Lactate Infusion in Anesthetized Rats Produces Insulin Resistance in Heart and Skeletal Muscles

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Plasma lactate is elevated in many physiological and pathological conditions, such as physical exercise, obesity, and diabetes, in which a reduction of insulin sensitivity is also present. Furthermore, an increased production of lactate from muscle and adipose tissue together with increased gluconeogenic substrate flux to the liver plays a primary role in enhancing hepatic glucose production (HGP) in diabetes. It has been shown that lactate may interfere with the utilization and oxidation of other substrates such as free fatty acids (FFAs). The aim of this study was to investigate if lactate infusion affects peripheral glucose utilization in rats. Animals were acutely infused with lactate to achieve a final lactate concentration of 4 mmol/L. They were then submitted to a euglycemic-hyperinsulinemic clamp to study HGP and overall glucose metabolism (rate of disappearance [Rd]). At the end of the clamp, a bolus of 2-deoxy-(1-^{3}H)-glucose was injected to study insulin-dependent glucose uptake in different tissues. The results show that lactate infusion did not affect HGP either in the basal state or at the end of clamp, whereas glucose utilization significantly decreased in lactate-infused rats (26.6 ± 1.1 *v* 19.5 ± 1.4 mg · kg^{-1} · min^{-1}, $P < .01$). A reduction in the tissue glucose utilization index was noted in heart (18.01 ± 4.44 *v* 46.21 ± 6.51 ng · mg^{-1} · min^{-1}, $P < .01$), diaphragm (5.56 ± 0.74 *v* 9.01 ± 0.93 ng · mg^{-1} · min^{-1}, $P < .01$), soleus (13.62 ± 2.29 *v* 34.05 ± 6.08 ng · mg^{-1} · min^{-1}, $P < .01$), and red quadricep (4.43 ± 0.73 *v* 5.88 ± 0.32 ng · mg^{-1} · min^{-1}, $P < .05$) muscle in lactate-infused animals, whereas no alterations were observed in other muscles or in adipose tissue. Therefore, we suggest that acute lactate infusion induces insulin resistance in the heart and some muscles, thus supporting a role for lactate in the regulation of peripheral glucose metabolism.

LACTATE IS THE END PRODUCT of glycolysis in many tissues and is also the major gluconeogenic substrate in the liver. In the fasting state, the brain, skin, erythrocytes, and skeletal muscle are the main organs of lactate production, which mostly derives from glucose and glycogen breakdown.[1] More recently, lactate release from adipose tissue has also been recognized as a relevant source of blood lactate, and increasing production from isolated adipocytes was reported in relation to fat cell size.[2] The metabolic fate of lactate is presently not completely understood; lactate may be taken up by different organs, but muscle and liver appear to be the major sites of lactate removal from the circulation. In resting conditions, lactate utilization by muscles appears to be largely dependent on oxidation,[3] whereas in the liver lactate can be oxidized as fuel for energy production, enter the gluconeogenic pathway, or be used for glycogen and lipid synthesis.[4] Lactate increases in the blood in many physiological and pathological conditions such as physical exercise, fasting, non–insulin-dependent diabetes mellitus, obesity, and hypertension.[5]

A glucose-sparing effect by nonglucidic fuels has been demonstrated for fatty acids and ketone bodies. Inhibition of glucose uptake and oxidation in muscles and heart by increased fatty acid oxidation or ketone bodies[6] has been explained by the observation that these substrates inhibit glucose oxidation at the level of pyruvate dehydrogenase.[7] In diabetes mellitus and obesity, the increased production of energy substrates such as free fatty acids (FFAs) and ketone bodies is capable of exerting metabolic effects that interfere with glucose metabolism. A fuel interaction has been suggested also for lactate and FFA, since lactate infusion resulted in a clear reduction of the release of FFA from fat stores and its oxidation in dogs.[8] Moreover, a previous study in dogs showed a suppression of glucose oxidation by lactate infusion in the heart.[9]

Specific lactate transporters have been described in skeletal and heart muscle,[10] and an increased lactate uptake and oxidation has been noted during hyperinsulinemia in human skeletal muscle[11] and in dog heart muscle.[12] At present, there is no clear evidence for an interaction between lactate and glucose for a common oxidative energy-producing pathway. In the rat heart, it has been shown that lactate interferes with glucose oxidation by shifting glucose metabolism toward glycogen synthesis, thus increasing its nonoxidative utilization.[13] Infusing lactate in healthy volunteers induced no changes in overall glucose appearance[14] or in whole-body or forearm muscle glucose utilization,[15] but in these experiments relatively low levels of blood lactate were reached.

A few years ago, DiGirolamo et al[5] hypothesized that in conditions in which insulin resistance and increased lactate concentration are present, namely obesity and diabetes, the latter is not only a consequence of the former, but may play a pathogenetic role in the perpetuation of insulin resistance. Thus, we speculate that increased lactate production may play a role in diminishing the insulin capacity to stimulate glucose metabolism. The present study was therefore performed to investigate the effect of sustained lactate infusion on overall glucose metabolism, also providing a quantitative in vivo measurement of insulin sensitivity in individual tissues in rats.

MATERIALS AND METHODS

Adult male Sprague-Dawley rats (Morini, Reggio Emilia, Italy) were purchased at a body weight of 160 to 180 g and housed at 24°C with a 7 AM to 7 PM light cycle. They had free access to water and chow pellets

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(Zoofarm, Padova, Italy) containing 5% cellulose, 2.5% fat, and 17.5% protein. At a weight of approximately 200 g, they were randomly assigned to either the control group or lactate group. All animals were studied after an overnight fast.

Surgery

In all animals after intraperitoneal anesthesia with phenobarbital (50 $mg \cdot kg^{-1}$), two indwelling catheters were inserted into the right jugular vein for infusion of the substances and a third catheter was inserted in the left carotid artery for blood sampling, as previously described in detail.[16] Patency of the arterial catheter was maintained by slowly infusing saline. A tracheotomy was performed to avoid respiratory problems. Body temperature of anesthetized rats was maintained at 37°C using a heating blanket connected to a rectal probe.

Glucose Clamp During Bicarbonate or Lactate Infusion

In six rats after an equilibration period of 0.5 hours, a baseline 30-minute saline infusion was started, followed by a primed-continuous infusion of L(+)-lactate sodium salt diluted in phosphate buffer (NaH_2PO_4 0.06 mol/L and Na_2HPO_4 0.0134 mol/L, pH 4.5) at a rate of 175 $\mu mol \cdot min^{-1} \cdot kg^{-1}$. A second group of six rats was infused with bicarbonate buffer at a rate of 43.75 $\mu mol \cdot min^{-1} \cdot kg^{-1}$; the dose of bicarbonate was calculated as 0.25 times the lactate dose. After the 30-minute lactate or bicarbonate infusion, a primed-continuous infusion of human insulin dissolved in 0.9% saline solution (Actrapid HM; Novo, Copenhagen, Denmark) at a rate of 3 mU/min was then begun and administered for 120 minutes.

Blood samples for determination of plasma glucose, FFA, lactate, alanine, and insulin were collected in basal conditions, after the 30-minute lactate infusion, and at the end of the clamp. Arterial blood (30 μL) was sampled at 5-minute intervals throughout the insulin infusion and immediately centrifuged for determination of plasma glucose. Glucose (20% wt/vol solution) was infused starting 1 minute after the beginning of insulin infusion. The glucose infusion rate (GIR) was adjusted to maintain plasma glucose at preinfusion levels, as previously described.[17] New steady-state conditions for plasma glucose and insulin concentrations were obtained between 30 and 40 minutes after beginning the clamp studies. During clamp studies, mean plasma glucose was kept constant at baseline values with a coefficient of variation less than 5%.

Glucose Turnover Rate

Starting 60 minutes before the insulin infusion and 30 minutes before lactate or bicarbonate buffer infusion, a group of 12 rats received a primed (2.25 μCi) infusion (0.15 μCi/min) of [3-^{3}H]-D-glucose (Amity-Amersham, Lipple, Chalfont, UK) that was maintained unchanged until the end of the study.

In a separate series of experiments, we measured the time course of variation of plasma [3-^{3}H]-D-glucose specific activity; steady state was reached within 20 minutes (data not shown).

Blood samples (100 μL) for [3-^{3}H]-D-glucose measurement were drawn every 10 minutes before and after the beginning of lactate or bicarbonate infusion and during the clamp. [3-^{3}H]-D-glucose specific activity was measured during the last 10 minutes in the baseline period, during lactate or bicarbonate infusion before the clamp, and at the end of clamp study. Blood samples for insulin (100 μL) were drawn at −60, −40, −30, −10, and 0 minutes and during the last 10 minutes of the clamp.

Overall glucose utilization was calculated as the mean GIR during the last 20 minutes of the clamp study plus any residual endogenous glucose production (EGP). The rate of EGP was measured by a radioisotopic dilution technique.[16]

Tissue Glucose Utilization Index

In a second series of experiments, glucose utilization in vivo within individual tissues was studied when a new steady state was reached during the insulin clamp study in a group of seven lactate- and seven bicarbonate-infused rats. Briefly, 30 μCi of the nonmetabolizable glucose analog 2-deoxy-[1-^{3}H]-D-glucose ([^{3}H]-2DG) was injected in 30 μl 0.9% NaCl solution as a bolus through a jugular vein according to a previously described method.[18,19] Blood samples for determination of plasma glucose and tracer were obtained from the arterial catheter 1, 3, 5, 10, 15, 20, and 30 minutes after bolus administration. At the completion of blood sampling, the rats were killed and the following tissues were quickly removed and collected in liquid nitrogen and kept frozen at −70°C for subsequent analysis: soleus, extensor digitorum longus, tibialis, diaphragm, white and red quadriceps, epididymal and inguinal white adipose tissue, and heart. The glucose utilization index was derived from the amount of 2-deoxy-[1-^{3}H]-glucose-6-phosphate ([^{3}H]2DGP) measured in various tissues as previously described.[18] This technique therefore uses the accumulation of [^{3}H]2DGP as an index of the glucose metabolic rate in individual tissues.

Analytical Procedures and Calculations

Glucose level was measured in heparinized samples during the hyperinsulinemic-euglycemic clamp every 5 minutes using the glucose oxidase method (Beckman Glucose Analyzer II; Beckman Instruments, Fullerton, CA).

Blood samples (50 μL) for determination of glucose, [3-^{3}H]-D-glucose, and [^{3}H]2DG specific activity were deproteinized with 250 μL $ZnSO_4$ and 250 μL $Ba(OH)_2$ and immediately centrifuged. After measurement of glucose concentration with a glucose oxidase kit (Boehringer, Mannheim, Germany), an aliquot of the supernatant was evaporated to dryness at 70°C, resuspended in water, and counted in scintillation fluid (Instagel; Packard, Groningen, The Netherlands) to determine [^{3}H]2DG plasma radioactivity. For evaluation of the glucose turnover rate, [3-^{3}H]-D-glucose specific activity was determined using an aliquot (200 μL) of the supernatant that was then dried and reconstituted with distilled water and counted. After correction for quenching, disintegrations per minute were determined for each sample by counting the ^{3}H activity using an LKB counter (model 1272; LKB Wallac, Turku, Finland). [3-^{3}H]-D-glucose content of the infusate was determined in the same assay, and the rate of tracer infusion was then calculated in disintegrations per minute.

Determination of [^{3}H]2DGP was performed taking into account its nonsolubility in the Somogy reagent system. Briefly, tissue solubilization was obtained by adding to each tube 0.5 mL NaOH (1N). After incubation at 80°C until complete digestion, the samples were neutralized with 0.5 mL HCl (1N). Two aliquots of the neutralized solution were added to 1 mL $HClO_4$ (1N) and to 1 mL $ZnSO_4/BaOH_2$, respectively.[18] Radioactivity was then calculated in the supernatant after centrifugation.

Plasma insulin concentration in basal conditions and in samples obtained during human insulin infusion was measured in the same assay by a radioimmunoassay technique using a double-antibody against both rat and human insulin.[20]

FFAs and lactate were assayed by enzymatic-spectrophotometric methods using commercial kits. Alanine level was measured with ion-exchange chromatography using an automatic system.

Calculation of the glucose turnover rate was made in both basal conditions and after lactate infusion. It was performed also in the new steady-state condition during the hyperinsulinemic-euglycemic clamp during either bicarbonate or lactate infusion. Taking into account that changes in plasma glucose specific activity (microcuries per milligram) could be dependent on changes in exogenous unlabeled glucose infusion (GIR in milligrams per minute) and on hepatic unlabeled glucose production (EGP in milligrams per minute), the total unlabeled glucose rate of appearance (Ra) in the blood compartment was

calculated as Ra (mg/min) = GIR + EGP, and then EGP = Ra − GIR. Ra was calculated as previously described in detail[16] from the plasma glucose concentration and specific activity and the tracer constant infusion rate (Ra times microcuries per minute) assuming a glucose space of 25% of body weight as previously determined.[21] During euglycemic hyperinsulinemia, glucose Ra equals total glucose disappearance (Rd) because euglycemia is maintained by the GIR. Therefore, it is possible to calculate EGP as Rd − GIR. Overall glucose utilization was calculated as the mean GIR during the last 20 minutes of the insulin clamp study plus any residual EGP.

Glucose clearance was calculated as Rd divided by blood glucose levels in the basal state and at the end of clamp study (milliliters per minute per kilogram). For measurement of the glucose utilization index, [^{3}H]2DGP concentration in the tissues was calculated as the difference between radioactivity after $HClO_4$ precipitation ([^{3}H]2DG + [^{3}H]2DGP) and radioactivity after Somogy precipitation ([^{3}H]2DGP). From this measurement of the accumulation of [^{3}H]2DGP per unit mass at 30 minutes, the steady-state plasma glucose concentration over the 30-minute period and the time course of the [^{3}H]2DG glucose utilization index were then calculated according to the equation described previously[18,19] and expressed as nanograms per milligram per minute.

Complete gas analysis was performed on additional arterial blood samples obtained in the basal condition and at the end of the clamp on an ABL520 Radiometer (Copenhagen, Denmark).

Blood sampling caused a slight decrease in hemoglobin content at the end of that clamp, which did not differ between the two groups.

Statistical Analysis

Values are expressed as the mean ± SEM. Statistical analysis was performed by ANOVA.

RESULTS

Effect of Lactate on Basal Biochemical Profile and Glucose Turnover

Basal plasma levels of lactate, glucose, insulin, FFA, pyruvate, and alanine did not differ between the lactate group and control group. Moreover, mean basal insulin levels were unaffected by lactate infusion (Table 1).

During sodium lactate infusion, arterial lactate increased from the fasting value of 0.89 ± 0.07 to 3.13 ± 0.20 mmol/L before the clamp, and continued to increase to a value of 3.93 ± 0.61 mmol/L at the end of the clamp (Fig 1).

HGP, as expressed by the glucose turnover rate, was not affected by lactate infusion (12.7 ± 1.3 *v* 12.9 ± 1.6 mg · min^{-1} · kg^{-1}; Fig 2).

Lactate infusion caused a slight but significant increase in pH arterial values from 7.37 ± 0.03 to 7.59 ± 0.03 at the end of the clamp ($P < .05$). Lactate infusion also caused a small increase in plasma bicarbonate that became significant at the end of the study (24.03 ± 1.08 *v* 33.50 ± 0.49 mmol/L, $P < .05$).

Effect of Lactate Infusion During Hyperinsulinemic-Euglycemic Clamp

The hyperinsulinemic-euglycemic clamp was performed in 12 rats randomly assigned to the lactate group or control group. During the glucose clamp study, plasma glucose in the steady-state condition (last 20 minutes of each clamp) was 5.3 ± 0.1 and 5.9 ± 0.3 mmol/L in the bicarbonate- and lactate-infused groups, respectively, and was also well matched with the values recorded at the beginning of insulin infusion (Table 1). Insulin levels at the end of the clamp were not significantly different between the two groups (492 ± 63 *v* 612 ± 71 μU · mL^{-1}; Table 1).

During the clamp, FFA plasma concentration was clearly decreased in both groups of animals, but the insulin-induced suppression of FFA was less evident in lactate-infused animals (322 ± 38 *v* 209 ± 25 mmol/L, $P < .05$).

At the end of the clamp, a significant decrease of plasma alanine was observed in both groups (397 ± 33 and 377 ± 41 μmol/L *v* 296 ± 14 and 299 ± 20 μmol/L in the control group and lactate group, respectively, $P < .01$).

The estimation of glucose production showed that at the end of the clamp HGP was completely suppressed in both groups (Fig 2). Whole-body glucose metabolism (Rd) at the end of the clamp was clearly decreased in lactate-infused animals (26.6 ± 1.1 *v* 19.5 ± 1.4 mg · kg^{-1} · min^{-1}, $P < .01$; Fig 3). Basal glucose clearance did not differ between the two groups of rats, but was significantly lower at the end of the clamp during lactate infusion compared with bicarbonate infusion (27 ± 3 *v* 18 ± 1 ml · min^{-1} · kg^{-1}, $P < .05$).

Lactate Effect on In Vivo Glucose Metabolism in Individual Tissues

The glucose utilization index in the different muscles considered was lower in lactate- versus bicarbonate-infused rats in soleus muscle, red quadriceps muscle, and diaphragm (Fig 4).

No significant differences were evident between control and

Table 1. Metabolic Parameters Measured in the Basal Condition, After Buffer or Lactate Infusion, and at the End of the Euglycemic-Hyperinsulinemic Clamp in Anesthetized Sprague-Dawley Rats in the Control Group (n = 6) and Lactate Group (n = 6)

Group	Plasma Glucose (mmol/L)	Plasma Insulin (μU/mL)	Plasma Lactate (mmol/L)	Plasma FFA (mmol/L)	Plasma Alanine (μmol/L)
Basal conditions					
Control	6.1 ± 0.5	23 ± 12	0.90 ± 0.06	1,025 ± 58	397 ± 33
Lactate	6.6 ± 0.3	17 ± 3	0.89 ± 0.07	1,184 ± 226	377 ± 41
Before clamp					
Control	6.1 ± 0.5	19 ± 7	0.92 ± 0.06	1,162 ± 91	—
Lactate	6.6 ± 0.3	20 ± 3	3.13 ± 0.20*	1,206 ± 266	368 ± 18
End of clamp					
Control	5.3 ± 0.1	612 ± 71	1.02 ± 0.13	209 ± 25	296 ± 14‡
Lactate	5.9 ± 0.3	492 ± 63	3.93 ± 0.61*	322 ± 38†	299 ± 20‡

NOTE. Statistical analysis was performed by ANOVA.

*$P < .01$, †$P < .05$: *v* control group.

‡$P < .01$ *v* basal values.

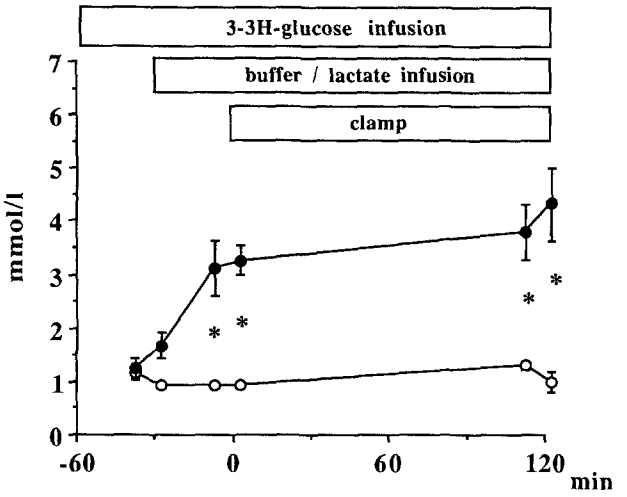

Fig 1. Arterial lactate concentration measured in buffer-infused (○) and lactate-infused (●) rats in the basal condition and during the euglycemic-hyperinsulinemic clamp. Each point represents the mean ± SEM of 6 experiments. Statistical analysis was performed by ANOVA. *$P \leq .01$.

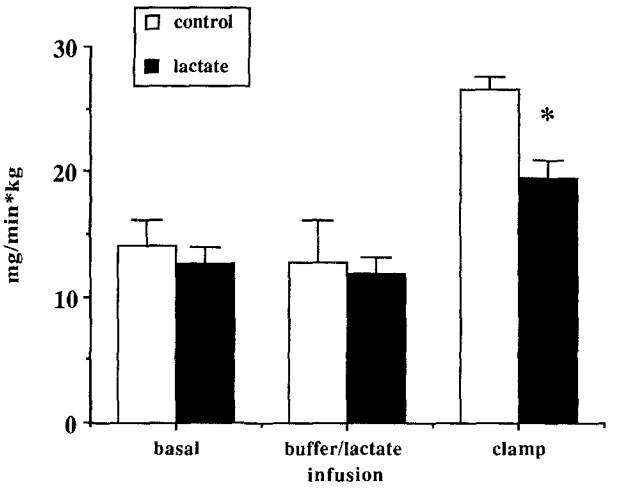

Fig 3. Glucose Rd assessed in the basal condition, after bicarbonate or lactate infusion, and during the euglycemic-hyperinsulinemic clamp in anesthetized Sprague-Dawley rats during bicarbonate (□) or sodium lactate (■) infusion. *$P < .01$.

lactate-infused animals either in epididymal and inguinal white adipose tissue (Fig 4) or in white quadriceps (3.05 ± 0.26 *v* 2.88 ± 0.69 ng · mg^{-1} · min^{-1}), tibialis (3.95 ± 0.53 *v* 4.29 ± 0.76 ng · mg^{-1} · min^{-1}), extensor digitorum longus (3.14 ± 0.41 *v* 3.28 ± 0.40 ng · mg^{-1} · min^{-1}), or white gastrocnemius (1.66 ± 0.19 *v* 2.08 ± 0.50 ng · mg^{-1} · min^{-1}).

[^{3}H]2DG uptake by heart muscle was clearly and significantly reduced in lactate-infused rats (Fig 4).

DISCUSSION

In pathological conditions characterized by insulin resistance, an increased plasma lactate concentration is often present.[5] Recently, an inverse correlation between plasma lactate levels and insulin sensitivity has been found in obese humans.[22] With the progression of obesity toward non–insulin-dependent diabetes mellitus, these changes in lactate metabolism become more substantial,[23] probably due to the increase in nonoxidative glycolysis.[24] All of these findings indicate that lactate metabolism abnormalities may be linked to insulin resistance by a cause-and-effect mechanism.

It is difficult at present to distinguish whether the abnormality of lactate metabolism may contribute to the appearance of insulin resistance or may be the consequence of decreased insulin sensitivity. Lactate may affect insulin sensitivity by acting on the liver or interfering with glucose metabolism in peripheral target organs of insulin action, particularly muscular tissue.

In our experimental conditions, lactate infusion in overnight-fasted rats leads to a fourfold increase in basal arterial concentrations of lactate. The high levels of lactate achieved and the particular model chosen (anesthetized rats) should be taken into account when reaching any clinical conclusion. In our experiments, we did not observe any change in basal or in glycemia HGP. This agrees with previous observations in which infusing

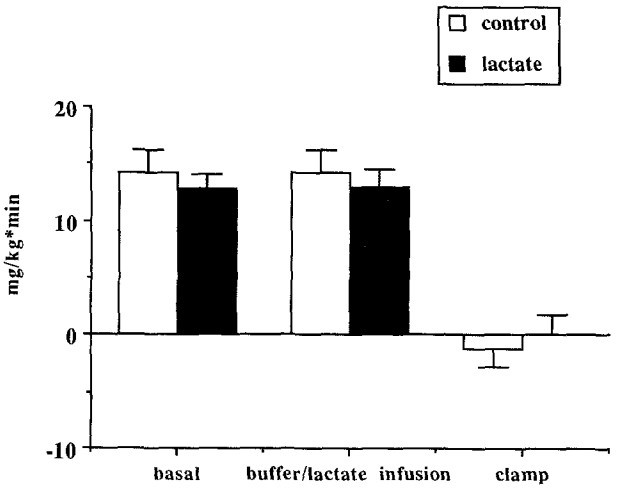

Fig 2. HGP assessed in the basal condition, after bicarbonate or lactate infusion, and during the euglycemic-hyperinsulinemic clamp in anesthetized Sprague-Dawley rats during bicarbonate (□) or sodium lactate (■) infusion.

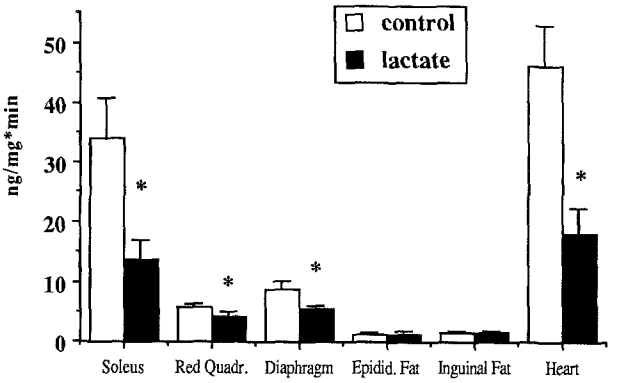

Fig 4. Glucose utilization index measured in different muscles, heart, and adipose tissue in bicarbonate-infused (□) and lactate-infused (■) rats at the end of the euglycemic-hyperinsulinemic clamp. Each bar represents the mean ± SEM of 7 experiments for each group. Statistical analysis was performed by ANOVA. *$P \leq .05$.

lactate in fasting humans produced no change in plasma glucose and in the rate of HGP.[14]

Plasma insulin was not significantly affected by hyperlactatemia, suggesting that lactate does not have any action on insulin secretion and metabolism. On the contrary, several years ago, we observed that lactate infused in anesthetized dogs caused a clear increase in insulin secretion.[25] Moreover, lactate may affect insulin metabolism in the liver by increasing hepatic insulin clearance.[26]

In our experiments, we did not observe any variations in HGP in the basal conditions or at the end of the clamp study. At this time, a normal insulin responsiveness in suppressing hepatic glucose Ra was also seen in lactate-infused rats. These results resemble those obtained by infusing lactate in healthy humans, in which no changes in overall glucose Ra were found.[14] In our experience, on the contrary, peripheral glucose disposal assessed by clamp techniques was significantly reduced by lactate infusion, suggesting that an enhanced availability of this end product of glucose metabolism may affect insulin-induced glucose uptake and utilization. Our data only partially agree with previous results obtained by Issekutz et al[8] in unanesthetized dogs, in which a glucose-sparing effect of lactate was identified in a reduction of its oxidation while plasma glucose removal was increased. Indeed, data obtained by Ferrannini et al[15] in healthy subjects disclosed no changes in whole-body or forearm muscle glucose utilization, probably because of the relatively low levels of blood lactate reached in these experiments. However, it is noteworthy that in those experiments muscle tissue was responsible for disposal of one fifth of the lactate load and that lactate had a strong capacity to stimulate whole-body energy expenditure; this suggests not only oxidation of the fuel but also triggering of synthetic processes such as glycogen formation and/or de novo lipid synthesis. These data taken together confirm the concept that lactate, far from being simply a final metabolic product, may be considered a readily available energy fuel.

In both humans and animals, the insulin resistance associated with obesity and diabetes is mainly located in skeletal muscle.[21,27,28] As a general rule, when insulin-mediated whole-body glucose disposal in vivo is considerably reduced, skeletal muscle is the quantitatively predominant site of insulin resistance.[27]

To determine the relative contribution of the different tissues to the decreasing effect of lactate on whole-body glucose utilization, we measured the glucose utilization index in a wide array of skeletal muscles with mostly white, mostly red, and mixed muscle fiber types. We observed a marked reduction of glucose utilization by red muscles and heart, but not by white adipose tissue. Specific lactate transporters have been reported in both skeletal and heart muscles,[10,29] and hyperinsulinemia was found to increase lactate utilization.[11,30] In a condition characterized by a great availability of lactate, it is possible to speculate that this fuel could be used to the detriment of an adequate glucose utilization. Among the different types of muscle considered, lactate infusion seems to affect mostly red oxidative fiber–rich muscles. This is not an unexpected result, because lactate transport is greater in sarcolemmal vesicles from red rat muscle than in vesicles from white muscles.[31]

Several recent in vitro and in vivo studies have shown that adipocytes from rats and humans possess the capacity to produce[32,33] and use lactate. However, in the present study, lactate infusion did not affect insulin-mediated glucose utilization by white adipose tissue.

Our results show for the first time with in vivo experiments the presence of a metabolic competition between lactate and glucose mainly at the muscular level, substantiating previous experiments showing that at increasing lactate concentrations in the medium there is a decline in glucose utilization by isolated rat soleus.[34] We did not calculate the lumped constant for each tissue, and thus we cannot measure the relative amount of glucose taken up by each tissue. Nevertheless, approximately less than half the muscle mass and heart would account for the 25% decrease in whole-body glucose disposal. This seems unlikely, and it is possible that other insulin-sensitive or insulin-independent tissues may contribute to the phenomenon. As a matter of fact, it has been observed that elevated lactate levels were able to decrease glucose utilization also in the lung.[35] We did not perform any tracer study to further investigate the metabolic fate of lactate within the tissues, but it is possible that the glucose-sparing effect of lactate may be mediated by the preferential utilization of lactate or by a direct or indirect interference with glucose metabolism. The fate of this substrate, when taken up by muscles, is still unclear, with the majority being oxidized after transformation into pyruvate or converted to alanine. A fuel interaction has been suggested also for lactate and FFA, and it has been reported that lactate displays a clear inhibitory effect on FFA delivery from fat stores and oxidation in dogs.[8] Our results show that lactate infusion did not modify FFA plasma levels in basal conditions, whereas, at the end of the clamp insulin infusion suppressed FFA in both control and lactate-infused animals, but in the latter group FFA remained significantly more elevated than in controls. We cannot derive any conclusion about the FFA turnover rate, but the difference between the two groups was not important enough to support a role for FFA in reducing glucose utilization.

Substrate competition was also reported between glucose and amino acids. Ferrannini et al[36] reported that infusion of a mixed amino acid solution in healthy volunteers reduced insulin-mediated glucose disposal. In our experiments, we measured the alanine concentration, which was reduced by euglycemic hyperinsulinemia but was not affected by lactate infusion. This fact excludes the possibility that lactate's effect on glucose metabolism may be mediated by increased concentrations of alanine.

In our study, we found a clear reduction of insulin-induced glucose utilization also in cardiac muscle. The heart muscle must continuously function independently from changes in substrate availability and whole-body energy needs, so its energy requirement can be provided from the oxidation of different substrates.[37] During supraphysiological hyperinsulinemia obtained by a euglycemic clamp, increased arterial levels of lactate and pyruvate with a concomitant decrease in FFA, glycerol, and β-hydroxybutyrate were reported. As a consequence a greater myocardial uptake of glucose, lactate, and pyruvate was observed with shifting of fat utilization, which occurs in basal conditions, to carbohydrate oxidation without

changes in oxygen consumption.[30] Competition between carbohydrate, fat, and protein substrates was described also in the heart,[6,7] but so far, few data on a possible competition between lactate and glucose have been detailed. In rat heart, it has been shown that lactate interferes with glucose oxidation, shifting glucose toward glycogen synthesis and thus increasing its nonoxidative disposal.[13,38]

Our results essentially show a decreased uptake and phosphorylation of glucose at the muscular level, but they do not offer a mechanistic explanation of the phenomenon. Glucose clearance is a process that involves both transport and phosphorylation. In skeletal muscle, glucose transport is mediated by GLUT4, whereas phosphorylation is catalyzed by hexokinase II.[39] Both of these reactions are limiting steps for glucose utilization. We cannot derive any conclusion about the possible influence of lactate on GLUT4 expression and translocation or on hexokinase II activity.

In summary, our results strongly suggest that hyperlactatemia obtained in normal rats by infusing lactate decreases whole-body glucose disposal without affecting HGP. The reduction in heart and red skeletal muscle of insulin-induced glucose utilization may justify, at least in part, the blunted overall glucose disposal. Our data and other results[5] are in keeping with the hypothesis that lactate may be important in the development of carbohydrate intolerance and insulin resistance. Moreover, lactate and FFA overproduction from the enlarged fat mass of obese patients may secondarily interfere with peripheral glucose metabolism, leading to insulin resistance and accelerating the progression of obesity toward diabetes.

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REFERENCES

1. Kreisberg RA: Glucose-lactate inter-relations in man. N Engl J Med 287:132-137, 1972

2. Crandall DL, Fried SK, Francendese AA, et al: Lactate release from isolated rat adipocytes: Influence of cell size, glucose concentration, insulin and epinephrine. Horm Metab Res 15:326-329, 1983

3. Gladden LB, Crawford RE, Webster MJ: Effect of lactate concentration and metabolic rate on net lactate uptake by canine skeletal muscle. Am J Physiol 266:R1095-R1101, 1994

4. Ahlborg G, Felig P: Lactate and glucose exchange across the forearm, legs and splanchnic bed during and after prolonged leg exercise. J Clin Invest 69:45-54, 1982

5. DiGirolamo M, Newby FD, Lovejoy J: Lactate production in adipose tissue: A regulated function with extra adipose implications. FASEB J 6:2405-2412, 1992

6. Randle PJ, Newsholme EA, Garland PB: Regulation of glucose uptake by muscle. Effects of fatty acids, ketone bodies, and pyruvate, and of alloxan-diabetes and starvation, on the uptake and metabolic fate of glucose in rat heart and diaphragm muscles. Biochem J 93:652-665, 1964

7. Kruszynska YT, McCormack JG, McIntyre N: Effects of nonesterified fatty acid availability on insulin stimulated glucose utilization and tissue pyruvate dehydrogenase activity in the rat. Diabetologia 33:396-402, 1990

8. Issekutz B Jr, Shaw WAS, Issekutz AC: Lactate metabolism in resting and exercising dogs. Am J Physiol 40:312-319, 1976

9. Drake AJ, Haines JR, Noble MIM: Preferential uptake of lactate by the normal myocardium in dogs. Cardiovasc Res 14:65-72, 1980

10. Roth D, Brooks G: Lactate transport is mediated by a membrane-bound carrier in rat skeletal muscle sarcolemmal vesicles. Arch Biochem Biophys 279:377-385, 1990

11. Consoli A, Nurjahan N, Gerich JE, et al: Skeletal muscle is a major site of lactate uptake and release during hyperinsulinemia. Metabolism 41:176-179, 1992

12. Young L, Zarst B, Barrett E: Physiological hyperinsulinemia stimulates lactate extraction by heart muscle in the conscious dog. Metabolism 38:1115-1119, 1989

13. Williamson JR: Effects of insulin and diet on the metabolism of L(+)-lactate and glucose by the perfused rat heart. Biochem J 83:377-383, 1962

14. Jenssen T, Nurjhan N, Consoli A, et al: Failure of substrate-induced gluconeogenesis to increase overall glucose appearance in normal humans. J Clin Invest 86:489-497, 1990

15. Ferrannini E, Natali A, Brandi LS, et al: Metabolic and thermogenetic effects of lactate infusion in humans. Am J Physiol 265:E504-E512, 1993

16. Terrettaz J, Jeanrenaud B: In vivo hepatic and peripheral insulin resistance in genetically obese (fa/fa) rats. Endocrinology 112:1346-1351, 1983

17. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: A method for quantifying insulin secretion and resistance. Am J Physiol 237:E214-E223, 1979

18. Ferré P, Burnol AF, Leturque A, et al: Glucose utilization in vivo and insulin-sensitivity of rat brown adipose tissue in various physiological and pathological conditions. Biochem J 233:249-252, 1985

19. James DE, Jenkins AB, Kraegen W: Heterogeneity of insulin action in individual muscles in vivo: Euglycemic clamp studies in rats. Am J Physiol 248:E567-E574, 1985

20. Herbert W, Lau KS, Gottlieb CW, et al: Coated charcoal immunoassay of insulin. J Clin Endocrinol Metab 25:1375-1384, 1965

21. Smith D, Rossetti L, Ferrannini E, et al: In vivo glucose metabolism in the awake rat: Tracer and insulin clamp studies. Metabolism 36:1167-1174, 1987

22. Lovejoy J, Newby FD, Gebhart SSP, et al: Insulin resistance in obesity is associated with elevated lactate levels and diminished lactate appearance following intravenous glucose and insulin. Metabolism 41:22-27, 1992

23. Ohlson LO, Larsson B, Bjorntorp P, et al: Risk factor for type 2 (non–insulin-dependent) diabetes mellitus. Thirteen and one-half years of followup of participants in a study of Swedish men born in 1913. Diabetologia 31:798-805, 1988

24. Del Prato S, Bonadonna RC, Bonora E, et al: Characterization of cellular defects of insulin action in type 2 (non–insulin-dependent) diabetes mellitus. J Clin Invest 91:484-494, 1993

25. Federspil G, Zaccaria M, Pedrazzoli S, et al: Effects of DL-lactate on insulin secretion in anesthetized dogs. Diabetes 29:33-36, 1980

26. Pagano C, Rizzato M, Lombardi AM, et al: Effect of lactate on hepatic insulin clearance in perfused rat liver. Am J Physiol 270:R682-R687, 1996

27. DeFronzo RA, Jacot E, Jequier E, et al: The effect of insulin on the disposal of intravenous glucose: Results from indirect calorimetry and hepatic and femoral venous catheterization. Diabetes 30:1000-1007, 1981

28. Terrettaz J, Assimacopoulos-Jeannet F, Jeanrenaud B: Severe hepatic and peripheral insulin resistance as evidenced by euglycemic

clamps in genetically obese fa/fa rats. Endocrinology 118:674-678, 1986

29. Fishbein W: Lactate transporter defect: A new disease of muscle. Science 234:1254-1256, 1986

30. Ferrannini E, Santoro D, Bonadonna R, et al: Metabolic and hemodynamic effects of insulin on human hearts. Am J Physiol 264:E308-E315, 1993

31. Juel C, Honig A, Pilegaard H: Muscle lactate transport studied in sarcolemmal giant vesicles: Dependence on fibre type and age. Acta Physiol Scand 143:361-365, 1991

32. Jansson PA, Smith U, Lonnroth P: Evidence for lactate production by human adipose tissue in vivo. Diabetologia 33:253-256, 1990

33. Newby FD, Sykes MN, Di Girolamo M: Regional differences in adipocyte lactate production from glucose. Am J Physiol 255:E716-E722, 1988

34. Pearce FJ, Connett RJ: Effect of lactate and palmitate on substrate utilization of isolated rat soleus. Am J Physiol 238:C149-C159, 1980

35. Fisher AB, Dodia C: Lactate and regulation of lung glycolytic rate. Am J Physiol 246:E426-E429, 1984

36. Ferrannini E, Bevilacqua S, Lanzone L, et al: Metabolic interactions of amino acids and glucose in healthy humans. Diabetes Nutr Metab 3:175-186, 1988

37. Carlsten A, Hallgren B, Jagenburg R, et al: Myocardial metabolism of glucose, lactic acid, amino acids and fatty acids in healthy human individuals at rest and at different work loads. Scand J Clin Lab Invest 13:418-428, 1961

38. Russel RR, Van Nguyen TB, Mrus JM, et al: Fasting and lactate unmask insulin responsiveness in isolated working rat heart. Am J Physiol 263:E556-E561, 1992

39. Katzen HM: The multiple forms of mammalian hexokinase and their significance to the insulin action. Adv Enzyme Regul 5:335-355, 1967