)	97 \pm 4	0.115 \pm 0.011	21.7 \pm 1.8
Diabetic (9)	97 \pm 6	0.138 \pm 0.017	17.9 \pm 2.8

Values are means \pm SE.

Abbreviations: CH, crude homogenate; SF, sarcolemmal fraction; PI K⁺-pNPPase, purification index of sarcolemmal enzyme K⁺-*p*-nitrophenylphosphatase.

for lactate transport in the control group was estimated to be 368 nmol/min/mg protein with a substrate $K_{1/2}$ of 26.6 mmol/L. In the STX-diabetic group, V_{\max} was determined to be 312 nmol/min/mg protein. Therefore, STZ-diabetic maximal lactate transport was reduced by 15%, whereas the determined $K_{1/2}$ was 25% higher than the control group.

Effect of Osmotic Forces

Figure 3 shows the effect of osmolarity on 1 mmol/L L-(+)-lactate uptake at equilibrium in SV from control and STZ-diabetic groups. A 2-way ANOVA showed that decreasing the intravesicular space by higher external sucrose concentration results in less L-(+)-lactate accumulation in vesicles from the 2 groups at pH 7.4 ($P < .05$). Moreover, lactate uptake at equilibrium was significantly higher in the control group when compared with the STZ-diabetic group ($P < .05$). Thus, while osmotic pressure exerted the same effect on the 2 groups, we observed the same general decrease in lactate transport in the STZ-diabetic group in these experiments.

Oxidative Stress

Fifteen days of STZ-induced diabetes resulted in an increase in RG Gpx activity in the STZ-diabetic group relative to the control group (84.7 \pm 15.01 v 48.03 \pm 3.1 μ mol \cdot min⁻¹ \cdot mg⁻¹ protein, $P < .05$), indicating an increased production of free radicals. Similarly, MDA content of the same skeletal muscle was increased in the STZ-diabetic group compared with the control group (100.3 \cdot 13.5 v 64.3 \pm 8.7 nmol \cdot g⁻¹ww, $P < .05$).

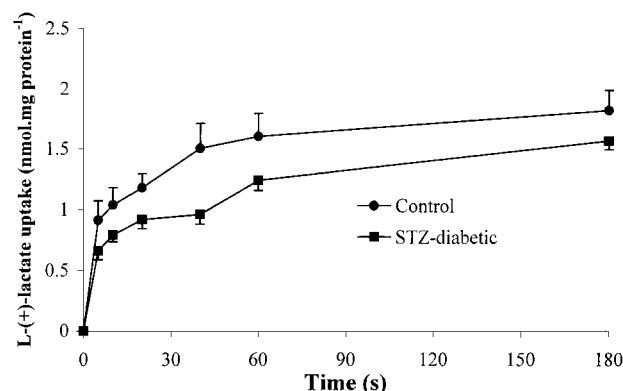


Fig 1. Time course of 1 mmol/L L-(+)-lactate uptake into vesicles from control and diabetic rats. Data are means \pm SE of 3 different membrane preparations. All assays were performed in duplicate in buffered sucrose, pH, 7.4, on both sides of membranes.

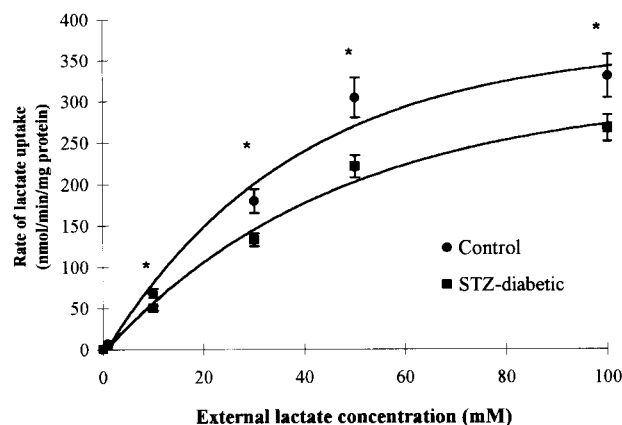


Fig 2. Kinetics of rate of lactate uptake_{10s} into vesicles from control and STZ-diabetic groups. The figure shows the initial rate of lactate influx from experiments with lactate only at the outside (zero-trans conditions). The curves depicted are the best Michaelis-Menten fit to the data. Values are means \pm SE; $n = 6$ to 8 different membrane preparations. All assays were performed in duplicate at pH 7.4. *, $P < .05$ control v STZ-diabetic.

.05) (Fig 4). These results pointed out that even in basal conditions, control rats showed a basal rate of lipid peroxidation in red skeletal muscle.

DISCUSSION

The purpose of the present study was to investigate whether type 1 diabetic state was characterized by altered muscle lactate exchanges using the subcellular model of SV. The major finding was that 15 days of STZ-induced diabetes decreased the rate of initial lactate uptake_{10s} at external lactate concentrations ranging from 1 to 100 mmol/L in a nonmetabolizing system of skeletal muscle SV. This was associated with an increased oxidative stress, as indicated by enhanced Gpx activity and MDA content in red skeletal muscle.

Diabetes models in rats, including STZ-induced diabetes, are all known to result in an increased rate of lactate appearance in basal conditions.^{9,24} In the present study, we found an increase in plasma and lactate concentration, which is in accordance with previous studies on the widely accepted concept of impaired metabolism of carbohydrate fuel in skeletal muscle.²⁴ In type 1 diabetes, the mechanisms leading to increased blood lactate concentration are more complex and could result from increased proteins degradation and reduction in mitochondrial activity. It may also result, in part, from increased glucose conversion to lactate in adipocytes as shown by Newby et al.²⁵ These investigators found that epididymal adipocytes of older

Table 3. Apparent Kinetic Constants for Lactate Transport in Control and STZ-Diabetic SV

Group	V_{max} nmol/min/mg protein	$K_{1/2}$
Control	368	26.6
STZ-diabetic	312	33.2

Abbreviations: V_{max} , maximal velocity; $K_{1/2}$, concentration at 50% V_{max} .

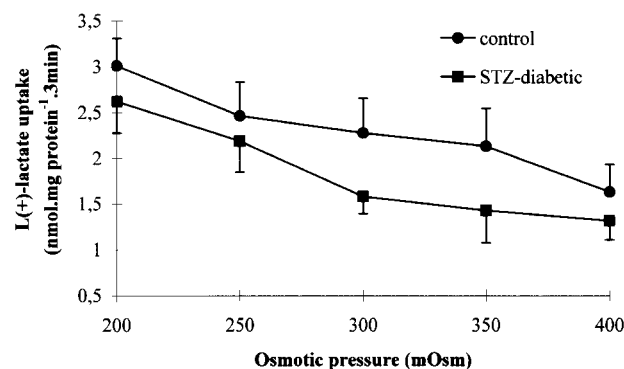


Fig 3. Effect of medium osmolarity on L(+)-lactate uptake into vesicles from control (●) and STZ-diabetic (■) rats. Assays were performed in duplicate at pH 7.4 with external sucrose concentrations adjusted to give the indicated osmolarities. Values are means \pm SE on 4 different membrane preparations.

(6- to 8-month-old, weighing 400 to 500 g), moderately obese STZ-diabetic rats have a 60% increase of glucose conversion to lactate when compared with young rats.²⁵ In the present study, the 13- to 14-week-old rats had a body weight of 371 ± 6 g. Although the 30% increase in blood lactate concentration was not significant, we believe that the relative and absolute amount of glucose converted into lactate increased in adipocytes of our STZ-diabetic rats. Moreover, Mondon et al.²⁴ found that low-dose (38 mg/kg) STZ-induced diabetes in rats was characterized by reduced pyruvate dehydrogenase (PDH) activity and increased lactate production in skeletal muscle and adipose tissue. These changes, in the presence of insulin deficiency, could contribute to enhanced hepatic glucose production.²⁴ We can speculate that the high-dose STZ-induced diabetes in the present study further reduced the PDH activity, leading to increased lactate production.

We investigated lactate transport into SV from control and STZ-diabetic rats and found a decreased transport at all external lactate concentrations in the diabetic group. The properties of lactate transport in our SV were similar to the standard results in isolated membranes.^{18,26,27} In the present study, the

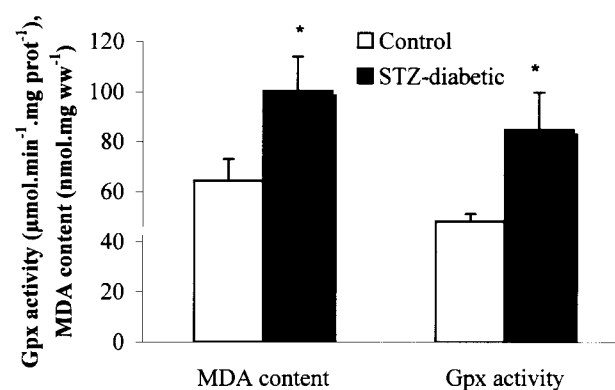


Fig 4. Effect of short-term diabetes on Gpx activity and MDA concentration. Data are means \pm SE. *, $P < .05$ control v STZ-diabetic.

SV of the 2 groups appears to be sealed, because they both showed identical sensitivity to changes in osmotic conditions. In equilibrium conditions, STZ-diabetic SV also showed reduced lactate accumulation. Lower accumulation of lactate in the equilibrium condition could also indicate that SV from the STZ-diabetic group are smaller than those of the control group. It has been reported that when uptake measurements are made in initial rate conditions, the uptake is only dependent on the transporter activity unlike that which occurs in the equilibrium conditions, in which the amount of accumulated substrate in the vesicles is dependent on both transporter activity and vesicle volume.²⁸ These findings suggest that diabetes could either influence the sarcolemma isolation procedure or induce some membrane alterations that might perturb the lactate transport properties or contribute to membrane fragility, leading to a decrease in lactate accumulation. Because the protein yields and the purification indices were similar in both groups, it is unlikely that diabetes could have affected the purification. As discussed later, diabetes is known to increase free radical concentrations and levels of lipid peroxidation responsible for membrane alterations that could contribute to this loss of membrane integrity.²⁹ We already reported that our system exhibits saturation with the increasing external lactate concentration. Moreover, previous studies from our laboratory have shown that these purified vesicles have detectable lactate transport properties when using the same sarcolemmal isolation procedure. They were sensitive to pH and the monocarboxylate transport inhibitor α -cyano-4-hydroxycinnamate and presented some *trans*-stimulatory properties at high external L⁻(+)-lactate concentrations^{16,17} Several lines of evidence, such as the presence of GLUT1 glucose transporter in sarcolemmal fractions from skeletal muscles,³⁰ support the view that this model is representative of the native plasma membrane. Despite the widely recognized problem of the low sarcolemmal recovery from skeletal muscle, isolated SV constitute a nonmetabolic system that allows detailed studies of membrane properties. One could argue that the decreased lactate transport in the STZ-diabetic group may be explained by some differences in the SV fraction. In fact, the STZ-diabetic group appeared with the greater amount of protein, while the enzyme K⁺-pNPPase enrichment was lower. We have not performed measurements of K⁺-pNPPase activity with and without deoxycholate to determine the proportions of right-side-out vesicles. However, this has already been performed with such a model in our laboratory,¹³ and the results were similar to those of previous studies.^{18,27}

The $K_{1/2}$ value determined for the STZ-diabetics was 25% higher than that of control (from 26.6 to 33.2 mmol/L), whereas the V_{max} value was only 84% of control. One explanation for the decreased maximal velocity in the STZ-diabetic group is a decreased number or density of the sarcolemmal membrane transporters. The $K_{1/2}$ values for the 2 groups are in accordance with those found in the literature for muscle.³¹

Few studies have dealt with lactate exchanges in diabetes. Metcalfe et al⁶ showed an enhanced carrier-mediated lactate uptake by isolated hepatocytes in both starved and STZ-induced diabetic fed rats compared with control fed rats. However, the diabetic fed animals were in a negative state of energy balance and the investigators could not state with certainty

whether diabetes per se had an effect distinct from that of starvation, given the qualitative similarity of the hormone profiles in the 2 states. The mechanisms of the change in lactate transport reported in their study remain unknown, but the investigators suggested a hormonal induction of carrier synthesis or mobilization.

One could speculate the presence of different MCT isoforms in liver and muscles, which could be differently regulated. Indeed, from the study of Price et al,⁸ we know that at least 2 isoforms of the transporter exist in mammalian muscle. The MCT-1 isoform is expressed predominantly in oxidative muscles,¹¹ and the MCT-4 isoform is found in both oxidative and glycolytic muscles⁷; while the main isoform observed in rat liver is the MCT-2 isoform. A recent study from Jackson et al³² failed to show an increase in both mRNA and protein expression of MCT-1 and MCT-2 isoforms after 24 or 48 hours of starvation in rat hepatocytes. These investigators also failed to show any increase in the maximal rate of lactate transport in rat hepatocytes.³²

In the present study, we can speculate that the decreased lactate uptake observed in SV of diabetic rats should result in a decreased lactate clearance explained mainly by a decreased oxidation as observed by Berger et al.³ In addition to a 75% decrease in lactate oxidation of STZ-diabetic rats, they noted an increased lactate and alanine release. Because the lactate uptake was not measured, it cannot be excluded that the uptake had decreased. The fact that an increased lactate production and release occur at the same time of a decreased lactate uptake in skeletal muscle of diabetic rats is not contradictory. Muscles possess at least 2 members of the monocarboxylate family: MCT-1 and MCT-4. Moreover, because of the predominant expression of MCT-4 in fast-twitch muscles, it has been suggested that this isoform expression may reflect the muscle's requirement for lactate efflux,⁷ whereas MCT-1 expression correlates with the requirement for lactate influx for oxidation.³³ Thus, we can speculate that the decreased lactate uptake measured in SV may reflect an overall downregulation of MCT-1 expression even if *in vitro* lactate exchanges across SV appear to be symmetric in their response to lactate gradients. Further experiments using specific antibodies directed against the lactate transporter isoforms will be necessary to investigate the quantitative and qualitative role of each known transporter in muscles of diabetic rats.

Because the hypothalamo-hypophyseal-thyroid axis is altered in STZ-induced diabetes,^{34,35} it seems important to consider the results of Juel et al.³⁶ Indeed, these investigators pointed out that thyroid hormones may influence sarcolemmal lactate transport. They showed with giant vesicles that hypothyroid rats possess a lower lactate transport, while hyperthyroid ones have a higher lactate transport than euthyroid rats, even if this effect was considered nonspecific because of the amount of other membrane proteins also affected by thyroid hormones.³⁶ A previous study from Rondeel et al³⁴ noted that after 2 weeks of diabetes, plasma thyroid-stimulating hormone (TSH), thyroxine (T4), and triiodothyronine (T3) was decreased about 40% to 60% when compared with control rats, as well as a reduced hypothalamic secretion of thyrotropin-releasing hormone (TRH).³⁴ It must be pointed out that the inhibitory effect of STZ-induced diabetes on TRH secretion is probably not due to

hyperglycemia,³⁴ but seems to be due to impaired TRH release from the median eminence, which may be related to the lack of insulin.¹⁸ Thus, taking into account these results, it is difficult to state with certainty on the combined effects of STZ treatment and decreased thyroid hormone levels on the SV lactate uptake, although the protein content of the SV fraction was not different between groups.

Oxidative stress has already been described in diabetes.²⁹ Although the present results provide no evidence for a direct relationship between oxidative stress and decrease lactate transport, some interesting findings exist in regard to transport activity in such diabetes model. Khamaisi et al⁹ reported that lipoic acid (LA) treatment in STZ-diabetic rats can restore insulin-stimulated glucose transport by normalizing GLUT4 transporters, as well as muscle lactate concentration. LA may interfere with protein degradation induced by changes in sulfhydryl groups/disulfide bands ratio (SH/S-S) present in diabetes responsible for increased susceptibility in proteolytic process. Indeed, in vitro free radicals, hydrogen peroxide, and ketoaldehydes produced by glucose autooxidation appear to be primary mediators of protein modifications and the peroxidation of protein-associated lipids. Here, we can only hypothesize that like GLUT4 transporters, a glucose-induced peroxidation of the lactate transporter occurs at the sarcolemmal membrane.

Because a basal level of MDA concentration exists, we cannot conclude that antioxidant defenses, including Gpx activity, are overwhelmed in such a model.

In conclusion, we found a decrease in total lactate uptake_{10s} in SV of diabetic rats at every external lactate concentration investigated using the model of SV. We also noted that the decrease in lactate transport was associated with increased production of free radicals. In addition, the decreased lactate uptake in SV of diabetic rats may result in a decreased lactate clearance. Such alteration, associated with an increased lactate production by extramuscular tissues such as adipose tissue, may lead to the increased basal blood lactate concentration often encountered in diabetes. We propose that the mechanisms underlying increased lactate production may also lead to changes in the different MCTs involved in muscle lactate transport. Further experiments are needed to determine whether oxidative stress alters transport activity.

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