Lactate infusion to normal rats during hyperglycemia enhances in vivo muscle glycogen synthesis

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Lactate infusion to normal rats during hyperglycemia enhances in vivo muscle glycogen synthesis. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R2072–R2079, 1997.—Both hyperglycemia and hyperinsulinemia stimulate whole body and muscle glucose disposal. To define the impact of increased lactate concentration (4–5 mM) on muscle glucose disposal during hyperglycemia, we studied anesthetized normal rats infused with either sodium lactate or sodium bicarbonate as control. Animals were studied under hyperglycemic clamp (13 mM) using [3-3H]glucose (study 1) and 2-deoxy-[1-3H]glucose (study 2) to assess glucose rate of disappearance (Rd), glycolytic flux (GF), glycogen synthesis, and glucose utilization index by different tissues. Moreover, in study 3, the effect of lactate on the pattern of plasma insulin response to hyperglycemia was evaluated. In study 1, lactate infusion resulted in an increased Rd (38.7 ± 1.7 vs. 32.3 ± 1.3 mg·min⁻¹·kg⁻¹; P < 0.01), which was explained by an enhanced rate of glycogen synthesis (23.0 ± 1.7 vs. 14.7 ± 1.2 mg·min⁻¹·kg⁻¹; P < 0.001), whereas GF was unchanged. In study 2, lactate-infused animals showed an increased 2-deoxyglucose disposal and a stimulated glycogen synthase activity as well as an increased glycogen accumulation at the end of the study in several skeletal muscles. In study 3, lactate did not induce any change in either early or late insulin response to hyperglycemia. In conclusion, our results show that muscle glycogen deposition may be enhanced by elevated lactate levels under hyperglycemic conditions and support a role for lactate in the regulation of glucose homeostasis.

LACTATE HAS LONG BEEN CONSIDERED a dead-end metabolite that results from an inadequate balance between anaerobic glycolysis and the oxidative processes of the tricarboxylic acid cycle. The main fate of lactate has been thought to be supply three carbon precursors to hepatic gluconeogenesis (Cori cycle). In the past two decades, evidence has accumulated indicating that lactate can be consumed by heart (9) and skeletal muscle (7, 8) and may therefore represent a relevant source of energy. Therefore, there is evidence that lactate, when present together with glucose or fatty acids, is preferentially oxidized, whereas the other two substrates are channeled through the glycogen and fat storage pathways (21).

Although the metabolic interactions between fatty acids and carbohydrates have been extensively studied in vivo and in vitro in muscle (13, 22), it is less clear how lactate interacts with other energy substrates. Studies from Ahlborg and co-workers (2) in humans indicated that the elevation of plasma lactate concentration induces a reduction of both glucose and fatty acid oxidation. Moreover, fatty acid and glycerol release from adipose tissue was also inhibited. The interactions between lactate and glucose are less clear. In vitro studies from the last decade indicate that lactate may promote glycogen deposition in liver (32) and skeletal muscle (20). From these studies a novel role as a promoter of glycogen deposition in liver was proposed for lactate (31). At present, few data are available in intact organisms. Ferrannini et al. (12) reported that lactate has a strong thermogenic effect when infused during euglycemic hyperinsulinemic clamp. Furthermore, increased lactate uptake and incorporation into glycogen was observed during hyperlactatemia in resting rats (26). More recently, data from our laboratory (30) have shown that acute and chronic hyperlactateemia produces insulin resistance.

Lactate levels rise in conditions characterized by an increased glucose utilization, such as the postprandial phase or exercise, independently of variations in plasma glucose concentrations. Elevated lactate concentration may also be observed in pathophysiological states, particularly when insulin resistance is present, i.e., in obesity and diabetes (18). In these conditions, increased plasma lactate is associated with normal or increased glucose levels.

Therefore, a major goal of these studies was to investigate in vivo the effect of increased lactate concentration in association with acutely elevated blood glucose. The model of the hyperglycemic clamp was used in rats, and parameters of in vivo glucose metabolism were evaluated. A quantitative estimation of in vivo glucose uptake by different tissues was also given. A second goal was to further investigate the pathways of intracellular glucose metabolism by measuring the effect of lactate on glycolysis and glycogen synthesis.

MATERIALS AND METHODS

Animals

Twelve-week-old male Sprague Dawley rats (200–220 g body wt) were used in the protocol. Animals were purchased from Charles River Italy (Calco, Como, Italy) and were housed in our animal quarter with a 12-h light cycle for at least 1 wk before the experiments. They had free access to water and to a standard animal chow (Zoopharma, Padua, Italy).

The protocol was approved by the Ethical Committee of the University of Padua, and experiments were performed in...
agreement with the rules of laboratory animal care and the Italian law on animal experimentation.

Surgical Procedure

After an overnight fast, rats were anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg), and two indwelling catheters were inserted: one in the right jugular vein for infusion of glucose, lactate, and tracers, and the other in the left carotid artery for blood sampling, as previously described in detail (28).

Body temperature was maintained at 37°C throughout the study by means of a heating blanket connected to a rectal probe. No additional pentobarbital sodium was administered after the clamp was started.

Experimental Designs

Study 1. To evaluate the effects of elevated lactate levels during hyperglycemia on overall glucose metabolism, endogenous glucose production (EGP), and whole body glycolytic flux (GF) in anesthetized rats, after a 30-min post-surgery recovery, a primed continuous infusion of sodium lactate (175 μmol/min prepared in a phosphate buffer, pH 6.5) was started and continued throughout the experiment to elevate plasma lactate from basal values to 4–5 mM (n = 7). Corresponding equivalents of sodium bicarbonate were infused in the control animals (n = 7). After 30 min of lactate infusion (time 0), a glucose solution (20%) was infused to acutely raise plasma glucose to ~13 mM by means of a variable glucose infusion during the first 5 min (70, 57, 46, 37, 30 and 25 mg·min⁻¹·kg⁻¹ at minutes 0, 1, 2, 3, 4, and 5, respectively) as previously described (28). Plasma glucose was monitored at 5- to 10-min intervals, and glucose infusion rate (GIR) was modified accordingly to maintain plasma glucose concentration at 13.0–13.5 mM for 120 min.

At time 0, a primed continuous infusion of [3-13H]glucose infusion (0.15 μCi/min; Amersham, Arlington Heights, IL) was started and initiated, and a stable glucose specific activity was reached within 30–40 min (Fig. 1).

Arterial blood samples (250 μl) were collected before the beginning of the experiment (time –30), after a 30-min lactate infusion (time 0), and at the end of clamp (time 120) to measure plasma glucose, insulin, and lactate concentration. Urine produced during the experiment was collected at the end of the study by means of suprapubic puncture.

Moreover, blood samples (50 μl) were collected every 10 min during the study. Fifty microliters of whole blood were precipitated in 250 μl ZnSO4 and 250 μl Ba(OH)2 and were immediately centrifuged. Aliquots of the supernatant were used to measure glucose concentration and [3-13H]glucose radioactivity after evaporation to dryness. Tritiated water for calculation of glycolysis was measured as described below. At the end of the study, animals were killed by a lethal dose of pentobarbital sodium. Glucose rate of disappearance (Rg) and EGP were calculated during the last 60 min of hyperglycemic clamp, when stable glucose specific activity and GIR were achieved, as previously described (28). GF was calculated as previously reported (23) and is described in detail in Whole Body GF In Vivo.

Study 2. To evaluate the effect of lactate and hyperglycemia on glucose utilization in individual tissues and glycogen content in skeletal muscle, a primed continuous infusion of lactate (n = 8) or bicarbonate (n = 8) at the same rates as in study 1 was started after a period of recovery from the surgical procedure and continued throughout the experiment. After 30 min (time 0), hyperglycemia was induced as in study 1 and was maintained throughout the study by means of a variable glucose infusion. After 90 min, when steady GIR was reached, a bolus of 2-deoxy-[1-3H]glucose (2-DG) (30 μCi, Amersham) was injected through the jugular vein, and arterial samples (50 μl) were collected at times 1, 3, 5, 10, 15, 20, and 30 min after 2-DG injection. Aliquots of arterial plasma were collected at times 30 and 0 and at the end of clamp for determination of insulin, lactate, and free fatty acid (FFA). Thirty minutes after 2-DG injection, animals were killed by injection of a lethal dose of pentobarbital sodium and tissues were rapidly collected and stored in aluminum tongs precooled in liquid nitrogen for determination of 2-DG and 2-deoxy-[1-3H]glucose 6-phosphate (2-DG-6-P) content and for glycogen determination. Tissues were frozen in liquid nitrogen and stored at –80°C for subsequent analyses. The following tissues were collected: diaphragm, white and red quadriceps, white and red gastrocnemius, tibialis, extensor digitorum longus (EDL), epididymal adipose tissue, and subcutaneous adipose tissue. 2-DG and 2-DG-6-P content were measured as described (13, 15). Briefly, blood samples for measurement of 2-DG specific activity were precipitated in 250 μl ZnSO4 and 250 μl Ba(OH)2 and were immediately centrifuged. Aliquots of the supernatant were used for glucose and radioactivity determination. Tissues were dissolved in 1 N NaOH in a shaking water bath at 80°C and were neutralized with 1 N HCl. Two separate aliquots were precipitated in ZnSO4-Ba(OH)2 or perchloric acid. The radioactivity in the protein-free supernatant was then measured on a β-counter (1215 Rackbeta Helsinki, Finland) after addition of 4 ml of scintillation fluid (Insta-Gel, Packard, Meriden, CT). Tissue glycogen content was measured in red gastrocnemius and red quadriceps, whereas glycogen synthase activity was determined in vitro on red quadriceps samples as described in Glycogen Synthase Activity.

Study 3. To evaluate the effect of lactate infusion on peripheral insulin response to hyperglycemia, we carried out hyperglycemic clamps, as previously described in rats (28). The surgical procedure was the same as in studies 1 and 2. After 30 min of recovery from surgery, an infusion of lactate (n = 8) or bicarbonate (n = 8) was started and continued.

![Graph](http://ajpregu.physiology.org/DownloadedFrom/10.230.32.5-August28,2017)

**Fig. 1.** [3-13H]glucose specific activity measured in arterial blood during hyperglycemic clamp. A priming dose of tracer (1.5 μCi/min) was infused during first 2 min, followed by a constant infusion (0.15 μCi/min). Arterial blood was collected at times indicated and was immediately precipitated in Somogy reagent. Supernatant radioactivity was measured after evaporation to dryness. Determinations were performed at 10-min intervals throughout study. dpm, Disintegrations/ min.
throughout the experiments. After 30 min (time 0 min), a 20% glucose solution was infused and hyperglycemic clamp was performed as in studies 1 and 2. Blood samples (100 µl) for insulin and glucose determination were collected at times −30 and 0 min to assess basal glycemia and insulinemia; at times 1, 2, 4, and 5 min to assess early insulin response to hyperglycemia; and then at 10-min intervals to the end of the study to evaluate late insulin response and plasma glucose. The experiments were stopped at time 120 min. Early (0–5 min), late (10–120 min) and total (0–120 min) insulin responses were calculated as the area under the curve (AUC) of insulinemia. Early and late phases of insulin response were separated arbitrarily according to the pattern of insulin response. At time 5 min, the early insulin peak was ended, and therefore early insulin secretion was defined as 0–5 min and late phase as 10–120 min.

Whole Body GF In Vivo

Essentially all of the tritium on the C-3 position of [3-3H]glucose is lost to water during glycolysis at the triose-isomerase step. Although a small amount of tritiated water is formed also during fructose 6-phosphate cycling and pentose isomerase step. Although a small amount of tritiated water is lost to water during glycolysis at the triose-phosphate step. The rate of tritiated water production during the last 60 min of the study was essentially 65% of the body weight and that blood water was 69% of whole body water mass. The rate of tritiated water production during the last 60 min of the experiments was essentially constant, as confirmed by linear regression of time and tritiated water specific activity (r² = 0.98 and r² = 0.97 in control and lactate experiments, respectively). This technique for in vivo estimates of GF was first validated on rats (23) and humans (3) and was confirmed (24) and adapted to dogs (5).

Glycogen Determination

Glycogen storage was evaluated by two different techniques: first, by the difference between the rate of overall glycogen disposal and the rate of GF, as previously described (22). This technique was found to strongly correlate with both glycogen synthase activity and incorporation of [3-3H]glucose into glycogen (24). Second, glycogen content in muscle samples was measured at the end of the clamp study by ethanol precipitation on chromatography paper and digestion with amyloglucosidase as described by Chan and Exton (4).

Glycogen Synthase Activity

Glycogen synthase activity in skeletal muscle was measured by a modification (10) of the method of Thomas et al. (29) and was based on the measurement of incorporation of radioactive activity into glycogen from UDP-[U-14C]glucose. Tissue samples (28–37 mg) from red quadriceps were homogenized in 1.6 ml buffer containing 60% glycerol, 10 mM EDTA, and 50 mM NaF, added to the probe wash (1.6 ml, 10 mM EDTA, 50 mM NaF), and centrifuged for 20 min at 2,000 g (+4°C). Aliquots of the supernatant were then added to a buffer containing rabbit liver glycogen type III (2.5 mg/ml), 0.02 M EDTA, 0.05 M tris(hydroxymethyl)aminomethane·HCl, 0.26 M NaF, 7.2 mM glucose 6-phosphate, 106–170 disintegrations/min (dpm) of UDP-[U-14C]glucose and cold UDP-glucose at final concentrations of 0.125, 0.25, 0.5, 1.0, and 2.0 mM. After exactly 10 min at 37°C, the reaction was stopped by transferring the mixture to filter paper (to precipitate radiolabeled glycogen), dried, and dropped in 66% ethanol. Data were linearized as Eadie-Hofstee plots and were fitted using linear regression. The Km for UDP-glucose was calculated as the reciprocal of the slope, whereas the maximal velocity (Vmax) was the y-intercept divided by the slope.

Reagents and Analytic Procedures

[3-3H]glucose and 2-DG were purchased from Amersham. Plasma glucose was determined by the glucose oxidase method (Glucose Analyzer 2, Beckman Instruments, Palo Alto, CA). Lactate and FFA were measured using commercial kits (Boehringer, Mannheim, Germany) with enzymatic spectrophotometric techniques. Insulin was measured with a radioimmunoassay using rat insulin as standard and an antibody raised against rat insulin (Linco, St. Charles, MO). In study 1, pH was measured after induction of anesthesia and at the end of the study in arterial blood samples (250 µl) on a pH analyzer (ABL 520 Radiometer, Copenhagen, Denmark).

Calculations and Statistical Analysis

In study 1, glucose Rd during clamp was calculated as the ratio of the tracer infusion rate (dpm/min) and glucose specific activity (dpm/mg). The rate of whole body glucose disposal (M) is expressed by the equation M = Rg − SpC − UrL, where SpC is the space correction during the last 60 min of clamp resulting from over- or underfilling of the glucose space and is calculated from the equation SpC = ΔSpC/Δt × V, where ΔSpC is the change of glucose concentration during the last 60 min of the study, Δt is 60 min, and V is the distribution volume of glucose, which is assumed to be 25% of body weight. UrL is the glucose urinary loss during the study. SpC and UrL were calculated as the difference between M and exogenous GIR. In study 2, glucose utilization index (GUI) was calculated as previously described (13).

All data are expressed as means ± SE. Elaboration of data and calculations was made on an electronic spreadsheet (Microsoft Excel 5.0). Statistical analysis was performed using analysis of variance and linear regression analysis. Values of probability <0.05 were considered statistically significant.

RESULTS

Study 1

Biochemical profiles at the different steps of experiments are summarized in Table 1. Plasma lactate was increased by lactate infusion from baseline values of 0.75 mM, reaching the concentrations of 4.25 ± 0.24 and 5.33 ± 0.45 mM before and after the clamp study, respectively. After elevation of plasma glucose at 13.0–13.5 mM, hyperglycemia was kept essentially constant throughout the study (coefficient of variation <5%) in both groups of animals.

Glucose Rd was averaged during the last 60 min of hyperglycemia, when steady-state glucose concentration, glucose specific activity, and GIR were achieved. Lactate-infused animals displayed a greater Rd compared with control animals (Fig. 2). Glucose clearance
was also significantly enhanced by lactate (0.166 ± 0.011 vs. 0.134 ± 0.006 ml/min, P < 0.01). Moreover, in lactate experiments, GF was unchanged, whereas GS was significantly increased (Fig. 2).

EGP was comparable in the two groups (12.6 ± 1.0 vs. 10.4 ± 1.1 mg·min⁻¹·kg⁻¹ in control and lactate groups, respectively), whereas GIR was increased by lactate infusion (28.3 ± 1.7 vs. 19.4 ± 1.6 mg·min⁻¹·kg⁻¹, P < 0.01).

When regression analysis was performed using lactate levels at the end of clamp and parameters of glucose metabolism, a statistically significant correlation was found with Rd (r = 0.56, P < 0.01) and GS (r = 0.59, P < 0.01). No correlation was found between plasma insulin at the end of clamp and Rd or glycogen synthesis.

Table 1. Biochemical profile of animals

<table>
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<tr>
<th></th>
<th>n</th>
<th>pH</th>
<th>Glucose, M</th>
<th>Lactate, M</th>
<th>FFA, M</th>
<th>Insulin, µIU/ml</th>
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<td>Baseline values</td>
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<tr>
<td>Control</td>
<td>7</td>
<td>7.37 ± 0.03</td>
<td>5.2 ± 0.3</td>
<td>0.89 ± 0.11</td>
<td>1.194 ± 273</td>
<td>23.5 ± 12.4</td>
</tr>
<tr>
<td>Lactate</td>
<td>9</td>
<td>7.40 ± 0.03</td>
<td>5.5 ± 0.2</td>
<td>0.75 ± 0.21</td>
<td>1.103 ± 116</td>
<td>17.7 ± 2.8</td>
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<tr>
<td>Before clamp</td>
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<td></td>
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<tr>
<td>Control</td>
<td>7</td>
<td>7.37 ± 0.03</td>
<td>5.3 ± 0.3</td>
<td>0.75 ± 0.10</td>
<td>1.288 ± 175</td>
<td>19.2 ± 6.7</td>
</tr>
<tr>
<td>Lactate</td>
<td>9</td>
<td>7.37 ± 0.03*†</td>
<td>7.3 ± 0.3*†</td>
<td>4.25 ± 0.24*†</td>
<td>1.082 ± 128</td>
<td>20.4 ± 3.2</td>
</tr>
<tr>
<td>End of clamp</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Control</td>
<td>7</td>
<td>7.55 ± 0.04†</td>
<td>13.4 ± 0.3†</td>
<td>2.18 ± 0.19†</td>
<td>330 ± 74†</td>
<td>120 ± 34†</td>
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<tr>
<td>Lactate</td>
<td>9</td>
<td>7.58 ± 0.03†</td>
<td>13.2 ± 0.2†</td>
<td>5.33 ± 0.45†</td>
<td>272 ± 76†</td>
<td>122 ± 26†</td>
</tr>
</tbody>
</table>

Values are means ± SE of 7–9 animals. Biochemical profile of animals measured after induction of anesthesia (baseline values), after 30-min lactate or bicarbonate infusion and before glucose infusion (before clamp), and at end of hyperglycemic clamp (end of clamp). Insulin concentration at end of clamp represents mean of last 30 min of study. Animals were infused with sodium lactate (175 µmol/min) or corresponding equivalents of sodium bicarbonate. FFA, free fatty acids. pH was measured in arterial blood after induction of anesthesia and at end of the study. Statistical analysis was performed with analysis of variance. *P < 0.05 compared with control group. †P < 0.01 compared with baseline value.

Study 2

GUI was measured 30 min after a bolus injection of 2-DG. Kinetic of disappearance of 2-DG from circulating blood is shown in Fig. 3. GUI was not significantly different between the two groups in subcutaneous adipose tissue and in epididimal adipose tissue (Fig. 4). In skeletal muscle, a diffuse pattern of increased glucose utilization was present in the lactate-infused group (Fig. 4). GUI in particular was significantly increased in diaphragm (P < 0.05), red quadriceps (P < 0.05), red gastrocnemius (P < 0.05), EDL (P < 0.05), and tibialis (P < 0.05). Lactate-infused group showed an increased muscle glycogen content at the end of the study in both red gastrocnemius (26.1 ± 3.6 vs. 13.4 ± 2.9 mg/g wet wt, P < 0.01) and in red quadriceps (P < 0.005, Fig. 5).

The kinetic analysis of glycogen synthase activity measured in vitro showed a reduced K_m for UDP-glucose in lactate-infused animals (132 ± 15 vs. 176 ± 3 µM, P < 0.02) and an increased V_max (0.069 ± 0.008 mg·min⁻¹·g⁻¹·mg⁻¹) in lactate-infused group (P < 0.05) compared with control group.
No changes were observed in fractional velocity (17.9 ± 4.5 vs. 12.5 ± 1.7%), glucose-6-phosphate-independent $K_m$ (1.35 ± 0.14 vs. 1.26 ± 0.05 µM), and glucose-6-phosphate-independent $V_{max}$ (0.35 ± 0.18 vs. 0.26 ± 0.07 µmol·g⁻¹·min⁻¹).

When regression analysis was performed, no significant correlation was found between plasma insulin at the end of clamp and GUI in skeletal muscle.

Study 3

This study was undertaken to evaluate if lactate infusion might affect peripheral insulin response to hyperglycemia. Basal glucose was comparable between the two groups of rats and was acutely increased to 13.0–13.5 mM in both groups of animals. The dynamic of insulin response in arterial plasma is shown in Fig. 6. No effect of lactate was observed in insulin response to hyperglycemia during the study. Also, when compared as AUC, early (0–5 min), late (10–120 min), and total (0–120 min) insulin secretion were similar in the two groups (early, 881 ± 112 vs. 792 ± 157 µIU·ml⁻¹·5 min⁻¹; late, 20,739 ± 4,376 vs. 20,697 ± 3,794 µIU·ml⁻¹·110 min⁻¹; total, 21,148 ± 4,422 vs. 21,093 ± 3,870 µIU·ml⁻¹·120 min⁻¹).

Fig. 4. Glucose utilization index (GUI) as assessed by 2-DG technique in control (open bars) and lactate (closed bars) groups (see MATERIALS AND METHODS for details). Samples of muscles and adipose tissues were rapidly collected and frozen in liquid nitrogen at end of study. GUI was calculated as accumulation of 2-DG 6-phosphate in each tissue. Each bar represents mean ± SE of 8 experiments. Diaph, diaphragm; gastr, gastrocnemius; quadr, quadriiceps; EDL, extensor digitorum longus; subcut, subcutaneous. *$P < 0.05$. Statistical analysis performed with ANOVA.

Fig. 5. Muscle glycogen content, maximal velocity ($V_{max}$) and $K_m$ of glycogen synthase enzyme activity measured in red quadriceps specimens harvested at end of study 2 from lactate-infused (closed bars) and control (open bars) rats. ww, Wet weight. *$P < 0.05$, #$P < 0.02$, and §$P < 0.005$ compared with control group. Statistical analysis performed with ANOVA.

Fig. 6. Plasma insulin response under hyperglycemia in control (open circles) or lactate-infused (closed circles) rats. Each point represents mean ± SE of 8 values. No statistically significant differences were found. Early (0–5 min) and late (10–120 min) insulimetic responses were separated arbitrarily. Time when insulin concentration started increasing again after exhaustion of early phase was considered cut-off point between early and late insulin responses.
DISCUSSION

The present study was undertaken to examine the effect of hyperlactatemia in association with hyperglycemia on in vivo glucose metabolism in rats. Results reported here essentially demonstrate that an increased availability of lactate, in association with hyperglycemia, enhances overall glucose disposal and glucose utilization in skeletal muscle, as confirmed by the increased 2-DG uptake and phosphorylation in various muscles.

The study was also designed to elucidate the intracellular pathways of glucose disposal. Whole body glycogen synthesis was estimated to be enhanced by lactate infusion, and this fact was confirmed by an increased glycogen content in skeletal muscle and possibly explained by an enhanced activity of glycogen synthase. These observations support a role for lactate in the regulation of overall glucose metabolism and muscle glycogen deposition under hyperglycemia. On the contrary, we cannot draw any conclusion on the influence of lactate on hepatic glucose metabolism because, in our experimental conditions, EGP may be affected by pentobarbital sodium anesthesia (6). Nevertheless, even if our results show a still-active EGP despite hyperglycemia and hyperinsulinemia, no difference was found between the lactate- and bicarbonate-infused groups.

These data are different from those reported by our laboratory and by others using euglycemic hyperinsulinemic clamps in which lactate induced a reduction of glucose disposal and utilization (30). Although results from the two models are not comparable quantitatively because of different insulin levels, a qualitative comparison of the results shows a clearly divergent effect of lactate on glucose utilization under normo- and hyperglycemia. In fact, glucose R_d was reduced by lactate under normoglycemia and increased under hyperglycemia. Thus it is likely that glucose concentration itself may be responsible for this divergent effect of lactate.

Such metabolic interactions between lactate and glycogen metabolism have been extensively investigated in liver using different in vitro models (1, 32). It was reported by several authors that the incorporation of glucose into hepatic glycogen is minimal in the absence of lactate or other gluconeogenic precursors (1). The presence of three-carbon precursors such as lactate, amino acids, or fructose strongly enhances glycogen formation through the so-called direct pathway, i.e., direct incorporation of glucose into glycogen. The molecular mechanism for this phenomenon is poorly understood, but an inhibitory effect of gluconeogenic precursors on phosphorylase activity has been postulated (31). In our experiments, sodium lactate and bicarbonate infusion resulted in a slight increase in blood pH that was comparable in the two groups. Moreover, preliminary experiments in our laboratory showed that glucose metabolism in bicarbonate-infused rats is not different from that of saline-infused rats (data not shown). Therefore, the effects of lactate cannot be explained by relevant pH changes or be evident compared with only bicarbonate-infused animals.

Previous studies reported that lactate may also affect gluconeogenesis in skeletal muscle. It was found in isolated rat soleus that when muscle was incubated with carbohydrates and lipids together with lactate at increasing concentrations, the lactate became the prime source of carbons for oxidative metabolism (21). Moreover, lactate induced a substantial redistribution of intracellular glucose utilization from glycogenesis to glycogen synthesis. These data and others (25) suggest that lactate may play a relevant role in the modulation of glycogen deposition not only in liver but also in striated muscle. More recently, Ryan and Radziuk (26) reported that during lactic acid infusion for 3 h in resting rats there is an increased uptake of lactate from the circulation into muscle. In this condition, glucose concentrations and muscle glycogen rose. In the presence of sufficient additional glucose to reproduce circulating glucose levels obtained during lactate infusion, both glucose and lactate stimulated soleus glycogen synthesis to the same extent. The authors further observed that, at rest under conditions of elevated circulating lactate, glucose overtook lactate, gluconeogenesis occurred mostly from lactate. In soleus and gastrocnemius muscles, lactate yields to an increase in gluconeogenesis, but this process derives not only from circulating lactate but also to a minor extent from intramuscular substrates.

In our experimental conditions, initial muscle glycogen content was depleted because of fasting, and therefore most of the glycogen measured at the end of the experiments was formed during the clamp study. We observed an increased glycogen formation when glucose and lactate were infused together. On the other hand, we suggest that the significant increase in glycogen formation flux is linked to the increased glucose uptake that we observed when infusing lactate during hyperglycemic clamp. We did not measure the direct incorporation of glucose into glycogen, and we cannot determine the contribution of infused or intracellular lactate to glycogen formation. Nevertheless, our results show for the first time in vivo that a stimulatory effect of a gluconeogenic precursor, i.e., lactate, on glycogen formation from glucose may also be operative in vivo in skeletal muscle under hyperglycemia. Concerning the mechanism(s) by which lactate exerts this effect, our data provide direct evidence that the mechanism of increased glycogen accumulation relies mostly on enhanced glycogen synthase activity rather than solely on inhibition of phosphorylase, as postulated for the liver. The clear redistribution of the intracellular fate of radiolabeled glucose may further elucidate this mechanism. In fact, whereas in control animals GF and GS represent 54 and 46%, respectively, of total glucose disposal, in lactate-infused animals, these proportions were inverted, with GF and GS accounting for 41 and 59%, respectively. Such a redistribution of glucose suggests two possible scenarios. In the first scenario, increased lactate concentration reduces glycolysis, which in turn makes more glucose available for glycogen synthesis. This, however, would not explain the
increase in glucose uptake in the lactate-infused animals. Alternatively, increased lactate concentration stimulates the activity of glycogen synthase; the increased activity of the enzyme in turn pulls more glucose to form glycogen, making it possible for more glucose to enter the cell. Although both mechanisms may be present, the stimulated activity of glycogen synthase, the increased glucose uptake, together with an unchanged overall glycolysis (as absolute flux) suggest that lactate-induced activation of glycogen synthase is the prevalent mechanism.

To exclude that the increment of glucose metabolism observed during lactate infusion could be mediated by an increased insulin response to hyperglycemia, a specifically designed protocol was carried out. The effect of lactate on in vivo peripheral insulin response to hyperglycemia was evaluated. Both the insulin levels reached at the end of the study and the pattern of insulin response to hyperglycemia were similar in the two groups of animals. Some reports from the 1970s and 1980s investigated isolated pancreas in vitro and in vivo in dog (11) and showed that lactate could increase insulin secretion. Moreover, recent studies (19) reported that lactate may enhance hepatic insulin extraction from the portal circulation in perfused rat liver. Taken together, these observations suggest that lactate may actually influence both insulin secretion from pancreas and hepatic insulin clearance, thus limiting the effect of lactate, if physiologically relevant, on portal insulin concentration.

Many studies have focused on the regulation of glycogen repletion, in particular during the starved-to-refeeding transition (5, 20, 21, 26, 27). Sugden et al. (27) reported that glycogen resynthesis and glucose uptake increase in parallel during refeeding after fasting, whereas the reactivation of pyruvate dehydrogenase is delayed. During the starved-to-fed transition, the rise of insulin and the reduced release of lipid fuels from adipose depots are the major regulators of glucose utilization and glycogen deposition in skeletal muscle. Our results suggest that lactate, the concentration of which rises in response to feeding, may also participate in this regulation.

In conclusion, it has been reported from these studies that lactate, although contributing to the provision of substrate for glucose metabolism, may also regulate glucose disappearance from circulation and glycogen deposition in skeletal muscle under hyperglycemia. It should be emphasized that, in the presence of hyperlactatemia, an increased glycogen formation results from the stimulation of glycogen synthase activity. Our findings provide evidence that lactate, as previously demonstrated in the liver, may also enhance glycogen formation from glucose in skeletal muscle.

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