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EFFECT OF CHRONIC UREMIA ON FRUCTOSE 2,6-BISPHOSPHATE GLYCOLYTIC AND GLUCONEOGENIC ENZYMES IN RAT LIVER

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SUMMARY: The level of fructose 2,6-bisphosphate and the maximal activities of key gluconeogenic and glycolytic enzymes were determined in the liver of a rat model of chronic uremia and in ad libitum-fed control and pair-fed control animals. Fructose 2,6-bisphosphate was decreased in uremia and its level negatively correlated with the concentration of blood urea nitrogen. The changes in gluconeogenic enzymes in uremic rats were not different from those in the pair-fed controls. However, pyruvate kinase was decreased in uremia when compared to both controls. These studies offer a possible mechanism for the role of the liver in the carbohydrate intolerance of uremia.

Abnormal carbohydrate metabolism in chronic uremia was first recognized by Neubauer in 1910 (1). Although there is a general agreement for decreased peripheral glucose utilization in uremia (2), the role of the liver is still unclear.

Liver slices from chronic uremic and sham-operated rats showed no difference in the incorporation of labeled glucose into glycogen (3). Liver slices from normal rats incubated with uremic serum demonstrated a significant inhibition of glucose utilization with an increase in de novo glucose production from endogenous substrates (4). Increased glucose output was also demonstrated in perfused liver from uremic rats (5).

The two major studies in humans that have attempted to resolve this important question have also provided conflicting results. Rubenfeld and Garber (6), using isotope dilution techniques, demonstrated increased liver glucose production and utilization and an increased gluconeogenesis from alanine. In contrast, DeFronzo et al (7), using [3-^{3}H]-glucose, have demonstrated that glucose production and utilization in the liver are normal in uremic humans.

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In this study we have measured the level of fructose 2,6-bisphosphate, a powerful metabolite in the regulation of glycolysis and gluconeogenesis (8), and the activity of key enzymes in carbohydrate metabolism in the liver from chronic uremic rats. In the experimental design, specific consideration was given to the nutritional status of the animal, which proved to be fundamental for the interpretation of the data.

MATERIALS AND METHODS

Experimental Model of Chronic Uremia: We have recently described the uremic rat model used in this study (9). Briefly, male Sprague Dawley rats weighing ~ 200 g were anesthetized with ether. The flank of the rat was entered and the left kidney was separated from the adrenal gland and perirenal fat. The kidney was placed into a vinyl chamber measuring 0.9 cm^3, which was closed completely except for a 3 mm diameter aperture for the renal pedicle. The enclosed kidney was replaced in the retroperitoneal space and the flank closed. Seven days after this operation, the right kidney was removed using a right flank approach leaving the right adrenal gland intact. The sham-operated controls were operated using the same technique to enter the retroperitoneal space. The kidneys were manipulated, but not removed, and a 1 cm^2 piece of vinyl was placed in the left retroperitoneal space.

Experimental Protocol: Male Sprague-Dawley rats with initial weights of ~200 g were used for all experiments. They were maintained in a constant temperature (30°C) animal room with a fixed artificial light cycle (7:00 a.m. to 7:00 p.m.). All animals were placed in individual cages and were fed standard Purina Chow (Ralston-Purina Co., St. Louis, MO).

The study animals included three experimental groups: group I consisted of uremic rats fed ad libitum; group II consisted of sham-operated rats pair-fed with the uremic rats; group III consisted of sham-operated rats fed ad libitum. The amount of chow given daily to any individual animal in group II was equal to the amount of chow that the uremic animal ate during the preceding 24 hours.

All animals were fasted for ~3 hours before killing 4 weeks after surgery. The liver was quickly excised and rapidly frozen between aluminum tongs that had been cooled in liquid nitrogen, and stored at -70°C. Blood was obtained for measurement of blood urea nitrogen, glucose, and immunoreactive insulin.

Extraction of Liver and Assay of Fructose-2,6-bisphosphate: Fructose-2,6-bisphosphate was assayed according to the method of Van Schaftingen et al (10). Freeze-clamped liver was homogenized in 0.05 M NaOH and heated to 80°C for 5 min. The homogenate was centrifuged and 1.0 ml of the supernate was neutralized with 0.05 ml of 400 mM HEPES and sufficient 2.5 M acetic acid to bring the solution to pH 7-8. The resulting mixture was centrifuged and the supernate was used in the assay. Pyrophosphate:fructose-6-phosphate phosphotransferase was purified from potato tubers by the method of Van Schaftingen (10), and the activity of the enzyme was assayed spectrophotometrically at 340 nm in a buffer containing 50 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 0.5 mM pyrophosphate, 1 mM fructose-6-phosphate, 0.15 mM NADH, 50 µg/ml aldolase, 1 µg/ml triosephosphate isomerase, and 10 µg/ml glycerol-3-phosphate dehydrogenase. Stimulation of enzyme activity by the extract was compared with that by standard quantities of fructose-2,6-bisphosphate.

Extraction of Liver and Assay of Enzyme Activities: Liver was extracted [the

ratio of tissue to extraction medium was 1:5 (w/v)] in a Polytron homogenizer at setting 4 for 15 secs. The homogenate was centrifuged for 2 mins (Eppendorf model 5412 centrifuge) and the supernatant was used for all enzyme assays. For glucokinase, glucose-6-phosphatase, fructose-1,6-bisphosphatase and phosphoenolpyruvate carboxykinase (PEPCK) homogenates were prepared in a buffer containing 300 mM sucrose, 50 mM triethanolamine HCl (pH 7.2), and 1.0 mM EDTA. Extracts for the assay of phosphofructokinase (PFK) and pyruvate kinase were prepared by homogenization of freeze-clamped liver in a buffer containing 50 mM triethanolamine-HCl (pH 7.4), 100 mM KF, and 1.0 mM EDTA.

Enzymes were assayed either spectrophotometrically at 25°C (with a Gilford recording spectrophotometer, model 240) or radiochemically at 30°C (with a Beckman scintillation counter, model 233). Fructose 1,6-bisphosphatase, PFK, pyruvate kinase and PEPCK were assayed spectrophotometrically, while glucokinase and glucose 6-phosphatase were both assayed radiochemically as previously described (11).

RESULTS AND DISCUSSION

This new experimental model of chronic uremia using a vinyl chamber to prevent hypertrophy of the remnant kidney leads to a severe degree of chronic uremia. The uremic rats were weak, lethargic, had coarse yellowish hair that tended to fall out, and some had gross and fine tremors of their limbs that were consistent with severe uremia. As shown in Table I, the uremic rats gained little weight over the 4 week experimental period because food intake was decreased. Table I also shows the blood urea nitrogen was approximately four times greater in the uremic rats. In addition, all groups were euglycemic but the uremic animals had increased serum immunoreactive insulin and glucagon (12). We have previously shown, using the same rat model of chronic uremia, that the liver was resistant to insulin with regard to amino acid transport and lipid synthesis (9,13) due to an undefined post-insulin binding defect(s). Thus, this model appears appropriate to investigate key

TABLE I

Morphometrics, Liver, and Serum Measurements from Uremic, Sham-Operated, Pair-Fed, and Ad Libitum-Fed Controls

	Uremic	Pair-Fed Control	Ad Lib-Fed Control
Initial body weight (g)	205 ± 15	207 ± 20	190 ± 15
Final body weight (g)	226 ± 15	220 ± 10	350 ± 20
Rat chow intake/28 d (g)	450 ± 30	476 ± 37	820 ± 40
Blood urea nitrogen (mg/dl)	68 ± 10	15 ± 2	18 ± 2
Glucose (mg/dl)	150 ± 10	135 ± 15	142 ± 17
Insulin (μU/ml)	35 ± 10	10 ± 1	22 ± 5
Glucagon (μg/ml)	231 ± 22	65 ± 11	155 ± 17

Data are means ± SE. The data on plasma glucagon was published previously (Ref 12).

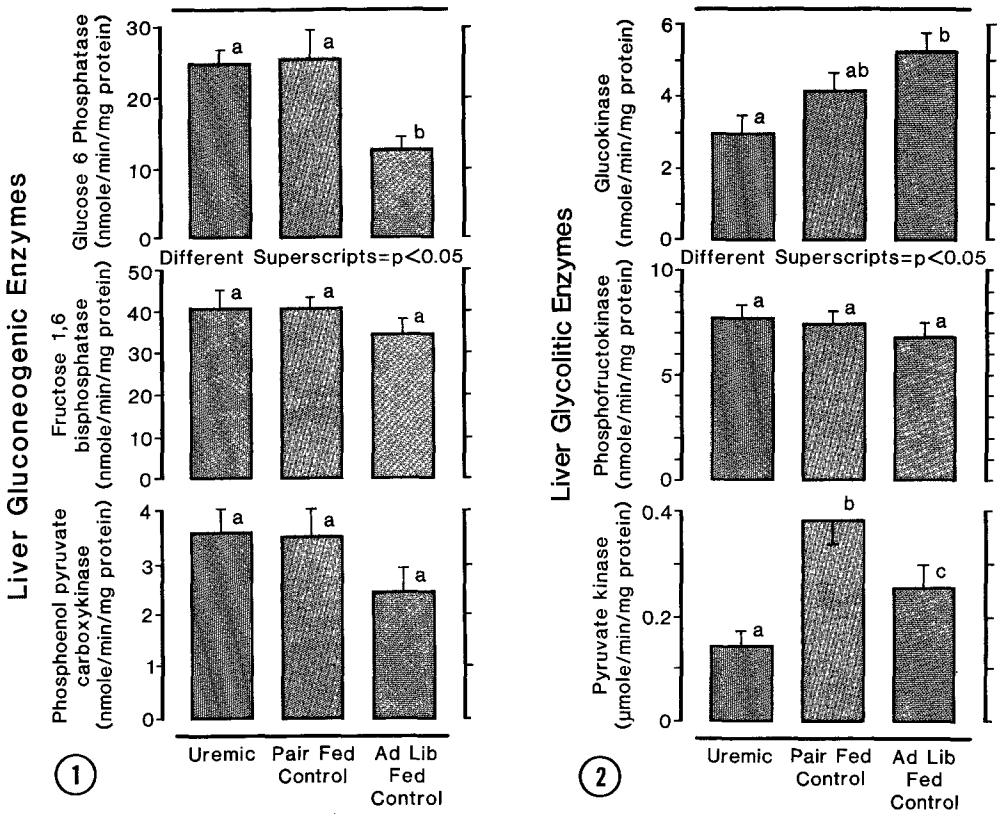

Figure 1. Maximal Activity of Gluconeogenic Enzymes in Liver from Uremic, Pair-Fed Control, and Ad Libitum-Fed Control Rats.

The data represent the means (± standard error) from 9 different animals in each group.

Figure 2. Maximal Activity of Glycolytic Enzymes in Liver From Uremic, Pair-Fed Control, and Ad Libitum-Fed Control Rats.

The data represent the means (± standard error) from 9 different animals in each group.

liver enzymes of carbohydrate metabolism and one of their most important regulators, fructose 2,6-bisphosphate.

Figure 1 shows the maximal activity of three gluconeogenic enzymes. These enzymes were increased in the uremic animals and the pair-fed controls when compared to the ad libitum-fed controls. These changes, which were statistically significant for glucose 6-phosphatase, could account for the well known increase in gluconeogenesis during semi-starvation (14).

We have previously shown a significant increase in the basal rate of aminoisobutyric acid (AIB) uptake in hepatocytes from both the uremic and the pair-fed controls (13). Similar increases have been reported in the liver

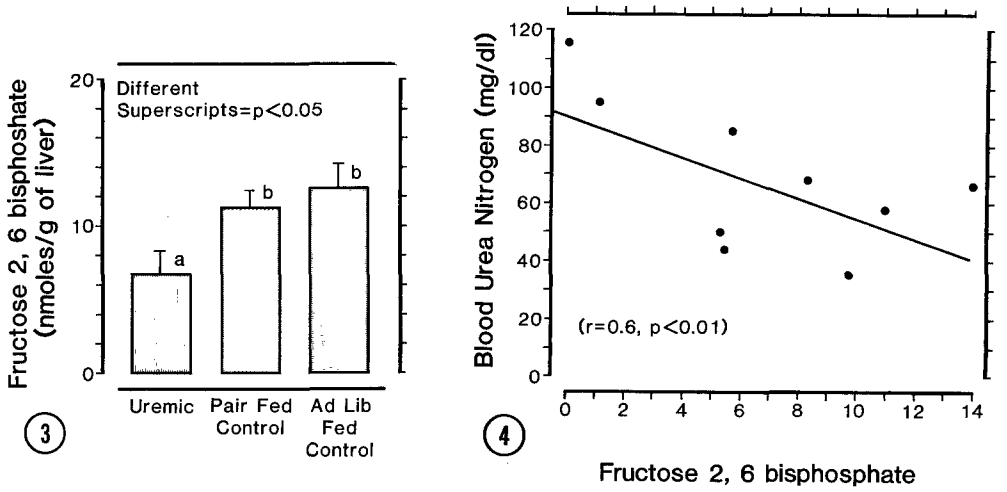

Figure 3. Level of Fructose 2,6-Bisphosphate in Liver From Uremic, Pair-Fed Control, and Ad Libitum-Fed Control Rats.

The data represent the means (± standard error) from 9 different animals in each group.

Figure 4. Correlation Between Blood Urea Nitrogen and Liver Fructose 2,6-Bisphosphate in Uremic Rats.

Data from individual experiments from Table I and Figure 3 are replotted.

from fasted and diabetic rats (15,16). This has been attributed to the appearance of a high affinity transport system for amino acid, which may play a regulatory role in the control of gluconeogenesis. These data emphasize the importance of the pair-fed control group in the experimental design since the changes observed are solely due to the decreased food intake that is a common feature of the uremic syndrome but not to uremia itself.

In contrast, Figure 2 shows that pyruvate kinase was decreased in uremia when compared to both the pair-fed and ad libitum-fed controls. The same trend was observed for glucokinase. Thus, our data showing changes in the maximal activity of key enzymes, owing to changes in enzyme concentration via effects on protein synthesis and/or degradation, could account for the abnormal carbohydrate metabolism in the liver in uremia. However, in addition to changes in enzyme concentration, the key gluconeogenic and glycolytic enzymes can be regulated by changes in the concentration of allosteric regulators. One of the newly discovered regulators is fructose 2,6-bisphosphate, which is a potent stimulator of phosphofructokinase (17) and an inhibitor of fructose 1,6-bisphosphate (14). The maximal activities of these enzymes were not affected by fructose 2,6-bisphosphate, but the

concentration of substrate required to give half-maximum activity was changed. Figure 3 demonstrates that the level of fructose 2,6-bisphosphate was decreased in uremia when compared to both the pair-fed and ad libitum-fed controls. Furthermore, the level of fructose 2,6-bisphosphate negatively correlates with the blood urea nitrogen, as shown in Figure 4.

Fructose 2,6-bisphosphate has been implicated as an important factor in the regulation of hepatic carbohydrate metabolism in diabetes (18,19), starvation (20), and exercise (21). The changes in fructose 2,6-bisphosphate depend on the activity of the bifunctional enzyme 6-phosphofructose 2 kinase/ fructose 2,6-bisphosphatase responsible for its synthesis and degradation. The regulation of this enzyme is a complex process which is dependent on the insulin/glucagon ratio (18,22). In uremia, in contrast to diabetes (18,19), starvation (20) and exercise (21), both hormones are increased. However, the liver (9,13) and the peripheral tissues (2) are resistant to insulin. Thus, the abnormal functional insulin/glucagon ratio in uremia is probably responsible for the decreased fructose 2,6-bisphosphate level which coupled to the changes in maximal activities of gluconeogenic and glycolytic enzymes offers a possible mechanism to the abnormal carbohydrate metabolism in the liver of uremic animals.

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