Glycolytic and gluconeogenic enzyme activities in renal cortex of diabetic rats

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DIABETES MELLITUS is accompanied by striking alterations in glycogen content (17, 39), glycoprotein composition and synthetic rates (10, 44, 45), mucopolysaccharide composition (11), and gluconeogenic rates (15, 20) in renal cortex of experimental animals. Alterations in blood glucose concentration and glycogen content in renal cortex, however, have not been correlated with changes in the activities of the important glycolytic, pentose phosphate pathway, and gluconeogenic enzymes in diabetic animals. In this study we have measured changes in glycogen content, glycolytic, NADPH-generating, and gluconeogenic enzyme activities in renal cortex of diabetic rats and examined correlations between these changes and blood glucose levels. Since induced acidosis, per se, increases the activities of the renal pentose phosphate pathway dehydrogenases (18, 31), we have examined correlations between blood pH and glycolytic and NADPH-generating enzyme activities in alloxan-diabetic rats. Also, since the diabetic state may alter the intracellular distribution of hexokinase (3), we have studied the compartmentation of hexokinase in renal cortex of these diabetic animals. Changes in renal cortical enzyme activities were compared with those of liver, another important gluconeogenic tissue, and with jejunal mucosa, a tissue with gluconeogenic capabilities (1, 2) and many transport functions resembling those of kidney (12).

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats weighing 175-250 g obtained from Hortoe Laboratories, Berkeley, Calif., were maintained in individual cages with mesh floors and fed a standard diet of Purina laboratory chow for at least 1 week prior to use. All animals were allowed free access to water and to food unless otherwise indicated. All comparisons were made between age-matched control and experimental groups. Alloxan diabetes was induced by the intraperitoneal injection of 100-125 mg of alloxan per kilogram body weight after a 60-hr fast and the animals were sacrificed 25-35 days later. The severity of the diabetic state was estimated by failure of normal growth, measurement of glycosuria (Tes-Tape, Eli Lilly and Company, Indianapolis, Ind.), and blood glucose measurements. Blood glucose estimations were performed in duplicate by the glucose oxidase method using Glucostat reagent (Worthington Biochemical Corporation, Freehold, N. J.) or Dextrostix and reflectance meter measurements (Ames Co., Elkhart, Ind.). Preliminary studies indicated an excellent correlation between blood glucose values obtained by the two methods, as others have noted (26).

Tissue preparations. Rats were killed by cervical fracture, the abdomen was opened, and the kidneys removed and weighed. Most enzyme estimations were performed on fresh tissue preparations, but some tissues were frozen at -70 C for 1-3 weeks. The activities and the intracellular distribution of renal cortical enzymes were found not to be altered by freezing when fresh tissue samples were assayed and then assays were performed on samples from the same kidneys that had been frozen for 3 weeks. The kidneys were slit and a portion of the renal cortex removed, weighed, and homogenized in 20 vol of 50 mM Tris, 100 mM KCl, 1 mM EDTA, 5 mM MgCl2, and 5 mM mercaptoethanol, pH 7.5, in a Kontes Duall grinder. An aliquot of homogenate was taken for hexokinase assay and the remain-
glycogen solutions were transferred to centrifuge tubes and centrifuged at 105,000 \( \times g \) for 60 min in a Spinco model L ultracentrifuge at 4 C. Liver and jejunal mucosal samples were obtained, homogenized, and centrifuged in a similar fashion, as previously described (6). Assays for other enzymes were performed on the 105,000 \( \times g \) supernatant or total pellet as indicated. All tissue preparations were carried out at 4 C.

**Blood pH and PCO\(_2\)** measurements. When blood pH measurements were performed, light anesthesia was induced by the intraperitoneal injection of approximately 50 mg/kg body wt of sodium methohexital. The abdomen was opened and blood was obtained from the abdominal aorta in a syringe containing heparin. Animals were killed by exsanguination and tissues were removed for enzymes assays. Arterial blood pH and PCO\(_2\) measurements were performed in duplicate on a Radiometer pH meter.

**Enzyme assays.** Hexokinase, phosphofructokinase, pyruvate kinase, glucose-6-phosphate (G-6-P) dehydrogenase, 6-phosphogluconate (6-P-G) dehydrogenase, malic enzyme, and fructose diphosphatase activities were assayed at 28 C in a Gilford recording spectrophotometer, model 2400. Hexokinase activity was assayed in the homogenate and in the 105,000 \( \times g \) supernatant and pellet by the method of Vinuela, Salas, and Sols (50) using a glucose concentration of 10 \( \mu M \) for renal and jejunal hexokinase and 100 \( \mu M \) for hepatic glucokinase. The following enzymes activities were measured in the 105,000 \( \times g \) supernatant by previously described methods: phosphofructokinase (25), pyruvate kinase (14), G-6-P dehydrogenase (30), 6-P-G dehydrogenase (38), malic enzyme (36), and fructose diphosphatase (46). Pyruvate carboxylase and phosphoenolpyruvate (PEP) carboxykinase activities were measured by a modification (1) of the method of Ballard and Hanson (8), these assays being performed on the 105,000 \( \times g \) supernatant and on the freeze-dried 105,000 \( \times g \) pellet. All enzymic assays were performed in triplicate and were linear with respect to time and protein concentration. NaH\(_{14}\)CO\(_3\) was obtained from Auerhans/Seattle Corp., Arlington Heights, Ill., and other enzymes, substrates and reagents from Sigma Chemical Company, St. Louis, Mo.

**Glycogen measurements.** Modifications of previously described techniques (23, 27) were made to measure the small amount of glycogen in the renal cortex of normal rats. Tissues were digested in 1 ml of 30% KOH per 300 mg tissue at room temperature. Samples were then heated for 20 min in boiling water; after cooling, 1.3 vol of 95% ethanol was added. Following the second centrifugation, the glycogen precipitates were dried by air and blood was obtained from the abdominal aorta in a syringe containing heparin. Animals were killed by exsanguination and tissues were removed for enzymes assays. Glycogen (Sigma Chemical Company) which had been treated in an identical fashion to the tissue extracts including digestion in KOH, precipitation with ethanol, hydrolysis with H\(_2\)SO\(_4\), and resuspension in NaOH were used.

**Protein concentrations.** Protein concentrations were measured by the method of Lowry et al. (32). Bovine serum albumin, dissolved in the homogenizing buffer used for the original sample, was used as a standard.

**RESULTS**

**Body and organ weights.** These age-matched animals had identical weights at the initiation of the study, but at sacrifice control rats weighed 338 \( \pm \) 8 g (mean \( \pm \) se, \( n = 42 \)) whereas diabetic rats weighed 263 \( \pm \) 9 g (\( n = 33, P < 0.001 \)). Diabetes was accompanied by significant renal hypertrophy, and a positive linear correlation (\( r = +0.89, P < 0.001 \)) was observed between kidney weight and blood glucose values in these diabetic rats. The total weight of both kidneys in 20 control rats was 2.6 \( \pm \) 0.1 g (mean \( \pm \) se) or \( \pm 0.73 \)\( \pm \) 0.02 g/100 g rat weight, while kidney weights in 15 diabetic rats with a mean blood glucose value of 402 \( \pm \) 15 mg/100 ml averaged 3.1 \( \pm \) 0.1 g or 1.04 \( \pm \) 0.03 g/100 g rat weight (P vs. controls < 0.001).

**Blood glucose and tissue glycogen concentrations.** Blood glucose values in 23 control rats were 100 \( \pm \) 2 mg/100 ml (mean \( \pm \) se). The diabetic rats selected for enzyme comparisons (Tables 1-3) had blood glucose values above 400 mg/100 ml. Mean glucose values for these rats were 502 \( \pm \) 14 mg/100 ml (\( n = 33, P < 0.001 \)). Renal cortical glycogen concentrations were approximately 30-fold higher in these diabetic rats (1,545 \( \pm \) 315 \( \mu \)g/g wt, \( n = 21 \)) than in control animals (54 \( \pm \) 2, \( n = 14, P < 0.001 \)). On the other hand, liver glycogen was significantly lower in these diabetic rats, 9.0 \( \pm \) 2.5 mg/g liver (\( n = 14, P < 0.01 \)), than in control animals, 62.2 \( \pm \) 4.4 (\( n = 14, P < 0.01 \)).

Renal cortical glycogen accumulation in diabetic rats tended to parallel the degree of hyperglycemia. In 24 diabetic rats with blood glucose values ranging from 156 to 640 mg/100 ml there was a linear correlation between the blood glucose and renal cortical glycogen concentrations (\( r = +0.42, P < 0.05 \)).

**Protein concentrations of renal cortex.** Diabetic rats had lower protein concentrations in the homogenate and the supernatant fraction, whereas fasted rats had higher values in these two fractions than observed for renal cortex from control rats (Table 1). Values for all enzyme activities except hexokinase are reported as specific activity (nmoles/min per mg protein). However, enzyme activities expressed per gram wet weight were compared and are discussed in the results section.

**Glycolytic enzyme activities in renal cortex.** Total hexokinase activity was only slightly increased in diabetic rats when compared with control animals, but there were significant alterations in the activities of the pellet and supernatant fractions (Table 1). Diabetic rats had significantly lower hexokinase activity in the pellet fraction and significantly higher activity in the supernatant fraction. These changes were observed both when hexokinase activity was expressed per gram renal cortex and as specific activity. As a result, the percentage of hexokinase activity in the supernatant...
The protein concentration and hexokinase activity in renal cortex are shown in Table 1. The protein concentration was significantly higher in diabetic rats (70.6 ± 3.0 %, mean ± se) compared to control rats (59.8 ± 2.5 %, mean ± se) (P < 0.001). The hexokinase activity was also significantly higher in diabetic rats (21.9 ± 1.0 nmol/min per mg protein) compared to control rats (14.6 ± 0.6 nmol/min per mg protein) (P < 0.001).

The correlation between renal cortical enzyme activities and blood pH and blood glucose is shown in Table 2. The activities of G-6-P dehydrogenase and 6-P-G dehydrogenase were not significantly correlated with blood pH or blood glucose. However, the activities of pyruvate kinase and hexokinase were significantly correlated with blood pH (r = +0.60, P < 0.05, n = 12) and blood glucose (r = -0.67, P < 0.005, n = 16). The decrease in malic enzyme activity showed a significant linear correlation with the decrease in blood pH (r = +0.60, P < 0.05, n = 12), but enzyme activity was not correlated with blood Pco2 values. In addition, diabetic rats with blood pH values below 7.3 had significantly lower values for renal cortical malic enzyme activity.

Since a negative linear correlation between blood pH and the degree of hyperglycemia in diabetic rats was observed (r = -0.67, P < 0.005, n = 16), we examined correlations between enzyme specific activities and blood glucose values. There were no significant correlations between hyperglycemia and hexokinase, pyruvate kinase, and G-6-P dehydrogenase activities in renal cortex. However, decreases in malic enzyme activity in diabetic rats were correlated, in a linear manner, with increases in blood glucose values (r = -0.66, P < 0.001, n = 26). Of the gluconeogenic enzymes, only soluble PEP carboxykinase activity was correlated, in a positive linear fashion, with the severity of the hyperglycemia in diabetic animals (r = +0.70, P < 0.002, n = 43).

Comparisons of enzyme activities in renal cortex, liver, and renal cortex was similar in diabetic and control rats while specific activity was significantly higher in the diabetics (Table 2). Pyruvate carboxylase activity in the supernatant fractions was identical in diabetic and control rats.

Correlation between renal cortical enzyme activities and blood pH and blood glucose. Animals with blood pH values above 7.3 had slightly higher hexokinase, pyruvate kinase, and G-6-P dehydrogenase specific activities than did the rats with lower pH values (Table 3). Thus, the significant increase in specific activities of these enzymes (Tables 1 and 2) could not be related specifically to the acidosis in these animals. Blood Pco2 values in 11 of these diabetic rats with blood pH values of 7.26 ± 0.04 (mean ± se) were 36.0 ± 3.7 mm Hg while Pco2 values in 8 of these control rats with blood pH values of 7.42 ± 0.02 (P vs. diabetic < 0.01) were 41.9 ± 1.6 (NS). When the specific activities of these renal cortical enzymes were compared with blood pH and Pco2 values, no significant correlations were observed. In sharp contrast, the decrease in malic enzyme specific activity showed a significant linear correlation with the decrease in blood pH (r = +0.60, P < 0.05, n = 12), but enzyme activity was not correlated with blood Pco2 values. In addition, diabetic rats with blood pH values below 7.3 had significantly lower values for renal cortical malic enzyme activity.

Table 1. Protein concentration and hexokinase activity in renal cortex

<table>
<thead>
<tr>
<th>Protein concentration, mg/g wet wt</th>
<th>Control (n = 18)</th>
<th>Diabetic (n = 15)</th>
<th>72-hr Fast (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td>134 ± 3</td>
<td>137 ± 4</td>
<td>169 ± 2</td>
</tr>
<tr>
<td>105,000 × g Pellet</td>
<td>75.2 ± 3.4</td>
<td>80.1 ± 2.6</td>
<td>102 ± 3.1</td>
</tr>
<tr>
<td>105,000 × g Supernatant</td>
<td>78.4 ± 2.0</td>
<td>63.7 ± 3.9</td>
<td>62.9 ± 2.7</td>
</tr>
</tbody>
</table>

Table 2. Enzyme activities in renal cortex of diabetic rats

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Control</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphofructokinase</td>
<td>23.1 ± 1.0 (14)</td>
<td>25.6 ± 1.4 (11)</td>
</tr>
<tr>
<td>Pyruvate kinase</td>
<td>240 ± 21 (30)</td>
<td>310 ± 24 (31)*</td>
</tr>
<tr>
<td>G-6-P dehydrogenase</td>
<td>29.3 ± 1.1 (42)</td>
<td>37.4 ± 1.9 (23)*</td>
</tr>
<tr>
<td>6-P-G dehydrogenase</td>
<td>22.7 ± 1.1 (11)</td>
<td>26.2 ± 1.1 (10)*</td>
</tr>
<tr>
<td>Malic enzyme</td>
<td>12.3 ± 0.6 (39)</td>
<td>7.8 ± 0.5 (22)*</td>
</tr>
<tr>
<td>Fructose diphosphatase</td>
<td>95 ± 3 (14)</td>
<td>108 ± 6 (11)</td>
</tr>
<tr>
<td>PEP carboxykinase</td>
<td>113 ± 12 (11)</td>
<td>230 ± 16 (16)*</td>
</tr>
<tr>
<td>Pyruvate carboxylase</td>
<td>147 ± 9 (11)</td>
<td>182 ± 7 (16)*</td>
</tr>
</tbody>
</table>

Values are given as means ± se with number of animals in parentheses. Enzyme activities are expressed as nmoles/min per mg protein and values for glucose-6-phosphate dehydrogenase and fructose diphosphatase represent nmoles NADPH produced, while values for other enzymes represent nmoles substrate utilized. Pyruvate carboxylase activities were measured in the 105,000 × g (total) pellet, while other enzyme activities were measured in the 105,000 × g supernatant. Significant differences from control values are *P < 0.001, +P < 0.01, and CP < 0.05.
jejunal mucosa. Distinct patterns of enzyme responses were observed for each of these tissues (Fig. 1). Diabetic rats had significantly lower hepatic activities of the important, rate-limiting enzymes of glycolysis, namely, hexokinase, phosphofructokinase, and pyruvate kinase. In sharp contrast, the activities of these enzymes were similar or significantly higher in renal cortex and jejunal mucosa of diabetic rats. After a 72-hr fast the activities of these enzymes in liver and jejunal mucosa were significantly lower than values for control rats, whereas renal cortical activities of hexokinase and pyruvate kinase were similar in fasted and control rats.

Three reactions of the supernatant fraction, those catalyzed by G-6-P dehydrogenase, 6-P-G dehydrogenase, and malic enzyme, are considered to be important sources for the NADPH generated in the cytosol of mammalian tissues (49), and it has been suggested that these enzymes might function as a coordinated group (37). In renal cortex, however, malic enzyme activity behaved differently than reported for other tissues in that diabetes produces significant increases in G-6-P dehydrogenase and 6-P-G dehydrogenase specific activities, whereas malic enzyme activity was significantly lower than control values. In addition, fasting was associated with a significant decrease in G-6-P dehydrogenase activity whereas malic enzyme activity was similar to control values. On the other hand, the changes observed in the aerobic glycolytic enzyme activities, (G-6-P dehydrogenase and 6-P-G dehydrogenase), were very similar to those noted for the three anaerobic glycolytic enzymes in response to diabetes and fasting in all three tissue studies.

PEP carboxykinase was the only gluconeogenic enzyme which was significantly increased in both liver and kidney of diabetic animals. The increase in PEP carboxykinase activity showed an excellent linear correlation with the degree of hyperglycemia in both liver (35) and renal cortex (vide supra). This activity in these two tissues of the same diabetic animals were closely correlated (r = + .81, P < 0.005, n = 23). Jejunal mucosa also contains significant levels of activities for the important gluconeogenic enzymes (1, 6), but in sharp contrast to renal cortex and liver, jejunal mucosal activity for PEP carboxykinase was not increased by diabetes. On the other hand, after a 72-hr fast jejunal PEP carboxykinase activity was twofold higher than in control animals.

### DISCUSSION

The diabetic state is accompanied by renal hypertrophy and by significant alterations in glycoen content and the specific activities of several glycolytic, NADPH-generating, and gluconeogenic enzyme activities. The renal hypertrophy we observed is similar to that reported by others (40, 44) and is closely correlated with the degree of hyperglycemia. The metabolic acidosis observed in these diabetic rats may also have contributed to the renal hypertrophy (31).

Glycogen accumulation in the renal tubules of diabetic patients has been recognized for almost a century (17). Studies in diabetic rats (7, 17, 22, 39) suggest, as do our data, that the renal accumulation of glycogen is related to the degree of hyperglycemia and presumably to the amount of glycosuria.1 The marked increase in renal cortical glycogen accumulation in the renal tubules of diabetic patients has been recognized for almost a century (17).

### TABLE 3  Blood pH and glucose values and renal cortical enzyme activities in diabetic rats

<table>
<thead>
<tr>
<th>Blood pH</th>
<th>Glucose mg/100 ml</th>
<th>Enzyme Activities in Supernatant Fraction nmoles/min per mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hexokinase</td>
</tr>
<tr>
<td>Control</td>
<td>7.42 ± 0.01</td>
<td>13</td>
</tr>
<tr>
<td>Diabetic pH &gt; 7.3</td>
<td>7.37 ± 0.01</td>
<td>7</td>
</tr>
<tr>
<td>P vs. control</td>
<td>0.05</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values given as means ± se. Significant differences are indicated: *P < 0.001, **P < 0.05 vs. diabetic group with pH > 7.3.
cogen that we and others (40, 47) have observed in these severely diabetic rats is consistent with the excessive accumulation of glucogen observed by others (7, 17) in the proximal tubules histologically.

Significant alterations in the intracellular location of hexokinase activity were observed in the renal cortex of these diabetic rats. The diabetic state appears to produce a release of particulate-bound hexokinase into the cytosol of adipose tissue (13), lactating mammary gland (51), and jejunal mucosa (4). The reduction in particulate activity and increase in supernatant activity in renal cortex that we noted is consistent with these observations. Particulate-bound hexokinase is largely localized to the mitochondria of kidney (28) as is pyruvate carboxylase (9). An alternate explanation for the increase in supernatant and decrease in particulate hexokinase activities is that diabetes may alter the fragility of mitochondria with release of bound hexokinase during tissue preparation. Against this explanation is our observation that soluble pyruvate carboxylase activity of renal cortex was identical in control and diabetic animals. Thus, our observations and those of others (13, 28, 51) are consistent with the hypothesis (3) that the diabetic state may produce a release of particulate-bound hexokinase with a resultant increase of hexokinase activity in the supernatant fraction.

The NADPH generating enzymes in renal cortex did not demonstrate the coordinated adaptive response to diabetes or fasting that was observed for liver and jejunal mucosa. The observations of others (24, 42, 48, 52, 53) have led to the suggestion that malic enzyme is the specific enzyme supplying NADPH for fatty acid synthesis. Thus, the reduction in malic enzyme activity in renal cortex of diabetic rats is consistent with reductions in liver (42, 52) and adipose tissue (53) and may reflect a decrease in fatty acid synthesis. On the other hand, the normal to increased levels of activity for the pentose phosphate pathway dehydrogenases observed in renal cortex of diabetic rats strongly suggests that other factors are operating to regulate the level of activities for the G-6-P and the 6-P-G dehydrogenases. Since the pentose phosphate pathway generates precursors for nucleic acid synthesis, the level of activities for these enzymes may be related to the observed renal hypertrophy. Consistent with this suggestion are the studies (18, 19) which indicate that the activities of the pentose phosphate dehydrogenases are increased in association with renal hypertrophy per se. Although the activities of renal cortical pentose phosphate dehydrogenases also are increased by induced acidosis (18, 31), we were unable to demonstrate significant correlations between the activities of these enzymes and blood pH in these diabetic rats.

An increase in the rate of gluconeogenesis in renal cortical slices (15, 20, 47) and in the intact kidney (43) of diabetic rats is well documented, and increased PEP carboxykinase activity in renal cortex has been reported previously (15, 29). The alterations in PEP carboxykinase activity appear to be the only alteration in the key gluconeogenic enzymes which correspond with reported changes (15) in the rate of gluconeogenesis. The excellent linear correlation between renal cortical PEP carboxykinase activity and blood glucose concentration suggests that the rate of renal gluconeogenesis is correlated with the severity of the diabetes.

Thus, our data and that of previous workers (15, 20, 33, 40, 44, 47) indicate that there are significant alterations in glucose metabolism in renal cortex of diabetic animals. The 30-fold higher levels of glucogen in renal cortex of diabetic rats indicates that either glucogen synthesis is accelerated or degradation is impaired. Despite the increase in gluconeogenesis (15, 20) in renal cortex of diabetic animals, our data suggest that the activities of the glycolytic and pentose phosphate pathways are maintained at least at normal rates. As recently reviewed (3), the cellular compartment in which glucogen is phosphorylated probably determines the rate of entry of glucose 6-phosphate into the glycolytic, pentose phosphate, glucogen synthetic, and glucuronate pathways as well as into the mucopolysaccharide and glycoprotein precursor pathways. A redistribution of hexokinase activity in renal cortex of diabetic rats may be an important determinant in the resulting glucogen accumulation, abnormalities in the composition and synthetic rates of glycoproteins (10, 44, 45), and abnormalities in mucopolysaccharide composition (11).

Hepatic glucogen metabolism in diabetic animals was also significantly altered, but in a manner that shows important differences from that observed for renal cortex. Gluconeogenesis is activated but the competing pathways, i.e., anaerobic glycolysis, pentose phosphate generation and glucogen synthesis, are correspondingly reduced, allowing the liver to function in releasing large amounts of glucogen in the diabetic state (31).

The jejunal mucosal metabolism of glucogen also was perturbed in diabetic and fasted rats. These alterations, however, were uniquely different from those observed for renal cortex or liver. Diabetes is associated with increased glucogen content (5), increased glycolytic and pentose phosphate pathway enzyme specific activities, and with a slight increase in the glycolytic rate (5) of jejunal mucosa. The increased glucogen content and enzyme activities in jejunal mucosa resemble the changes noted in renal cortex of diabetic rats. These concomitant changes may be related to the fact that both tissues are increased in weight (49) and that both tissues, which share similar transport functions, are presented with increased amounts of glucogen for transport in the diabetic state. Hepatic glucogen content and glycolytic enzyme activities, however, were reduced in diabetic animals. In contrast to the diabetic state, fasting produced reductions in the glycolytic and pentose phosphate pathway enzyme specific activities in all three tissues, with significant changes being observed in liver and jejunal mucosa. In addition, while diabetes was associated with significant increases in PEP carboxykinase activity in renal cortex and liver, no changes were observed in jejunal mucosal activity. These observations differ from those noted with fasting where PEP carboxykinase activity was significantly increased in both jejunal mucosa and liver. Thus, our data suggest that diabetes does not alter the rate of gluconeogenesis, whereas fasting is associated with a significant increase in gluconeogenesis in jejunal mucosa; these observations contrast with those for liver where both condi-

cortical glucogen values that we observed were similar to or threefold higher than values previously reported (40, 47).
tions increase gluconeogenic rates and enzyme activities (6, 34).

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