Control of Gluconeogenesis in Liver

V. EFFECTS OF FASTING, DIABETES, AND GLUCAGON ON LACTATE AND ENDogenous METABOLISM IN THE PERFUSED RAT LIVER*

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SUMMARY

The isolated perfused rat liver preparation was employed to study the effects of glucagon, fasting, and diabetes on the flow of exogenous lactate or endogenous substrates into the pathways of gluconeogenesis, glycogenesis, ketogenesis, lipogenesis, and the Krebs cycle in the liver. Measurements were made of the utilization of substrates and oxygen, the formation of glucose, glycogen, ketone bodies, and urea, and the incorporation of isotope from [14C]lactate into glucose, glycogen, protein, CO2, ketone bodies, cholesterol, and other lipids.

Gluconeogenesis from lactate was enhanced in livers treated with glucagon or from fasted or diabetic rats. The increased gluconeogenesis was associated with increased lactate utilization and was not the result of inhibition of alternative pathways of lactate metabolism. The disposition of isotope between glucose and glycogen indicated impaired glycogen synthesis in these livers. The changes in glucose and glycogen synthesis in livers from diabetic rats were reversed by insulin treatment in vivo.

CO2 was the major product of lactate metabolism in livers from fed rats, whereas glucose was the major product in livers treated with glucagon or from fasted or diabetic rats. Ketone bodies, fatty acids, and cholesterol were minor products of lactate metabolism in all situations examined. Glucagon, fasting, and diabetes had negligible effects on the oxidation of lactate to CO2, but inhibited the synthesis of fatty acid and cholesterol from this substrate. Fasting and diabetes also reduced lactate ketogenesis.

Urea production was increased about 2-fold by glucagon and diabetes and by about 30% by fasting. It was minimally changed by lactate. The alterations in ureogenesis indicated enhanced gluconeogenesis from endogenous protein by glucagon, fasting, and diabetes.

Ketone body production was increased about 10-fold in livers from fasted or diabetic rats perfused without lactate. The increase was apparently due to increased lipid utilization and decreased Krebs cycle activity. Glucagon produced a 2-fold increase in ketone bodies and it is suggested that these were mainly derived from protein.

Lactate markedly suppressed ketogenesis and increased respiration in livers from fasted or diabetic rats. Both effects were attributable to an increase in the Krebs cycle. Lactate also replaced lipid as the major fuel oxidized by livers from fed rats and it is suggested that it acted by diverting endogenous fatty acid from oxidation to esterification.

The metabolic changes observed in the present study are discussed from the viewpoints of underlying mechanisms and physiological implications. It is concluded that changes in the disposition of substrate (pyruvate or fatty acid) within the liver may be as important as changes in substrate supply in the alterations in gluconeogenesis and ketogenesis during fasting and diabetes.

The perfused rat liver preparation has been widely used to study the control of gluconeogenesis, ketogenesis, and other hepatic functions. However, there have been no systematic studies of the effects of diabetes and fasting on endogenous and lactate metabolism in this preparation. Such studies are desirable to elucidate further the role of the liver in the metabolic alterations during diabetes and fasting in vivo.

The present study was undertaken to define quantitatively the flow of lactate or endogenous substrates into the pathways of gluconeogenesis, glycogenosis, ketogenesis, lipogenesis, and the Krebs cycle in perfused livers from fed, fasted, and diabetic rats, and in glucagon-perfused livers from fed rats. To this end, rates of utilization of oxygen and substrates and rates of formation of glucose, glycogen, ketone bodies, and urea were measured. The incorporation of isotope from [14C]lactate into glucose, glycogen, protein, CO2, ketone bodies, cholesterol, and other lipids was also determined.

EXPERIMENTAL PROCEDURE

Liver Perfusion—The technique of liver perfusion and the perfusion apparatus have been described (3). The perfusion medium consisted of oxygenated Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 3% bovine serum albumin (Pentex) and 20% bovine erythrocytes prepared as reported previously (3).

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Treatment of Animals—Male rats of Sprague-Dawley strain weighing 95 to 135 g were used. They were fed ad libitum on Purina lab chow or fasted for 18 to 22 hours prior to perfusion. Alloxan diabetes was induced by the rapid intravenous injection of 60 mg of alloxan monohydrate per kg of body weight. Animals showing blood glucose levels greater than 11 mm were used 48 hours later.

General Analyses—Samples of medium were withdrawn at 0, 20, 40, and 60 min of perfusion and the supernatant, perfusate plasma was analyzed for glucose, urea, [14C]glucose, lactate, and pyruvate (3). At the end of the perfusion, with the pump still running, a sample of the liver was rapidly removed and frozen in a clamp (4) cooled in liquid N2. The sample was analyzed for glycogen, [14C]glycogen, and cyclic AMP by the methods used in our earlier studies (3, 5), and for protein radioactivity according to the method of Regen and Terrell (6).

Lipid Analyses—Lipids were extracted from about 1 g of frozen liver according to the procedure of Folch et al. (7) and were taken up in 4 ml of chloroform. Aliquots (0.2 ml) were dissolved in 10 ml of toluene scintillation fluid (containing 5 g of 2,5-diphenyloxazole and 0.5 g of 1,4-bis[2-(5-phenyloxazolyl)] benzene per liter) and radioactivity measured with a Tri-Carb scintillation spectrometer (Packard Instrument Co.). In these, and in all subsequent measurements, values were corrected for quenching with internal standards. Further portions (0.25 ml) of the chloroform extracts were subjected to thin layer chromatography on Silica Gel G prepared according to Stahl and obtained from Merck, Darmstadt, Germany. After characterization by comparison with authentic samples of palmitic acid, lecitin, cholesterol, mono-, di-, and trigalenic and cholesterol ester (Applied Science Laboratories, Inc., State College, Pa.), lipid bands were scraped off into stoppered tubes and extracted with 5 ml of chloroform except in the case of P-lipid where methanol was used. Aliquots (1 ml) of the extracts were placed in glass scintillation vials, evaporated to dryness, and counted in 10 ml of toluene scintillation fluid.

Portions (0.5 ml) of the initial chloroform extracts were evaporated to dryness and saponified for 1 hour at 80°C with 2 ml of ethanolic KOH (15 g of KOH in 100 ml of 25% ethanol). The saponified samples were diluted with 2.5 ml of water and extracted twice with 10 ml of petroleum ether to remove sterol. The samples were then acidified with 1.5 ml of 2 N H2SO4 and extracted twice with 10 ml of petroleum ether to remove fatty acids. Aliquots (1 ml) of the sterol and fatty acid extracts were added to 10 ml of toluene scintillation fluid for radioactivity determinations. The aqueous phases were neutralized with 2 M NaOH and 0.5-ml aliquots were counted in p-dioxane scintillation fluid (3) to determine radioactivity in glycerol. Aliquots (2 ml) of the triglyceride, P-lipid, and cholesterol ester fractions eluted from the silica gel chromatograms were also saponified and the fatty acids extracted with petroleum ether as described above. The petroleum ether extracts were evaporated to dryness, redissolved in 5 ml of chloroform, and assayed for fatty acid by the method of Itaya and Ui (8).

Ketone Body Analyses—Acetoacetate and β-hydroxybutyrate levels in perfuse plasma were determined by fluorimetric adaptations of the enzymatic methods of Williamson et al. (9). The radioactivity of ketone bodies was determined by a procedure based on that of Mayes and Felts (10). The initial stages of the analytical procedure of Bloom (11) were followed. Samples of the medium were deproteinized with ZnSO4 and Ba(OH)2 and 4 ml of the filtrate were reacted at 100°C for 30 min with H2SO4 and K2Cr2O7 in Teflon-sealed tubes. Aliquots (1 ml) of the oxidized samples were mixed with salicylaldehyde reagent for measurement of acetone as described by Bloom (11). Further volumes (4 ml) were placed in 50-ml Erlenmeyer flasks at ice temperature, 3.5 ml of 5 N NaOH were added, and the tubes were sealed with rubber stoppers with attached plastic hanging wells (Kontes) containing filter paper wicks and 0.5 ml of hydrazine lactate (prepared by adjusting the pH of hydrazine hydrate to 5 with NH4-lactic acid). The flasks were kept at room temperature for 24 hours. The hanging wells were cut off into p-dioxane scintillation fluid and the radioactivity of the trapped [14C]acetone was measured. Studies with [1-3-14C]acetone (Amersham-Searle) showed that the trapping of acetone by this procedure was more than 98% complete.

The oxidation of ketone bodies to acetone by H2SO4 and K2Cr2O7 was 60 to 80% complete. Acetoacetate and β-hydroxybutyrate standards were routinely included in the analyses and values for the [14C] content of the ketone bodies were corrected for incomplete conversion.

CO2 Production and O2 Consumption—The 14CO2 produced during perfusion was measured by drawing the gas leaving the oxygenation chamber through three aspirators in series, each containing 25 ml of 2 M NaOH. Studies with H14CO3 added to the medium showed almost complete recovery of label provided recirculation of the medium through the perfusion apparatus was continued for 10 min after ending the perfusion.

The oxygen consumption of the liver was calculated by measuring the oxygen tension in the medium entering and leaving the liver at 10, 30, and 50 min of perfusion. Samples were injected directly into the chamber of an oxygen electrode (Instrumentation Laboratories, Inc., Boston). Values were approximately constant throughout perfusion.

The oxygen content of influent or effluent medium was calculated according to the formula:

\[
\mu l \text{O}_2 \text{ml}^{-1} \text{ h}^{-1} = \frac{187 \cdot h \cdot s - 2 \cdot p}{40-100} \times 700
\]

where 187 is the \(O_2\) (in microliters per ml) combined with hemoglobin in fully oxygenated bovine blood of normal hematocrit (12). \(h\) is the hematocrit (milliliters of packed red cells per 100 ml of perfusion medium). \(s\) is the percentage saturation of bovine hematocrit, \(O_2\) at the measured \(O_2\) tension of the medium (derived from the oxygen dissociation curve of bovine blood in Reference 12). \(p\) is the oxygen tension of the medium (mm Hg).

The oxygen consumption of the liver was calculated with the formula:

\[
\mu moles O_2 \text{ min}^{-1} \cdot 100 \text{ g body weight}^{-1} = \text{mean difference between } O_2 \text{ content of influent and effluent medium (} \mu l \text{ml}^{-1} \cdot \text{flow rate (} \mu l \text{ml}^{-1}) \cdot 100/22.4 \text{ body weight of rat (g)}
\]

Chemicals—Lactic acid was obtained from Mann, and sodium L-[1-14C]lactate from Amersham-Searle. Glucagon was a kind gift from Eli Lilly Co. and was prepared as previously described (14). Protamine zinc insulin was a commercial preparation from Lilly.

Calculation of Results—The methods used for the calculation of glucose, [14C]glucose, and lactate production or utilization were those described previously (3) except that the corrections for erythrocyte glucose consumption or lactate production were...
TABLE I
Glycogen and lipid levels in unperfused livers

Livers from fed, fasted, and diabetic rats (95 to 135 g) were sampled and analyzed as described under "Experimental Procedure." Values are from six livers in each category. Glycogen values are in glucose equivalents and lipid values are in fatty acid equivalents.

<table>
<thead>
<tr>
<th>Liver donor</th>
<th>Fed</th>
<th>Fasted</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen</td>
<td>920 ± 49</td>
<td>6.1 ± 0.6</td>
<td>392 ± 88</td>
</tr>
<tr>
<td>P-lipid</td>
<td>201 ± 3</td>
<td>235 ± 20</td>
<td>258 ± 16</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>36 ± 4</td>
<td>50 ± 8</td>
<td>67 ± 8</td>
</tr>
<tr>
<td>Cholesterol ester</td>
<td>8.0 ± 1.0</td>
<td>0.0 ± 1.2</td>
<td>8.4 ± 0.8</td>
</tr>
</tbody>
</table>

omitted due to the replacement in the perfusion medium of rat erythrocytes by beef red cells (15). Pyruvate, ketone body, and urea production or utilization were calculated in the same manner as lactate production or utilization.

To estimate the changes in glycogen and tissue lipids during perfusion, the levels found at the end of perfusion were subtracted from those determined in unperfused livers taken from anesthetized rats paired for weight and age with those used for perfusion (Table I). This method was adopted because attempts to sample liver lobes during perfusion increased the variability of metabolic rates and frequently caused extensive leakage of medium from the cut portion of the liver.

Studies of the changes in glycogen and lipid levels during the surgical procedures required to establish a closed perfusion system indicated that the method resulted in a 10 to 20% overestimation of the breakdown of glycogen or lipid during perfusion.

Isotope incorporation, expressed as microatoms of 14C incorporated, was computed from the radioactivity of products and the specific activity of lactate carbon added.

All values are expressed on the basis of 100 g body weight represented as mean ± standard error of the mean. The student t-test was used to estimate the statistical significance of differences between means.

TABLE II
Metabolic changes during perfusions of fed, fasted, and diabetic livers perfused with or without lactate or glucagon

Livers from rats weighing 95 to 135 g were perfused for 1 hour with recirculating medium containing no added substrate or 20 mm L-[1-14C]lactate. Rats were fed ad libitum, fasted 18 to 22 hours overnight, or treated with alloxan as described under "Experimental Procedure." Glucagon was infused into the medium flowing to the liver at the rate of 15 pmoles per min. Values are from six perfusions in each category.

<table>
<thead>
<tr>
<th>Metabolic changes</th>
<th>Fed livers</th>
<th>Fasted livers</th>
<th>Diabetic livers</th>
</tr>
</thead>
<tbody>
<tr>
<td>No additions</td>
<td>Lactate</td>
<td>Glucagon</td>
<td>Lactate + glucagon</td>
</tr>
<tr>
<td>Glucose ± 6</td>
<td>+157</td>
<td>+227</td>
<td>+665</td>
</tr>
<tr>
<td>Glycogen*</td>
<td>−181</td>
<td>−162</td>
<td>−337</td>
</tr>
<tr>
<td>Lactate</td>
<td>−40</td>
<td>−354</td>
<td>−50</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>−1 ± 1</td>
<td>10 ± 2</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>Oxygen</td>
<td>−474 ± 24</td>
<td>−364 ± 24</td>
<td>−495 ± 30</td>
</tr>
<tr>
<td>Acetoacetate</td>
<td>+7.4 ± 0.9</td>
<td>+10.9 ± 0.8</td>
<td>+16.3 ± 3.0</td>
</tr>
<tr>
<td>β-Hydroxybutyrate</td>
<td>+3.3 ± 0.2</td>
<td>+8.5 ± 0.5</td>
<td>+4.8 ± 1.0</td>
</tr>
<tr>
<td>P-lipid*</td>
<td>−42 ± 13</td>
<td>−21 ± 11</td>
<td>−46 ± 15</td>
</tr>
<tr>
<td>Triglyceride*</td>
<td>−8 ± 4</td>
<td>−5 ± 4</td>
<td>6 ± 6</td>
</tr>
<tr>
<td>Cholesterol ester+</td>
<td>+5 ± 2</td>
<td>0 ± 2</td>
<td>+6 ± 6</td>
</tr>
<tr>
<td>Urea</td>
<td>+32 ± 2</td>
<td>+36 ± 3</td>
<td>+60 ± 3</td>
</tr>
</tbody>
</table>

* Glucose equivalents; standard errors of the mean refer to variations in values at end of perfusion.

† Fatty acid equivalents; standard errors of the mean refer to variations in values at end of perfusion.

omitted due to the replacement in the perfusion medium of rat erythrocytes by beef red cells (15). Pyruvate, ketone body, and urea production or utilization were calculated in the same manner as lactate production or utilization.

To estimate the changes in glycogen and tissue lipids during perfusion, the levels found at the end of perfusion were subtracted from those determined in unperfused livers taken from anesthetized rats paired for weight and age with those used for perfusion (Table I). This method was adopted because attempts to sample liver lobes during perfusion increased the variability of metabolic rates and frequently caused extensive leakage of medium from the cut portion of the liver. Studies of the changes in glycogen and lipid levels during the surgical procedures required to establish a closed perfusion system indicated that the method resulted in a 10 to 20% overestimation of the breakdown of glycogen or lipid during perfusion.

Isotope incorporation, expressed as microatoms of 14C incorporated, was computed from the radioactivity of products and the specific activity of lactate carbon added.

All values are expressed on the basis of 100 g body weight represented as mean ± standard error of the mean. The changes in glycogen and tissue lipids as shown in Table I are overestimates since they do not take into account the decreases in these substrates during surgical manipulation prior to perfusion.

As pointed out under "Calculation of Results," the changes in glycogen and tissue lipids shown in Table I are overestimates since they do not take into account the decreases in these substrates during surgical manipulation prior to perfusion.
and diabetic rats due to the high activity of gluconeogenesis (Table III). The amounts of glucose synthesized from lactate in livers from fed, fasted, or diabetic rats, computed from the isotope data of Table III assuming a sixteenth loss of label as \( ^{14}C \) due to randomization of isotope between C-1 and C-4 of oxalacetate formed during the conversion of pyruvate to P-pyruvate, were similar to the increases in glucose production after lactate addition observed experimentally (Table II).

Lactate increased the glycogen content of livers from fasted rats by an amount (Table II) equal to that computed from the incorporation of isotope (Table III). In livers from fed rats, glycogen synthesis from lactate (Table III) was too small to significantly affect glycogen disappearance (Table II). The labeling of glycogen was greatly reduced in livers treated with glucagon or obtained from diabetic rats despite an increase in total glycogen (glucose plus glycogen) radioactivity (Table III). These results indicate a severe impairment in glycogen synthesis. In livers from fasted rats, the incorporation of \(^{14}C \) into glycogen was the same as in livers from fed rats, but the ratio of \(^{14}C \) to \(^{14}C \)glucose synthesized was decreased by more than 50% (Table III).

Lactate utilization was increased by 28% in glucagon-treated livers, by 21% in livers from fasted rats, and by 80% in livers from diabetic rats (Table II). The extra lactate utilized was essentially equal to that needed for the extra glucose plus glycogen synthesized except in the case of livers from fasted rats (Table III). This indicates that inhibition of alternative pathways of lactate metabolism played a relatively minor role in the enhancement of lactate gluconeogenesis by glucagon and diabetes.

Effects of Fasting, Diabetes, and Glucagon on Lipid Metabolism in Livers Perfused with and without Lactate—In the absence of lactate, ketone body production was increased approximately 10-fold in livers from fasted and diabetic rats and 2-fold in livers perfused with glucagon (Table II). Lactate increased ketogenesis in livers from fed rats in the absence and presence of glucagon.

In livers from fasted and diabetic rats, on the other hand, lactate suppressed endogenous ketogenesis by about 70% (Table II). About 60% of the increased ketogenesis caused by lactate in control or glucagon-treated livers from fed rats could be attributed to ketone synthesis from lactate (Table III). Fasting and diabetes reduced the incorporation of \(^{14}C \) from lactate into ketone bodies by 48 and 33%, respectively (Table III).

Initial P-lipid and triglyceride levels were higher in livers from fasted and diabetic rats (Table I) and the disappearance of lipid fatty acid during perfusion was apparently greater in these livers (Table II). Lactate and glucagon produced no significant changes in the disappearance of lipid under any conditions (Table II), but there were large variations in the values. The incorporation of label into total liver lipids in the fed rats represented 7.5% of the \(^{14}C \)lactate utilized (Table III) and labeling of lipid glycerol accounted for about one-fourth of the total lipid radioactivity. Isotope was highest in P-lipid, triglyceride, and cholesterol, and was negligible in cholesterol ester and free fatty acid (Table III). Glucagon, fasting, and diabetes reduced the labeling of total lipids by decreasing the incorporation of \(^{14}C \) into the fatty acid and sterol moieties (Table III). The radioactivity in the P-lipid and triglyceride fractions was reduced to about the same extent as in the total lipids. The incorporation of isotope into serum lipids was equivalent to less than 1% of the \(^{14}C \)lactate utilized and was omitted from most analyses.

Effects of Glucagon, Fasting, and Diabetes on Protein Metabolism in Livers Perfused with and without Lactate—Urea production by livers perfused without substrate was approximately doubled by glucagon and diabetes and increased slightly by fasting (Table II). Addition of lactate to the perfusion medium had no effect on ureogenesis except in livers from fasted rats where it produced a small inhibition in agreement with earlier findings (3). Ureogenesis was necessarily associated with deamination of amino acids in these experiments since no other source of NH3 was available. The keto acids produced were probably a major source of glucose in livers from fasted or diabetic rats perfused without lactate, and of ketones in livers from fed rats.

The incorporation of label from \(^{14}C \)lactate into liver protein was reduced by 35% by glucagon and by about 50% by fasting and diabetes. Whether these changes were due to a reduction in the specific activity of amino acid precursors or an inhibition of net protein synthesis is unknown.

Effects of Glucagon, Fasting, and Diabetes on Oxygen Consumption and Krebs Cycle in Livers Perfused with and without Lactate—Oxygen consumption was not altered by glucagon, fasting, or diabetes in livers perfused without substrate (Table II). Lactate increased oxygen consumption by 19% in livers from fed rats, and by larger amounts in livers treated with glucagon or obtained from fasted or diabetic rats (Table II). The four conditions, the formation of \(^{14}CO_2 \) from \(^{14}C \)lactate was substantial and of similar magnitude (Table III). This indication that lactate oxidation was not decreased by glucagon, fasting, or diabetes was confirmed by an analysis of the sources of \(^{14}CO_2 \) in these experiments (Table IV). In this analysis, it was assumed that \(^{14}CO_2 \) not produced during carbohydrate, fatty acid, ketone body, or cholesterol synthesis arose from the complete oxidation of \(^{14}C \)lactate. The table shows that the complete oxidation of \(^{14}C \)lactate accounted for most of the \(^{14}CO_2 \) produced and was very similar in the four situations studied.

An analysis of the sources of oxygen utilization in the experiments is given in Table V. Considering first the changes in \(O_2 \) consumption used in the combustion of substrate to ketones and \(CO_2 \), it is seen that, in the absence of lactate, lipids apparently

### Table III

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Fed</th>
<th>Glucagon-treated</th>
<th>Fasted</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (umoles carbon incorporated/<em>g body wt</em>)</td>
<td>262 ± 9</td>
<td>643 ± 42</td>
<td>617 ± 31</td>
<td>846 ± 57</td>
</tr>
<tr>
<td>Glycogen (umoles carbon incorporated/<em>g body wt</em>)</td>
<td>83 ± 10</td>
<td>5 ± 2</td>
<td>81 ± 14</td>
<td>21 ± 4</td>
</tr>
<tr>
<td>CO₂ (umoles carbon incorporated/<em>g body wt</em>)</td>
<td>420 ± 24</td>
<td>449 ± 17</td>
<td>416 ± 13</td>
<td>438 ± 10</td>
</tr>
<tr>
<td>Ketone bodies (umoles carbon incorporated/<em>g body wt</em>)</td>
<td>21 ± 1</td>
<td>25 ± 2</td>
<td>31 ± 2</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>Lipid (umoles carbon incorporated/<em>g body wt</em>)</td>
<td>96 ± 6</td>
<td>61 ± 13</td>
<td>71 ± 2</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>Lipid glycerol (umoles carbon incorporated/<em>g body wt</em>)</td>
<td>19 ± 1</td>
<td>22 ± 2</td>
<td>16 ± 1</td>
<td>16 ± 1</td>
</tr>
<tr>
<td>Lipid fatty acid (umoles carbon incorporated/<em>g body wt</em>)</td>
<td>40 ± 6</td>
<td>24 ± 7</td>
<td>17 ± 4</td>
<td>19 ± 7</td>
</tr>
<tr>
<td>Sterol (umoles carbon incorporated/<em>g body wt</em>)</td>
<td>20 ± 4</td>
<td>9 ± 1</td>
<td>4 ± 1</td>
<td>20 ± 1</td>
</tr>
<tr>
<td>Protein (umoles carbon incorporated/<em>g body wt</em>)</td>
<td>25 ± 4</td>
<td>16 ± 6</td>
<td>15 ± 2</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>P-lipid (umoles carbon incorporated/<em>g body wt</em>)</td>
<td>27 ± 4</td>
<td>17 ± 3</td>
<td>7 ± 1</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Triglyceride (umoles carbon incorporated/<em>g body wt</em>)</td>
<td>18 ± 1</td>
<td>9 ± 2</td>
<td>7 ± 1</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Cholesterol (umoles carbon incorporated/<em>g body wt</em>)</td>
<td>19 ± 2</td>
<td>10 ± 2</td>
<td>6 ± 1</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Cholesterol ester (umoles carbon incorporated/<em>g body wt</em>)</td>
<td>2 ± 0.5</td>
<td>2 ± 0.3</td>
<td>1 ± 0.3</td>
<td>1 ± 0.3</td>
</tr>
<tr>
<td>Free fatty acid (umoles carbon incorporated/<em>g body wt</em>)</td>
<td>1 ± 0.5</td>
<td>0.3 ± 0.3</td>
<td>0.6 ± 0.3</td>
<td>1 ± 0.3</td>
</tr>
<tr>
<td>Substrate (umoles carbon incorporated/<em>g body wt</em>)</td>
<td>1000 ± 45</td>
<td>1211 ± 42</td>
<td>1200 ± 60</td>
<td>1536 ± 36</td>
</tr>
</tbody>
</table>

* Separated by thin layer chromatography.
* Substrate utilized, i.e. microatoms of lactate-C plus pyruvate-C removed.
the major fuel oxidized in livers from fed or fasted rats. In the presence of glucagon or in livers from diabetic rats, however, protein apparently became equal to lipid as a fuel source. Addition of lactate to livers from fed rats perfused without or with glucagon markedly suppressed the oxidation of lipid. In livers from fasted rats it appeared to reduce protein oxidation, whereas in livers from diabetic rats it did not cause much change in total endogenous oxygen consumption.

Considering next the citric acid cycle, Table V shows that, in livers perfused without lactate, fasting or diabetes caused a marked diminution, whereas glucagon had little effect. Addition of lactate produced a small increase in the cycle in livers from fasted rats which was not altered by glucagon. In livers from fasted or diabetic rats, however, lactate increased the rate of the cycle 3 to 4-fold. The utilization of lipid in the cycle was decreased slightly by glucagon and markedly by fasting and diabetes. Lactate greatly suppressed the oxidation of lipid in the cycle in livers from fed rats perfused without or with glucagon. On the other hand it markedly increased the utilization of lipid in the cycle in livers from fasted or diabetic rats.

Effect of Insulin on Gluconeogenesis in Diabetic Rats—To determine whether the action of alloxan on gluconeogenesis was due primarily to insulin lack, alloxan-treated rats were injected subcutaneously with protamine zinc insulin for 2 days. Table VI shows that this completely reversed the effects of alloxan on the output of glucose and the synthesis of [14C]glucose and [14C]glycogen from [14C]lactate in the perfused liver. In rats so treated, the blood glucose was reduced to normal (Table VI) in agreement with earlier findings (5).

DISCUSSION

Hepatic Gluconeogenesis during Fasting, Diabetes, and Glucagon Administration in Vivo—The present findings confirm earlier demonstrations that fasting and diabetes increase gluconeogenesis in liver slices (16) and in the perfused liver (5, 17). They are also consistent with findings in vivo of increased [14C]glucose synthesis from [14C]-labeled lactate, pyruvate, alanine, serine, and glycine in fasted or diabetic humans and rats (18-21).

It is probable that amino acids mobilized from muscle due to decreased protein synthesis are the principal substrates utilized in vivo for glucose synthesis during fasting or insulin insufficiency. Although the percentage of lactate and pyruvate converted to glucose is increased during fasting and diabetes (19-21), the major fuel oxidized in livers from fed or diabetic rats.

<table>
<thead>
<tr>
<th>Sources of O₂ in livers perfused with [14C]lactate</th>
<th>Fed livers</th>
<th>Glucagon-treated fed livers</th>
<th>Fasted livers</th>
<th>Diabetic livers</th>
</tr>
</thead>
<tbody>
<tr>
<td>[14C]O₂ produced in the Krebs cycle</td>
<td>100</td>
<td>100</td>
<td>176</td>
<td>160</td>
</tr>
</tbody>
</table>

Table IV

Values were computed from the data of Tables II to IV. O₂ utilized for lactate ketogenesis was computed on the basis of 0.5 molecule per atom of carbon incorporated. Lipid ketogenesis was computed as the difference between total ketogenesis and that due to lactate and protein (see below). O₂ consumed for lipid ketogenesis was computed as 1.75 molecules per molecule of ketone (average fatty acid chain length taken as 16). O₂ utilized for protein oxidation was computed on the basis of 4 molecules of O₂ per molecule of urea (see below). O₂ consumed during the complete oxidation of lactate was taken as equal to the CO₂ produced (Table IV). O₂ used for lipid oxidation was taken as the difference between total O₂ consumption and that attributable to ketogenesis and the oxidation of protein and lactate. O₂ consumed during the combustion of protein in the cycle was computed as 1.2 molecules of O₂ per molecule of urea (see below). O₂ utilized in the cycle during the combustion of lactate or lipid was assumed to be two-thirds of that used to completely oxidize these substrates. The values for protein ketogenesis and O₂ consumption for protein oxidation are taken from an unpublished analysis of the metabolism of endogenous protein in the perfused liver by J. H. Exton. 8

<table>
<thead>
<tr>
<th>Source of O₂ consumption</th>
<th>Fed livers</th>
<th>Glucagon-treated livers</th>
<th>Fasted livers</th>
<th>Diabetic livers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate ketogenesis</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Lipid ketogenesis</td>
<td>5</td>
<td>7</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Protein oxidation</td>
<td>128</td>
<td>144</td>
<td>240</td>
<td>284</td>
</tr>
<tr>
<td>Lactate oxidation to CO₂</td>
<td>0</td>
<td>285</td>
<td>0</td>
<td>285</td>
</tr>
<tr>
<td>Lipid oxidation to CO₂</td>
<td>341</td>
<td>104</td>
<td>246</td>
<td>84</td>
</tr>
<tr>
<td>Protein oxidized in cycle</td>
<td>38</td>
<td>43</td>
<td>72</td>
<td>85</td>
</tr>
<tr>
<td>Lactate oxidized in cycle</td>
<td>0</td>
<td>199</td>
<td>0</td>
<td>199</td>
</tr>
<tr>
<td>Lipid oxidized in cycle</td>
<td>227</td>
<td>69</td>
<td>164</td>
<td>56</td>
</tr>
<tr>
<td>Total oxidized in cycle</td>
<td>365</td>
<td>311</td>
<td>236</td>
<td>331</td>
</tr>
</tbody>
</table>

Table V

Values were computed from the data of Tables II to IV. O₂ utilized for lactate ketogenesis was computed on the basis of 0.5 molecule per atom of carbon incorporated. Lipid ketogenesis was computed as the difference between total ketogenesis and that due to lactate and protein (see below). O₂ consumed for lipid ketogenesis was computed as 1.75 molecules per molecule of ketone (average fatty acid chain length taken as 16). O₂ utilized for protein oxidation was computed on the basis of 4 molecules of O₂ per molecule of urea (see below). O₂ consumed during the complete oxidation of lactate was taken as equal to the CO₂ produced (Table IV). O₂ used for lipid oxidation was taken as the difference between total O₂ consumption and that attributable to ketogenesis and the oxidation of protein and lactate. O₂ consumed during the combustion of protein in the cycle was computed as 1.2 molecules of O₂ per molecule of urea (see below). O₂ utilized in the cycle during the combustion of lactate or lipid was assumed to be two-thirds of that used to completely oxidize these substrates. The values for protein ketogenesis and O₂ consumption for protein oxidation are taken from an unpublished analysis of the metabolism of endogenous protein in the perfused liver by J. H. Exton. 8

8 An Analysis of the Metabolism of Endogenous Protein in the Perfused Rat Liver by J. H. Exton is available as JBC Document Number 71M-1435, in the form of 1 microfiche or 14 pages. Orders for supplementary material should specify the title, author, and reference to this paper and the JBC Document number, the form desired (microfiche or hard copy), and the number of copies desired. Orders should be addressed to The Journal of Biological Chemistry, 950 Rockville Pike, Bethesda, Md. 20014, and must be accompanied by remittance to the order of the Journal in the amount of $2.50 per microfiche or 15¢ per page of hard copy, with a minimum charge of $2.50.
Effects of insulin on glucose production and gluconeogenesis in livers from alloxan diabetic rats

Rats were injected with alloxan as described under "Experimental Procedure" and used 48 hours later. Protamine zinc insulin (0.5 unit) was injected twice daily for 2 days prior to perfusion. Livers were perfused for 1 hour with recirculating medium containing 20 mM [U-14C]lactate.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>5.9 ± 0.20 ± 0.05</td>
<td>130 ± 13</td>
<td>220 ± 15</td>
<td>115 ± 4</td>
<td>14 ± 3</td>
</tr>
<tr>
<td>Alloxan</td>
<td>25 ± 4</td>
<td>1.00 ± 0.10</td>
<td>343 ± 19</td>
<td>222 ± 25</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>Alloxan plus insulin</td>
<td>4.0 ± 1.50 ± 0.08</td>
<td>176 ± 40</td>
<td>120 ± 4</td>
<td>15 ± 3</td>
<td></td>
</tr>
</tbody>
</table>

* Measured at the end of perfusion.

The present results suggest that decreased ketogenesis of fasted or diabetic animals is the result of several changes. Of primary importance is the increased supply of fatty acids to the liver. In addition, there is evidence for an increased rate of the β oxidation pathway in the liver (41-43) and a decreased rate of the citric acid cycle (44, 45). Other changes such as reduced utilization of acetyl-CoA for the synthesis of fatty acids and cholesterol and alterations in the enzymes of ketone body synthesis probably play minor roles.

The increased ketogenesis of fasted or diabetic animals is the resultant of several changes. Of primary importance is the increased supply of fatty acids to the liver. In addition, there is evidence for an increased rate of the β oxidation pathway in the liver (41-43) and a decreased rate of the citric acid cycle (44, 45). Other changes such as reduced utilization of acetyl-CoA for the synthesis of fatty acids and cholesterol and alterations in the enzymes of ketone body synthesis probably play minor roles.

The present results suggest that decreased Krebs cycle activity was a major factor in the enhancement of ketogenesis in livers from fasted or diabetic rats perfused without substrate (Table V). The data of Tables II and V also indicate increased utilization of lipid in such livers pointing to overproduction of acetyl-CoA as another factor.

Mechanisms Involved in Antiketogenic Action of Lactate—The findings confirm earlier observations of the large antiketogenic effect of lactate in perfused livers from fasted rats (3) and show a similar effect in diabetic livers. The effect of lactate appears to be due in part to increased oxidation of acetyl-CoA in the Krebs cycle (Table V). It is possible that the cycle is enhanced because of an increase in the mitochondrial oxalacetate level, but this is difficult to assess because of uncertainties regarding measurement of this intermediate and its distribution between cytoplasm and mitochondria. It has generally been found that total tissue levels of oxalacetate and related intermediates are normal or decreased in fasted or diabetic livers (for references, see Reference 38). Perfusion of livers with lactate has been found to cause a small increase in tissue oxalacetate (48).

The data of Table V suggest that lactate may also reduce ketogenesis by reducing the over-all production of acetyl-CoA from lipid. An effect of lactate to decrease β oxidation of endogenous fatty acids by increasing their esterification to triglyceride and P-lipid has been observed in unpublished studies in our laboratory. It is probable that the increased esterification is the result of increased levels of glycerol-1-P (49).

The antiketogenic action of lactate in the isolated liver is mimicked by other physiological substrates including phosphenolpyruvate carboxykinase activity. An increase in the supply of these substrates and other glycogenic amino acids may play a significant role in the control of ketogenesis in vivo. Their ability to reduce free fatty acid release from adipose tissue by forming glycerol-1-P (51) may reinforce their antiketogenic action in the liver.

Mechanisms Involved in Decreased Fatty Acid and Cholesterol Synthesis with Glucagon, Fasting, and Diabetes—The present findings indicate that fatty acid and cholesterol synthesis represent minor pathways of lactate metabolism in the perfused liver. The decreased incorporation of \(^{14}C\) into these compounds caused by glucagon, fasting, and diabetes probably reflects decreased rates of synthesis and reduced specific activity of \(^{14}C\)acetyl-CoA because of enhanced formation of unlabelled acetyl-CoA from lipid or protein. Fatty acid synthesis is depressed in livers from fasted or diabetic rats because of decreased levels of the rate-limiting enzyme, acetyl-CoA carboxylase, or increased levels of the inhibitor, fatty acyl-CoA (for references, see Reference 42). Decreased cholesterologenesis during fasting has also been reported and may reflect reduced activity of the rate-limiting enzyme \( \beta \)-hydroxy-\( \beta \)-methylglutaryl-CoA reductase (for references, see Reference 52).

Effects of Glucagon, Fasting, and Diabetes on Lactate Oxidation—The present findings provide little evidence of significant inhibition of lactate oxidation to acetyl-CoA by glucagon, fasting, or diabetes (Table V). Lactate utilization for processes other than pyruvate formation, gluconeogenesis, or glyceroneogenesis (Table III), and \(^{14}CO_2\) formed by \(^{14}C\)lactate oxidation (Table IV) were only slightly reduced.

It should be pointed out that the present results probably do not reflect the situation in vivo because of the high concentration of lactate employed. Studies with physiological levels of substrate indicate that glucagon and fasting substantially decrease lactate oxidation in the perfused liver.

Changes in Protein Metabolism with Glucagon, Fasting, and Diabetes—The effects of glucagon on the liver include stimulation of the uptake of certain amino acids through acceleration of their inward transport, or intracellular utilization, or both, and promotion of the release of other amino acids because of increased proteolysis (53). Increased proteolysis was probably the cause of the stimulation of ureogenesis by glucagon in the present study.

It might also have caused the decreased incorporation of \(^{14}C\) from lactate into protein.

The increased ureogenesis of fasting or diabetes observed in the present study was probably due to increased activity of the enzymes of the urea cycle (54) and to enhanced breakdown of liver protein (55). These changes may be related to the level of cyclic AMP in the diabetic or fasted liver (2, 5) since glucagon administration markedly increases the enzymes of the urea cycle (54) and diabetes and fasting cause changes in the levels of amino acids in rat liver (56, 57) which resemble those produced by glucagon (53).

Acknowledgment—We wish to thank C. R. Park for his very helpful suggestions concerning the manuscript.

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Control of Gluconeogenesis in Liver: V. EFFECTS OF FASTING, DIABETES, AND GLUCAGON ON LACTATE AND ENDOGENOUS METABOLISM IN THE PERFUSED RAT LIVER

John H. Exton, Jerry G. Corbin and Sandra C. Harper


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