

Hyperlactatemia reduces muscle glucose uptake and GLUT-4 mRNA while increasing (E1 α)PDH gene expression in rat

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Lombardi, Anna M., Roberto Fabris, Flavia Bassetto, Roberto Serra, Armelle Leturque, Giovanni Federspil, Jean Girard, and Roberto Vettor. Hyperlactatemia reduces muscle glucose uptake and GLUT-4 mRNA while increasing (E1 α)PDH gene expression in rat. *Am. J. Physiol.* 276 (*Endocrinol. Metab.* 39): E922–E929, 1999.—An increased basal plasma lactate concentration is present in many physiological and pathological conditions, including obesity and diabetes. We previously demonstrated that acute lactate infusion in rats produced a decrease in overall glucose uptake. The present study was carried out to further investigate the effect of lactate on glucose transport and utilization in skeletal muscle. In chronically catheterized rats, a 24-h sodium lactate or bicarbonate infusion was performed. To study glucose uptake in muscle, a bolus of 2-deoxy-[³H]glucose was injected in basal condition and during euglycemic-hyperinsulinemic clamp. Our results show that hyperlactatemia decreased glucose uptake in muscles (i.e., red quadriceps; $P < 0.05$). Moreover in red muscles, both GLUT-4 mRNA (–30% in red quadriceps and –60% in soleus; $P < 0.025$) and protein (–40% in red quadriceps; $P < 0.05$) were decreased, whereas the (E1 α)pyruvate dehydrogenase (PDH) mRNA was increased (+40% in red quadriceps; $P < 0.001$) in lactate-infused animals. PDH protein was also increased (4-fold in red gastrocnemius and 2-fold in red quadriceps). These results indicate that chronic hyperlactatemia reduces glucose uptake by affecting the expression of genes involved in glucose metabolism in muscle, suggesting a role for lactate in the development of insulin resistance.

pyruvate dehydrogenase; insulin resistance; substrate competition

A DECREASED ABILITY of insulin to regulate glucose utilization is a major feature of different pathological conditions, including obesity, hypertension, and non-insulin-dependent diabetes mellitus (NIDDM) (10, 18). In those states, an increase in blood lactate is often present, ranging from ~0.6 mmol/l in lean subjects to 0.8 and 1.1 mmol/l in obese and NIDDM patients (12), respectively. Lactate, the end product of nonoxidative glycolysis, derives from many sources. The major lactate production is coming from skeletal muscle, but brain, skin, and erythrocytes also contribute to it (26). Lately, it has been demonstrated that adipose tissue

may be a relevant source of blood lactate, the production of which is correlated to fat cell size (25).

Recently, Di Girolamo and colleagues (12, 32) hypothesized that the hyperlactatemia might not be simply a consequence of insulin resistance but might possibly play a pathogenic role in the maintenance of insulin resistance.

In previous studies, when rats were acutely infused with lactate, a decrease in insulin-dependent glucose uptake was observed mainly at the muscular level (48). To date, no mechanistic explanation has been proposed for this phenomenon. In humans (17, 50) and rodents (21, 49), the decreased whole body insulin-mediated glucose uptake associated with obesity and NIDDM has been localized at the level of glucose transport. This event is considered a rate-limiting step for glucose disposal in skeletal muscles.

At present, it is not clear whether the increased availability of a nonglucidic substrate may interfere with glucose transport activity, directly affecting the specific transporter gene expression. In animals fed a high-fat diet, a decreased insulin-stimulated glucose transport in skeletal muscle and adipose tissue has been observed (19, 22, 30, 46, 51). This fact may be the result of a series of events starting from the inhibition of pyruvate dehydrogenase (PDH) and phosphofructokinase activity and leading to an impairment in glucose transport or phosphorylation. As a matter of fact, in isolated adipocytes, free fatty acids (FFAs) have been found to reduce GLUT-4 gene expression (31).

Lactate has a similar influence on glucose uptake, but it is still unknown whether this phenomenon may occur at the level of glucose transport gene expression or elsewhere in the intracellular glucose utilization pathway.

Our aim was to investigate whether chronic hyperlactatemia may influence insulin-dependent glucose uptake acting at the glucose transport level. Moreover, it is well established that in insulin-resistant states, as a consequence of an increased FFA oxidation, the relative abundance of Krebs cycle intermediates may reduce both PDH (42) and phosphofructokinase activity (44, 52), thus decreasing glucose utilization through the glycolytic pathway. On the contrary, little is known about the competition between glucose and lactate, which could be oxidized by the muscle after conversion into pyruvate or in a lesser extent incorporated into glycogen via the gluconeogenic pathway (35). In both situations, PDH activity becomes the clue to under-

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standing the mechanism of the phenomenon. Therefore, we also studied the net effect on (E1 α)PDH mRNA, the catalytic subunit of the PDH enzymatic complex, in the same groups of animals.

MATERIALS AND METHODS

Adult male lean (*fa/?*) Zucker rats (Charles River, Lecco, Lombardia, Italy) were purchased at a body weight of 160–180 g and housed at 24°C with a 0700 to 1900 light cycle. They had free access to water and chow pellets. At a weight of ~200 g, they were randomly assigned to either a control or a lactate group. All animals were studied in the morning after 8 h of fasting.

The study protocol was approved by the institutional review board of the University of Padova (Padova, Italy).

Surgical procedure. All rats were anesthetized with an intraperitoneal injection of 500 mg/kg of chloral hydrate (Merck, Darmstadt, Germany). When surgical anesthesia was confirmed by the absence of corneal and toe-pinch reflexes, two Tygon catheters (ID, 0.1 mm; OD, 0.3 mm; Masterflex Cole-Parmer Instruments, Niles, IL) filled with a saline solution were inserted in the right femoral vein and artery, respectively, for venous infusion and blood sampling. At the end of anesthesia, the animals were placed in individual cages with a single slot for the way out of the catheters, which were maintained in continuous tension. The 24-h patency of the arterial catheter was maintained by a slow infusion of saline solution. The animals were freely moving.

Glucose clamp during bicarbonate or lactate infusion. In a first group of five rats, after a 12-h recovery from surgery, an infusion of L-(+)-lactate sodium salt diluted in phosphate buffer (0.06 M NaH₂PO₄ and 0.0134 M Na₂HPO₄) was started and continued at a rate of 130.6 $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ for 24 h. A second group of five animals was infused with sodium bicarbonate buffer at a rate of 32.65 $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$; the dose of bicarbonate was calculated as 0.25/lactate dose in millimoles per hour (33). Blood samples were collected before the beginning of the infusion for the determination of basal blood glucose, plasma insulin, FFA, and lactate concentration. After the 24-h lactate or bicarbonate infusion, a primed-continuous infusion of human insulin (Actrapid HM, Novo, Copenhagen, Denmark, dissolved in 0.9% saline solution) at a rate of 3 mU/min was performed and carried on for 120 min. Blood samples for plasma glucose, FFA, lactate, and insulin were collected before the beginning and at the end of clamp. Arterial blood was sampled at 5-min intervals throughout the clamp for determination of plasma glucose concentration. Glucose (20% wt/vol solution) was infused starting 1 min after the beginning of insulin infusion. The glucose infusion rate was adjusted to maintain plasma glucose at the preinfusion levels as previously described (11).

Tissue glucose utilization index. Glucose utilization in vivo within individual tissues was studied in a first group of eight rats in basal conditions, after a 24-h lactate or bicarbonate infusion. In a second group of 10 animals, treated with either lactate or bicarbonate, tissue glucose utilization index was assessed during the euglycemic-hyperinsulinemic clamp.

Briefly, 30 μCi of the nonmetabolizable glucose analog 2-deoxy-D-[1-³H]glucose (2-DG) were injected in 30 μl of 0.9% NaCl solution as a bolus through the femoral vein, according to a previously described method (15, 20). Blood samples (50 μl) for determination of plasma glucose and tracer concentrations were obtained from the arterial catheter 1, 3, 5, 10, 15, 20, 30, 40, and 60 min after bolus administration.

At the completion of blood sampling, rats were killed and skeletal muscles were quickly removed, collected in liquid

nitrogen, and kept frozen at –80°C for the subsequent analysis.

The glucose utilization index was derived from the amount of 2-deoxy-[1-³H]glucose 6-phosphate (2-DG-6-P) measured in various tissues as previously described (15). This technique therefore uses the accumulation of 2-DG-6-P as an index of the glucose metabolic rate in individual tissues.

Quantification of GLUT-4 and (E1 α)PDH mRNA. In a separate series of experiments, after a 12-h recovery period from surgery, eight rats were infused with bicarbonate or lactate for 24 h. Then they were killed and muscular tissues (red quadriceps and soleus for GLUT-4 and red quadriceps for PDH) were immediately removed, rinsed in NaCl solution, and frozen in liquid nitrogen. Total mRNA was isolated from muscular tissues with the guanidine thiocyanate method (7). The concentration of RNA was determined by absorbance at 260 nm, and the RNA was stored at –80°C. All the samples had a 260-to-280 absorbance ratio of ~2.0. The RNA (20 μg) was denatured and size fractionated on a 1% agarose, 2 M formaldehyde gel. After staining (0.5 $\mu\text{g}/\text{ml}$ ethidium bromide), the 28S and 18S ribosomal bands were visualized and photographed by ultraviolet transillumination to ensure that the RNA was intact and evenly loaded. The RNA was then transferred to Hybond-N, which was ultraviolet cross-linked. Northern blots and hybridization were performed as previously described (40). The cDNA probe of the rat GLUT-4 (1.5-kb insert) and the probe of the human PDH E1 α subunit (1.6-kb insert) were used. Quantification was performed by scanning densitometry.

Quantification of GLUT-4 and PDH protein. Muscles were homogenized with a buffer saline (1 mM NaHCO₃, 0.2 mM MgSO₄, 0.2 mM CaCl₂), and the pellet was then resuspended with a sucrose buffer (10 mM HEPES, 250 mM sucrose, 1 mM EDTA) and diluted with Laemmli buffer. The protein concentration of each sample was determined with the Bio-Rad assay (Bio-Rad, Munich, Germany) with bovine serum albumin as standard. The proteins (50 μg) were analyzed under denaturing conditions on a 10% SDS polyacrylamide gel. (29). After transfer to nitrocellulose, the membrane was blotted with a monoclonal antibody directed against GLUT-4 (Genzyme Diagnostic, Cambridge, MA). To detect the three subunits that form the PDH complex, E1(α + β), E2, and E3, a polyclonal antibody was utilized (34). The overall pattern of PDH complex of rat skeletal muscle showed six bands with apparent molecular mass values of 100, 76, 57, 48, 37, and 20 kDa. Among those we measured by scanning densitometry, the band of 57 kDa was the only one that exhibited variations. The detection was performed with an ECL phosphorylation detection system (Amersham International, Buckinghamshire, UK).

Analytical procedures and calculations. Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer; Beckman Instruments), and plasma insulin was measured by radioimmunoassay with rat and human standards (Techno Genetics, Milano, Italy; Linco Research, St. Charles, MO). Plasma lactate and FFA were determined with commercial kits (Boehringer Mannheim, Mannheim, Germany; Boehringer Mannheim, Tokyo, Japan). Blood pyruvate was spectrophotometrically determined with an enzymatic method (Boehringer Mannheim). Intracellular lactate concentration was determined in red gastrocnemius by means of the same commercial kit utilized for plasma samples after deproteinization with 6% perchloric acid. Glycogen content in red quadriceps was evaluated as previously described (37).

Blood samples (50 μl) for determination of 2-DG specific activity were deproteinized with 250 μl ZnSO₄ and 250 μl Ba(OH)₂ and immediately centrifuged. After measurement of

glucose concentration with a glucose oxidase kit (Boehringer), an aliquot of the supernatant was evaporated to dryness at 70°C, resuspended in water, and counted in scintillation fluid (Instagel, Packard) to determine 2-DG plasma radioactivity.

Determination of 2-DG-6-*P* was performed, taking into account its nonsolubility in the Somogy reagent system. Briefly, tissue solubilization was obtained by adding 0.5 ml of NaOH (1 N) into each tube. After incubation at 80°C until complete digestion, the samples were neutralized with 0.5 ml of HCl (1 N). Two aliquots of the neutralized solution were added to 1 ml of HClO₄ (1 N) and to 1 ml of ZnSO₄/BaOH₂, respectively (15). Radioactivity was then calculated in the supernatant after centrifugation.

For the measurement of the glucose utilization index, the 2-DG-6-*P* concentration in the tissues was calculated as the difference between the radioactivity after HClO₄ precipitation (2-DG + 2-DG-6-*P*) and the radioactivity after Somogy precipitation (2-DG-6-*P*). From this measurement of the accumulation of 2-DG-6-*P* per unit mass at 60 min, the steady-state plasma glucose concentration over the 60-min period and the time course of the 2-DG, the glucose utilization index was then calculated according to the previously described equation (15, 20) and expressed as nanograms per milligrams per minute.

Statistical analysis. All values are presented as means \pm SE. Statistical analysis was performed by ANOVA.

RESULTS

Metabolic parameters. The lactate infusion significantly raised plasma lactate in comparison with bicarbonate-treated rats. No significant differences in blood glucose, insulin, or FFA at the end of 24 h of lactate or bicarbonate infusion were observed (Table 1). Lactate infusion led to a sevenfold increase in blood pyruvate vs. controls (287 ± 18.5 vs. 39 ± 8.3 μ mol/l; $P < 0.01$).

Lactate infusion provoked a significant increase in pH arterial values from 7.37 ± 0.03 to 7.59 ± 0.03 at the end of clamp ($P < 0.05$). The lactate infusion provoked a small but significant increase in plasma bicarbonate at the end of the study (24.03 ± 1.08 to

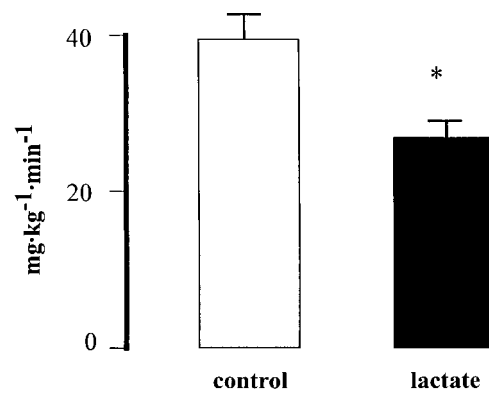


Fig. 1. Glucose infusion rate as assessed at end of clamp in lactate- or bicarbonate-treated rats. Each bar is mean \pm SE of 5 experiments for each group. * $P < 0.001$.

33.50 ± 0.49 mmol/l; $P < 0.05$). During bicarbonate infusion, pH and bicarbonate arterial values increased at a similar extent as in the lactate experiment (pH: 7.39 ± 0.15 to 7.51 ± 0.01 ; bicarbonate: 25.65 ± 1.65 to 34.75 ± 1.75 mmol/l).

At the end of clamp, there were no significant changes in blood glucose, whereas FFA plasma concentration was clearly decreased in both groups of animals (Table 1).

The intracellular lactate concentration in the red gastrocnemius significantly increased in lactate-treated rats (0.54 ± 0.02 vs. 0.23 ± 0.02 mg/g; $P < 0.01$) in comparison with controls; in the same group of animals, the intracellular glycogen content also exhibited a twofold increase in the red portion of quadriceps (19.15 ± 0.85 vs. 10.86 ± 1.2 mg/g; $P < 0.05$).

Euglycemic-hyperinsulinemic clamp. The glucose infusion rate at the steady state was reduced by 38% in lactate-treated rats compared with controls ($P < 0.001$; Fig. 1).

The index of glucose utilization assessed in basal condition showed no differences between lactate- and bicarbonate-treated animals in soleus (6.4 ± 0.9 vs. 9.2 ± 2.6), white (1.3 ± 0.3 vs. 1.4 ± 0.5) and red (1.7 ± 0.29 vs. 2.5 ± 0.4) gastrocnemius, white (0.9 ± 0.2 vs. 0.5 ± 0.1) and red (3.5 ± 0.5 vs. 3.4 ± 1.4) quadriceps, extensor digitorum longus (2.4 ± 0.3 vs. 1.3 ± 0.5), and tibialis (1.0 ± 0.2 vs. 0.6 ± 0.1 ng·mg⁻¹·min⁻¹) muscle.

The crucial step was to measure glucose uptake in individual tissues after an euglycemic-hyperinsulinemic clamp. In this condition, the glucose utilization index in control animals showed an increase of 72% compared with the glucose utilization index assessed in basal conditions (i.e., red quadriceps; $P < 0.05$). On the contrary, lactate-infused rats did not show any increase at the end of clamp vs. basal conditions. Moreover, in all red fiber muscles of lactate-treated rats, the glucose utilization index displayed a significant decrease compared with controls (i.e., 40% reduction in red quadriceps; $P < 0.05$; Fig. 2), whereas no difference was observed among white fiber muscles.

Quantification of GLUT-4 and (E1 α)PDH mRNA and protein in skeletal muscle. Lactate-treated rats showed a reduction in GLUT-4 mRNA of 60% in soleus ($P <$

Table 1. Metabolic parameters measured in basal conditions, after 24-h lactate or bicarbonate infusion, and at end of an euglycemic-hyperinsulinemic clamp, as assessed in conscious, lean Zucker rats

	Blood Glucose, mmol/l	Insulin, μ U/ml	Lactate, mmol/l	FFA, μ mol/l
<i>Basal conditions</i>				
Control	6.1 ± 0.5	31.3 ± 5.5	0.90 ± 0.06	798 ± 104
Lactate	6.6 ± 0.3	25.0 ± 2.8	0.95 ± 0.2	710 ± 43
<i>Before clamp</i>				
Control	5.9 ± 0.4	35.7 ± 7.2	1.24 ± 0.16	902 ± 108
Lactate	7.1 ± 0.4	28.6 ± 13.8	$4.2 \pm 0.87^\dagger$	631 ± 78
<i>End of clamp</i>				
Control	6.0 ± 0.3	441.1 ± 48.0	$1.98 \pm 0.11^*$	$214 \pm 73^\dagger$
Lactate	5.9 ± 0.2	420.7 ± 84.6	$4.76 \pm 1.18^\dagger$	$129 \pm 8^\dagger$

Values are means \pm SE of 5 experiments/group. Basal conditions, after 12-h recovery period; before clamp, after 24-h lactate or bicarbonate infusion; end of clamp, after 120 min of euglycemic-hyperinsulinemic clamp. * $P < 0.05$ after clamp vs. before clamp; $^\dagger P < 0.01$ after clamp vs. before clamp; $^\ddagger P < 0.05$ lactate vs. control.

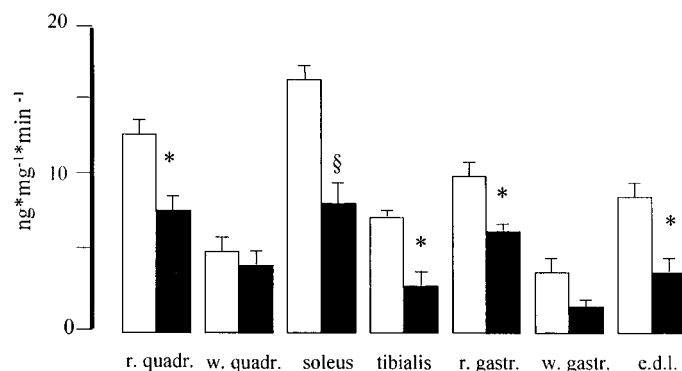


Fig. 2. Glucose utilization index measured in skeletal muscles in lactate- (solid bars) and bicarbonate- (open bars) infused rats at end of euglycemic-hyperinsulinemic clamp (see MATERIALS AND METHODS). Each bar is mean \pm SE of 5 experiments for each group. R. quadr., red quadriceps; w. quadr., white quadriceps; r. gastr., red gastrocnemius; w. gastr., white gastrocnemius; e.d.l., extensor digitorum longus. * $P < 0.05$. § $P < 0.001$.

0.05) and 30% in red quadriceps ($P < 0.05$) compared with controls (Fig. 3). The GLUT-4 protein levels appeared also significantly reduced (40% in red quadriceps; $P < 0.05$; Fig. 4).

On the contrary, the lactate-treated rats showed a 40% increase in the (E1 α)PDH mRNA levels in the red portion of the quadriceps muscle vs. the control group ($P < 0.001$; Fig. 5). PDH protein showed a fourfold increase in red gastrocnemius and a twofold increase in red quadriceps vs. controls (Fig. 6).

DISCUSSION

More than 30 years ago Randle et al. (41) introduced the concept of substrate competition between glucose and FFA. According to this hypothesis, increased lipid oxidation with elevated FFA suppresses glucose oxidation at the level of PDH (24, 27) and glycolysis at the level of phosphofructokinase (44, 52). Recently, evidence has been gathered about a similar competitive effect also between glucose and lactate (13, 14). In fact, lactate, which increases in blood circulation in obesity and NIDDM, may be taken up by peripheral tissues, where it can be converted into pyruvate (4, 8). The latter is added to the glucose-derived pyruvate, and both may be further oxidized in the tricarboxylic acid cycle. Alternatively, lactate can be converted into glucose or to fatty acids in liver cells (38).

We previously demonstrated that acute hyperlactatemia obtained in anesthetized rats is able to decrease whole body glucose disposal without affecting hepatic glucose production (HGP). The reduction of insulin-dependent glucose uptake was mainly localized in skeletal muscles, thus supporting the hypothesis that lactate may be important in the development of carbohydrate intolerance and insulin resistance (48).

In the present study, the 24-h lactate infusion led to a threefold increase in basal arterial concentrations of lactate, without affecting basal glycemia, insulin, or FFA plasma levels. During euglycemic-hyperinsulinemic clamp, total glucose disposal was significantly decreased in lactate-infused rats compared with con-

trols. It has been demonstrated that an accelerated flux of lactate to the liver may enhance gluconeogenesis without affecting HGP (8). In the present study we did not measure HGP, so we cannot exclude a residual endogenous glucose production. However, in a previous study, similar insulin levels were able to induce a suppression of HGP during lactate or bicarbonate infusion in anesthetized rats (48), which usually exhibit a higher HGP than conscious animals. Then we can presume that, in our experimental model, the glucose infusion rate is representative of overall glucose disposal.

Moreover, one should take into account that the lactate group had energy supply from the lactate infusion, whereas the controls were continuously starved. Because it has been reported that fasting may influence insulin sensitivity by increasing the insulin-dependent glucose transporter in muscle (6), we cannot rule out the possibility that the observed differences in

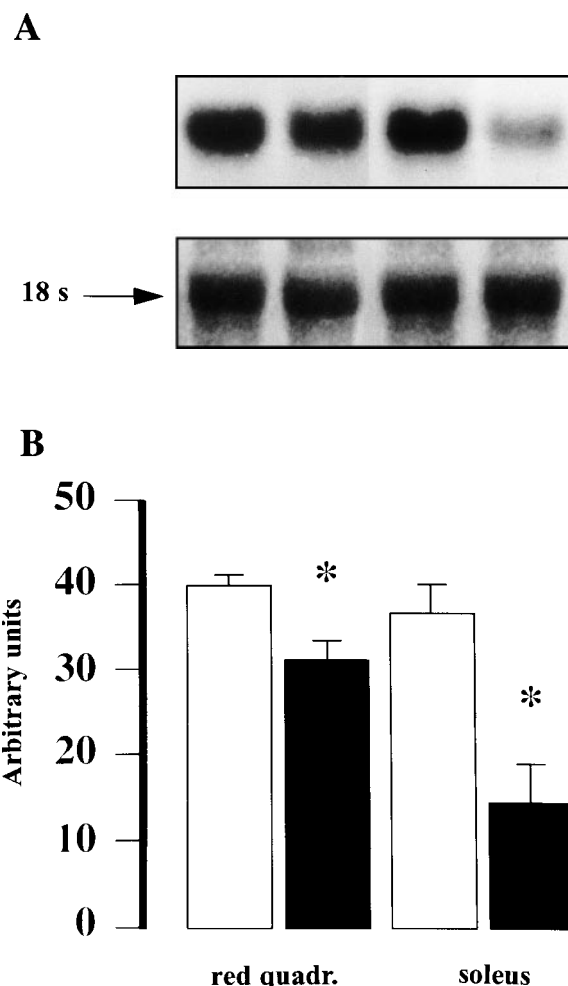


Fig. 3. Effect of 24 h of lactate (solid bars) or bicarbonate (open bars) infusion on GLUT-4 mRNA concentrations in red quadriceps (red quadr.) and soleus. Total RNA was isolated as described in MATERIALS AND METHODS. RNA (20 μ g) was applied to each lane. A: representative autoradiogram with each lane representing a single muscle preparation; top bands, red quadriceps and soleus GLUT-4 mRNA; bottom bands, 18S rRNA. B: GLUT-4 mRNA densitometric analysis ($n = 4$ /group) is mean \pm SE. * $P < 0.05$.

overall glucose disposal may be partially due to an increased insulin sensitivity in the control group. However, similar findings were also obtained in our previous experiment (48), during which no substrate supply was applied to any of the two groups of animals before an acute lactate infusion, thus suggesting an effect of lactate per se on insulin sensitivity.

As a general rule, when insulin-mediated glucose uptake is considerably reduced, the skeletal muscle is the predominant site of insulin resistance (9). Thus, to determine the relative contribution of the different muscles to the phenomenon, we measured the glucose utilization index in white and red fiber-type muscles.

Chronic lactate infusion did not affect the glucose uptake measured in basal condition in different skeletal muscles. On the contrary, in insulin-stimulated states, hyperinsulinemia failed to induce any significant increase in the tissue glucose utilization index in chronic lactate-infused rats, whereas a clear increase in muscle glucose utilization in control animals was

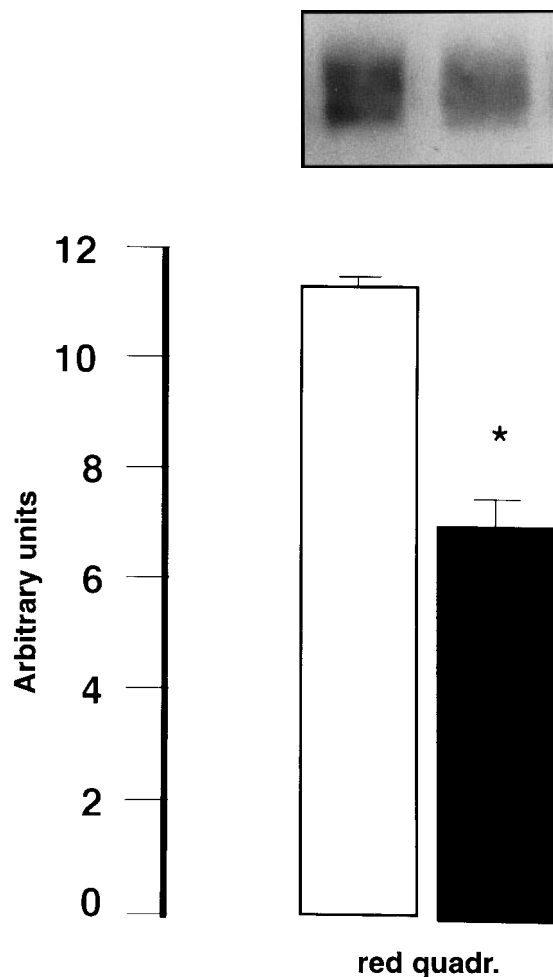


Fig. 4. Effect of 24 h of lactate (solid bars) or bicarbonate (open bars) infusion on GLUT-4 protein concentrations in red quadriceps. GLUT-4 protein was detected with a monoclonal antibody. Quantification was performed by scanning densitometry of autoradiograms from Western blot analysis. Top, representative autoradiogram with each lane representing a single muscle preparation. Data are means ($n = 4/\text{group}$) \pm SE. * $P < 0.05$.

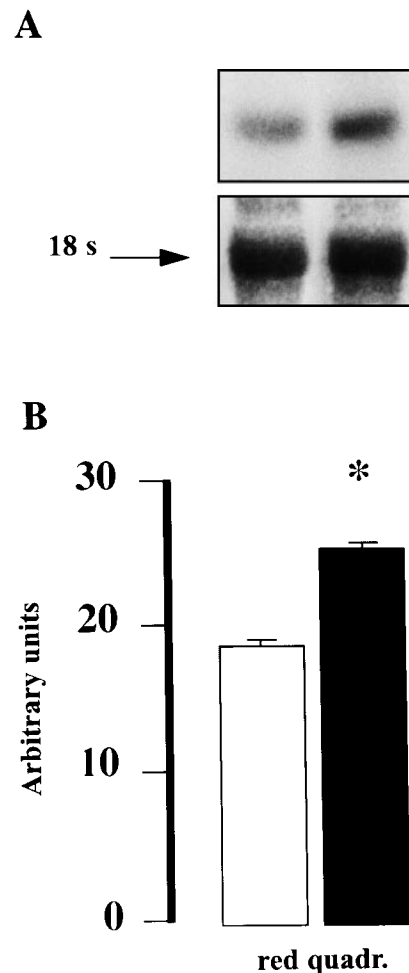


Fig. 5. Quantification of (E1 α)pyruvate dehydrogenase (PDH) mRNA in red quadriceps in lactate- (solid bars) or bicarbonate- (open bars) infused rats. A: representative autoradiogram with each lane representing a single muscle preparation; top bands, red quadriceps and soleus (E1 α)PDH mRNA; bottom bands, 18S rRNA. B: (E1 α)PDH mRNA densitometric analysis ($n = 4/\text{group}$) is mean \pm SE. * $P < 0.05$.

observed. These data confirm our previous report of a metabolic interference between lactate and glucose, mainly at the muscular level. This point has been substantiated in vitro in other experiments showing that, at increasing lactate concentrations in the medium, a decline in glucose utilization by isolated rat soleus is detected (39).

To further elucidate how from a mechanistic point of view this phenomenon may occur, the interference between lactate and glucose metabolism was assessed. Interferences might occur at different steps. Glucose clearance is a process that involves both transport and phosphorylation. In skeletal muscles, glucose transport is mediated by GLUT-4, whereas phosphorylation is catalyzed by hexokinase II (23). It is noteworthy that a selective overexpression of GLUT-4 in skeletal muscles of transgenic mice appears to significantly improve insulin sensibility and glucose uptake (28, 43) and that a reduction of GLUT-4 gene expression is able to induce insulin resistance at the adipose tissue level (45). However, skeletal muscle is the site of >80% of insulin-

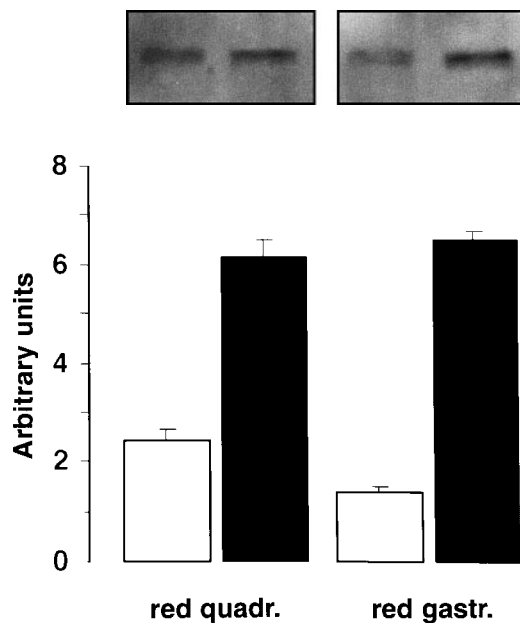


Fig. 6. Effect of 24 h of lactate (solid bars) or bicarbonate (open bars) infusion on (E1 α)PDH protein concentrations in red quadriceps and red gastrocnemius (red gastr.). (E1 α)PDH protein was detected with a polyclonal antibody. Quantification was performed by scanning densitometry of autoradiograms from Western blot analysis. Top, representative autoradiogram with each lane representing a single muscle preparation. Data are means ($n = 4/\text{group}$) \pm SE. $P < 0.01$.

mediated glucose uptake *in vivo* (9). Nonetheless, even if in several pathological conditions insulin resistance may be associated with normal GLUT-4 mRNA levels in skeletal muscles, it has been shown that a pretranslational suppression of GLUT-4 is present at muscular level in some experimental models of obesity (22).

Our data show that during chronic hyperlactatemia, in the presence of normal glucose and insulin plasma levels, a significant reduction of GLUT-4 mRNA exists in the muscle, together with a decrease in GLUT-4 protein levels. Likewise, in different experimental conditions, also characterized by increased lactate plasma levels, changes in GLUT-4 mRNA and protein have been observed. As a matter of fact, in the streptozotocin-induced diabetes an impaired glucose transport has been found in red fiber-type skeletal muscles, mainly due to a decrease in GLUT-4 protein and mRNA content (16). On the other hand, in fasting conditions, an increase in GLUT-4 mRNA occurs only in white muscles (5). These situations are characterized by decreased insulin and increased glucagon and lactate plasma levels, but GLUT-4 mRNA and protein content go clearly in opposite directions. The main differences between these two conditions are hyperglycemia in diabetes and the presence of maintained basal insulin levels during fasting. The toxic effect of hyperglycemia may justify, at least in part, the reduction of GLUT-4 mRNA and protein levels in skeletal muscle observed in streptozotocin-diabetic rats, because the correction of hyperglycemia by fasting (3) or vanadate treatment (47) was accompanied by an increase in GLUT-4 mRNA and protein. So far, no data are available on the possible toxic effect of chronic hyperlactatemia on GLUT-4 gene

expression. Our findings represent the first observation *in vivo* about an influence on GLUT-4 expression by an intermediate of glycolysis.

In our experiments, a twofold increase in glycogen content in lactate-infused animals at the muscular level was found. These data agree with those obtained in a previous study performed in our lab, in which anesthetized rats were submitted to an acute infusion of lactate and then studied during hyperglycemic clamp (37). Although the experimental design of the present study is quite different and we did not analyze muscle glucose 6-phosphate and the key enzymes involved in glycogen formation, the results we obtained do not exclude the possibility that the increased availability of lactate may lead to an increased synthesis of glycogen, probably through an accelerated gluconeogenic pathway.

Another topic of this study was to investigate the net effect of a chronic infusion of lactate on the catalytic subunit of the (E1 α)PDH mRNA. The data showing an enhanced gene expression of this catalytic subunit, confirmed by the protein assessment, are not completely unexpected: it may be the consequence of an increased availability of its substrate, that is, pyruvate. In fact, the intracellular pool of pyruvate is mainly determined by the rate of glycolysis, the rate of oxidation in the tricarboxylic cycle, and the equilibration between pyruvate and lactate (36). The decreased glucose uptake observed during hyperlactatemia might lead *per se* to a reduction of the glycolytic flux and its end products, such as pyruvate. In our experimental conditions, however, the increased availability of lactate provoked a rise in pyruvate levels as a consequence of the acceleration of the lactate-pyruvate interconversion. The enhanced (E1 α)PDH mRNA may thus not only reflect the preferential oxidation of the excessively formed substrate but may also represent another competitive pathway between glucose and lactate. This hypothesis agrees with the recent observation that in obese NIDDM patients, in which a significant rise in lactate plasma levels is present, a concomitant acceleration of the interconversion rate of lactate into pyruvate was noted (1). Moreover, to date and to the best of our knowledge, no data are available describing a direct effect of lactate on PDH gene expression.

With respect to the well-known FFA-glucose interaction, it was observed that lowering FFAs in NIDDM patients did not normalize insulin-stimulated glucose uptake, indicating that FFA could account for only a part of the insulin resistance in diabetes and that a major part, perhaps as much as 50%, was unrelated to fatty acids (2). Our results raise the possibility of a more complex interaction between substrates and in particular between glucose and lactate.

It must also be noted that an increased availability of lactate is able to reduce glucose uptake, mainly at the level of glucose transport. On the other hand, from the point of view of energy, lactate appears to be a more efficient substrate compared with glucose and can be directly oxidized via the PDH complex. It is possible to

suggest, also, that the (E1 α)PDH gene expression may be influenced by a chronic overflow of its specific substrate.

In conclusion, our data show that lactate, widely considered an end product of glycolysis, may play a competitive role vs. glucose, modulating the gene expression of key steps of glucose utilization, namely GLUT-4 and PDH, thus adding new insights in the development of insulin resistance.

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