Comparison of the Effects of Metabolic Acidosis and Acute Uremia on Carbohydrate Tolerance

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SUMMARY

In an attempt to assess the role of metabolic acidosis in the glucose intolerance of uremia, we have estimated insulin-mediated glucose uptake in normal dogs, in acidotic dogs with normal renal function, and in uremic dogs. Mean steady state plasma glucose and insulin levels were measured in eighteen dogs (pH 7.4 ± .01) during constant infusion of glucose and insulin, while endogenous insulin secretion was suppressed by infusion of propranolol and epinephrine. The dogs were then divided into three groups: moderate acidosis was produced by giving 7 gm. NH_4Cl/day for four days; severe acidosis, 12 gm. NH_4Cl/day for four days; and uremia (serum creatinine 15.7 ± 0.2 mg. per cent) by anastomosing both ureters to the vena cava. Duplicate infusions were then performed and the results compared to control values. Plasma insulin levels were identical, despite the presence of either acidosis or uremia. The mean increase in plasma glucose in moderate acidosis (pH 7.2 ± 0.1) over controls was 39 mg./100 ml. ($p < .05$); in severe acidosis (pH 7.0 ± .04) plasma glucose increased by 69 mg./100 ml. ($p < .01$). However, the mean increase in plasma glucose (149 mg./100 ml.) in uremia (pH 7.3 ± .03) was significantly greater ($p < .005$) than that following acidosis, although the pH did not significantly fall. Thus, although metabolic acidosis results in deterioration of glucose tolerance, the magnitude of the change does not seem able to account for the carbohydrate intolerance of uremia. DIABETES *21*:1109-15, November, 1972.

Considerable evidence indicates that glucose intolerance in patients with renal failure is improved by dialysis.[1-4] These observations strongly suggest that the glucose intolerance is due to the metabolic consequences of chronic renal failure, but provide little insight as to which of the protean manifestations of uremia might be responsible. As part of our continuing interest in the role of the kidney in insulin and glucose metabolism,[5-7] we have begun a systematic study of the effect of various metabolic abnormalities present in uremia on glucose homeostasis. In this report we describe the effect of ammonium chloride-induced metabolic acidosis on the ability of normal dogs to dispose of a glucose load. Our results confirm earlier studies indicating that metabolic acidosis produces deterioration of glucose tolerance.[8] However, the degree of deterioration was significantly less than that seen in dogs made uremic by diversion of both ureters into the vena cava, in spite of a lesser degree of acidemia in the uremic dogs. Thus, although metabolic acidosis can adversely affect glucose intolerance, the glucose intolerance of uremia does not appear to be due to the accompanying metabolic acidosis.

MATERIALS AND METHODS

1. *Experimental protocol*

A control evaluation of glucose tolerance (to be described in detail) was carried out in eighteen female mongrel dogs, weighing 16 to 27 kg. They were then randomly assigned to one of the following three treatment groups.

a) *Mild acidosis.* Six dogs received ammonium chloride, 7 to 10 gm./day, for three consecutive days.

b) *Severe acidosis.* Six dogs received ammonium chloride, 10 to 15 gm./day, for three consecutive days.

c) *Uremia.* Six dogs were anesthetized with sodium pentobarbital, and both ureters, with attached mucosa and muscularis, were removed from the bladder and anastomosed to the inferior vena cava just below the ovarian vein. The bladder was then removed following ligation of the urethra. In this manner uremia was produced without loss of renal mass. (An autopsy was performed after the studies were completed on the six dogs in whom bilateral ureteral diversion had been

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performed, and in no instance had hydronephrosis developed.)

2. *Evaluation of glucose tolerance*

Experiments were performed in awake dogs after a twenty-four hour fast. Glucose tolerance was estimated by a method previously described and validated,[9] in which experimental subjects receive a constant intravenous infusion of epinephrine, propranolol, glucose and crystalline insulin. By this procedure endogenous insulin secretion and hepatic glucose output are inhibited, and similar circulating levels of exogenous insulin are produced in each animal. Under these conditions the height of the steady state plasma glucose level which results from the glucose infusion is a direct measure of the experimental subjects' ability to take up glucose at similar plasma insulin levels. In these experiments we gave a loading dose of propranolol (.07 mg./kg.) and then infused glucose (17 mg./kg. body weight/min.), epinephrine (.08 μgm./kg./min.), propranolol (.0017 mg./kg./min.), and insulin (950 μU./kg./min.) for 130 minutes into the cephalic vein. Blood was drawn from a catheter placed in the saphenous vein before and every ten minutes (starting at ninety minutes) after the infusion was begun. The mean steady state plasma glucose and insulin response was determined from five samples drawn 90, 100, 110, 120 and 130 minutes after the beginning of the infusion. Following this, the dogs were randomized into one of the three experimental groups described earlier. Dogs were fed with a regular kennel diet, making sure that each dog received 100 gm. of glucose each day for four days before the control and experimental studies. (In the case of the uremic animals, this often had to be accomplished by intravenous infusion.) Fluid intake was held constant in the dogs with bilateral diversion to maintain normal serum sodium concentration, and polystyrene sulfonate was administered when serum potassium concentrations exceeded 6 mEq./L.

3. *Analytical procedures*

Blood was drawn into tubes containing either EDTA (insulin) or sodium fluoride (glucose). Plasma was separated immediately, quickly frozen in acetone-dry ice, and stored at —20° C. Circulating exogenous insulin concentration at each time point was determined in triplicate by the method of Hales and Randle,[10] and plasma glucose concentration by a modification of a glucose oxidase method adapted for the Technicon AutoAnalyzer.[11] The AutoAnalyzer was also used to measure serum creatinine,[12] serum sodium and potassium were determined by flame photometry, and pH was measured on arterial blood samples by an Astrup pH meter (Radiometer). The plasma immunoreactive insulin and glucose concentrations from both control and experimental studies were measured on the same day to minimize technical variation. Finally, statistical significances were based upon use of the t test.[13]

RESULTS

In table 1 the arterial pH and serum creatinine, sodium, and potassium for each dog are listed. The three experimental groups are easily distinguishable on the basis of their degree of acidosis; the mean pH of the group with moderate acidosis (7.21) was significantly greater ($p < .005$) than that of dogs with severe acidosis (7.05) and less ($p < .005$) than that of the group with uremia (7.30). The relatively modest degree of acidosis in dogs with bilateral ureteral diversion is likely related both to the relatively short period of uremia and to the effects of the high carbohydrate diet the dogs received. Serum creatinine ranged between 15 and 17 mg. per cent in all dogs four days after bilateral ureteral diversion. Ammonium chloride acidosis had no consistent effect on serum creatinine concentration and the somewhat higher creatinine values in dogs with severe acidosis is a function of higher basal values in this group. Serum potassium levels were somewhat elevated for the dogs with severe metabolic acidosis and even more so in those with uremia.

TABLE 1

Arterial pH and serum creatinine, sodium, and potassium concentrations

Treatment	Dog	pH	Creatinine (mg./100 ml.)	Na (mEq./L.)	K (mEq./L.)
Moderate acidosis	Va	7.19	0.7	144	4.0
	In	7.21	0.6	144	4.5
	Kr	7.20	0.9	145	3.8
	Hi	7.21	0.8	145	4.3
	Sa	7.25	0.8	145	3.8
	Em	7.19	0.8	142	3.6
Severe acidosis	Is	7.04	1.1	143	4.5
	He	7.12	0.9	141	4.6
	Ly	7.13	1.0	142	4.7
	Co	6.99	1.2	142	5.3
	Fr	6.89	1.2	139	6.0
	Ev	7.13	0.9	142	4.9
Uremia	Tr	7.26	15.2	139	5.9
	Sc	7.35	15.9	135	6.5
	Rh	7.31	16.4	143	6.0
	Me	7.29	15.4	137	6.3
	Oo	7.34	15.3	143	5.5
	Pa	7.27	15.7	139	6.5

In table 2 the mean (± SD) steady state plasma glucose and immunoreactive insulin level that was observed during the infusion is given for each of the eighteen dogs. (No changes occurred in fasting values of either glucose or insulin, and they have been omitted from this table for the sake of simplicity.) The constancy of the plasma levels of glucose and insulin during these studies is attested to by the relatively small standard deviations of the individual dogs' mean plasma glucose and insulin level. Table 2 also establishes the similarity of the circulating immunoreactive insulin levels during the control and experimental studies. In most cases the insulin levels were identical during both studies, and, in the few instances when significant differences did occur, the levels were more often higher during the experimental study. Thus, significant hyperglycemia following NH_4Cl administration or bilateral ureteral diversion cannot be attributed to lower levels of circulating insulin. Finally, from table 2, it is clear that both metabolic acidosis and uremia led to an increase in steady state plasma glucose concentration in every dog. The difference between control and experimental glucose concentration ranged from 6 mg./100 ml. to 185 mg./100 ml., seemed to be related to the degree of acidosis in the intact dogs, but significantly more severe hyperglycemia appeared to result from acute uremia in spite of lesser acidemia.

These general impressions are borne out by the statistical analysis. The hyperglycemic effect of moderate acidosis was significant at the $p < .05$ level ($t = 2.4$), severe acidosis at the $p < .01$ level ($t = 5.3$), and bilateral ureteral diversion at the $p < .005$ level ($t = 11.1$). The relative effect of the three treatments on glucose tolerance is illustrated in figure 1, in which mean differences between control and experimental studies for each of the three groups of animals are compared. It can be seen that the average difference between the control and experimental plasma glucose level of dogs with severe acidosis was almost twice that of dogs with moderate acidosis. However, there was considerable variation from dog to dog and this difference did not reach the level of statistical significance when the two groups were compared. Although the mean increase in plasma glucose level of the six dogs with severe acidosis was not significantly greater than the mean rise of the six dogs with moderate acidosis, when individual dogs were compared there did appear to be some relationship between degree of acidemia and severity of hyperglycemia. This point is illustrated in figure 2 and suggests that a rough correlation between degree of acidemia and worsening of glucose tolerance might exist. Finally, it can be seen in figure 1 that the degree of hyperglycemia in dogs with acute uremia was statistically significantly different from that of dogs

TABLE 2

Steady state plasma glucose and insulin concentrations

Treatment	Dog	Mean (+ SD) plasma glucose (mg./100 ml.)			Mean (+ SD) plasma insulin (μU./ml.)		
		Control	Experimental	Difference	Control	Experimental	Difference
Moderate acidosis	Va	84 ± 12	120 ± 11	36	68 ± 3	48 ± 2	−20
	In	154 ± 15	188 ± 15	34	78 ± 3	78 ± 7	0
	Kr	100 ± 2	216 ± 10	116	43 ± 3	72 ± 2	29
	Hi	125 ± 14	152 ± 20	27	86 ± 5	75 ± 5	−11
	Sa	155 ± 5	161 ± 2	6	89 ± 9	87 ± 2	− 2
	Em	145 ± 3	160 ± 6	15	80 ± 6	82 ± 2	2
Mean ± SE		127 ± 12	166 ± 13	39 ± 16	74 ± 7	74 ± 6	− .3
Severe acidosis	Is	85 ± 9	108 ± 7	23	73 ± 3	75 ± 4	2
	He	118 ± 13	191 ± 12	73	91 ± 3	94 ± 2	3
	Ly	95 ± 10	159 ± 11	64	60 ± 4	58 ± 2	− 2
	Co	156 ± 22	303 ± 20	147	97 ± 9	112 ± 2	15
	Fr	129 ± 5	212 ± 9	83	55 ± 4	56 ± 1	1
	Ev	104 ± 2	128 ± 29	24	70 ± 4	76 ± 6	6
Mean ± SE		115 ± 11	184 ± 28	69 ± 19	74 ± 7	78 ± 9	4
Uremia	Tr	98 ± 3	277 ± 5	179	77 ± 3	76 ± 6	− 1
	Sc	82 ± 10	242 ± 9	160	70 ± 7	78 ± 5	8
	Rh	105 ± 5	255 ± 4	150	59 ± 2	55 ± 1	− 4
	Me	112 ± 8	297 ± 6	185	80 ± 7	80 ± 10	0
	Oo	115 ± 2	222 ± 3	107	53 ± 2	49 ± 2	− 4
	Pa	165 ± 7	278 ± 3	113	78 ± 3	104 ± 6	26
Mean ± SE		113 ± 11	262 ± 11	149 ± 13	70 ± 3	74 ± 8	4

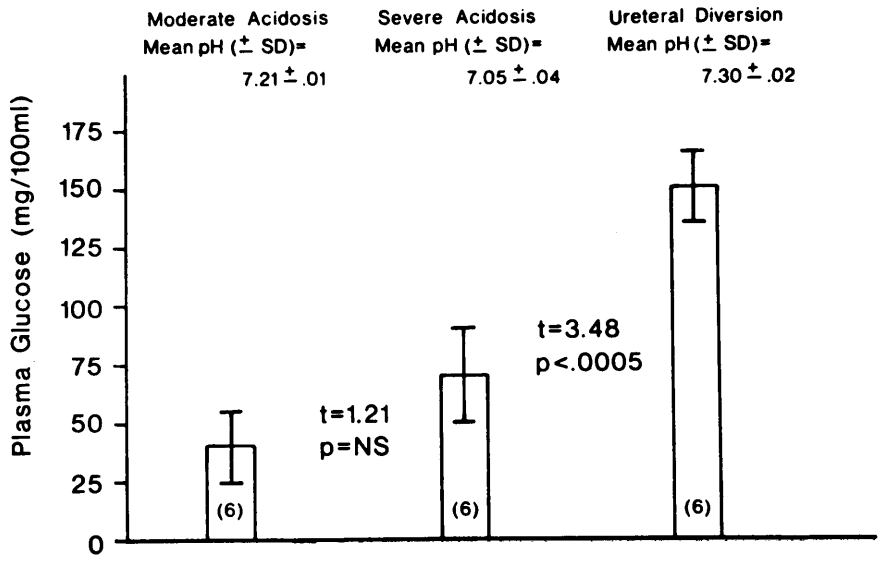

FIGURE 1

The mean difference in steady state plasma glucose concentration between the control and experimental study for each of the three experimental groups. The numbers in brackets refer to the fact that control and experimental studies were conducted on six dogs in each group.

with moderate or severe acidosis ($p < .0005$). Since these differences existed in spite of significantly less acidosis (see table 1), the studies strongly suggest that the glucose intolerance or uremia cannot be attributed to metabolic acidosis alone.

DISCUSSION

Although it has been known for some time that glucose intolerance can occur in patients with chronic renal failure,[14-17] considerable controversy still exists as to its pathogenesis. Studies have indicated that the abnormality in carbohydrate metabolism might be due to impaired insulin secretion,[2-4] resistance of peripheral tissues to insulin-mediated glucose uptake,[19-24] defective hepatic uptake and release of glucose,[25-27] and excessive secretion of growth hormone.[19,28-30] In light of today's information it is difficult to ascertain which of these factors is most responsible for the deterioration in glucose tolerance that occurs with uremia, and all are probably involved to a varying degree. Furthermore, even if it became evident which of these factors was most important, there is little agreement as to which aspect of the complex syndrome of uremia is primarily responsible for the glucose intolerance.

The reason for this lack of consensus is not entirely due to the complicated nature of the problem, and a good deal of the confusion that currently exists can be attributed to limitations of previous investigations. One major problem is entirely methodological, and simply relates to methods used to measure plasma glucose concentration. Falsely elevated plasma glucose will result if the usual AutoAnalyzer method,[31] which uses alkaline ferricyanide, is used in patients with uremia. On the other hand, elevated uric acid concentrations can interfere with measurement of plasma glucose by the glucose oxidase method, resulting in falsely low glucose values in uremic patients.[32] The other major problem is more conceptual and results from an effort to compare patient groups on the basis of their plasma immunoreactive insulin responses. For example, if a patient with uremia responds to an oral glucose challenge with a greater than normal plasma glucose and insulin concentration, it is frequently concluded that he is hyperglycemic *in spite* of secreting a greater than normal amount of insulin, i.e. that "insulin resistance" is the cause of the glucose intolerance. This may be a valid conclusion in patients with normal renal function, but the impaired removal of insulin from plasma of patients with renal failure[5,33] makes it impossible to conclude that elevated insulin levels are due to increased pancreatic insulin secretion, rather than to decreased insulin removal.

In order to avoid both of these problems we have used a somewhat different experimental model to study

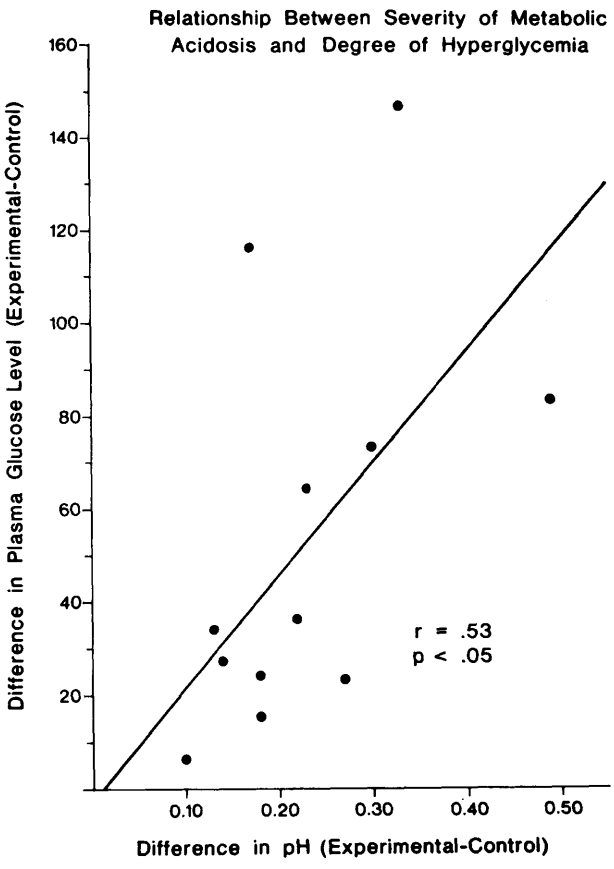

FIG. 2. The relationship between the fall in arterial pH and the increase in mean steady state plasma glucose concentration (Experimental study—Control study) resulting from oral administration of ammonium chloride. The regression line is based upon a least squares analysis.

the effect of uremia on glucose tolerance. In the first place, experimental uremia has been produced without loss of renal mass, and the removal rate of insulin from the plasma of dogs made uremic by ureteral diversion was normal.[7] Secondly, plasma glucose has been measured by the glucose oxidase method. Since dogs can convert uric acid to allantoin, hyperuricemia does not occur in uremic dogs; under these conditions, glucose oxidase provides a specific measure of plasma glucose. Finally, by suppressing endogenous insulin secretion and infusing equal amounts of glucose and insulin into each dog, we have a simple experimental model in which the height of the steady state plasma glucose level provides a direct comparison of the effect of various treatments. Thus, the mean steady state plasma glucose response to a constant intravenous infusion of crystalline insulin and 17 mg. of glucose per kilogram body weight per minute was 113 mg./100 ml. during a control study and 262 mg./100 ml. during the experimental study performed four days after the production of acute uremia. Since the mean steady state plasma immunoreactive insulin concentration was the same during both studies, it seems reasonable to attribute the 230 per cent increase in mean plasma glucose concentration to an increase in insulin resistance secondary to the development of uremia. Although the production of metabolic acidosis by the oral ingestion of NH_4Cl led to hyperglycemia in comparable studies, the mean increase in plasma glucose after three days of moderate acidosis (39 mg./100 ml.) or even three days of severe acidosis (69 mg./100 ml.) was much less dramatic. Thus, in spite of a lesser degree of acidemia, dogs with uremia demonstrated a much greater degree of insulin resistance. Obviously, the design of these experiments is such that no insights are provided as to the locus of this insulin resistance, and uremia could result in a decrease in the ability of insulin to promote glucose uptake in muscle, adipose tissue or liver, or to inhibit hepatic glucose output. However, the results do clearly indicate that acute uremia is associated with resistance to the action of insulin and support the notion that the insulin resistance of patients with chronic renal failure is not primarily due to the coexistence of metabolic acidosis.

Furthermore, since these studies have been carried out under conditions in which the circulating level of insulin is fixed (and equal) during both control and experimental studies, the results provide no information as to the possible effect of acidosis on insulin secretion. Consequently, one could still argue that metabolic acidosis plays a major role in the glucose intolerance of patients with uremia, and that this is achieved by inhibiting insulin secretion. However, in previous studies we have been unable to demonstrate any evidence of a primary *decrease* in insulin secretion during uremia.[6] Indeed, the rate of delivery of insulin into the general circulation seemed to be equal to or greater than normal during acute experimental uremia in dogs. The fact that insulin delivery rates were not greatly *increased* during the hyperglycemia of uremia might be interpreted as evidence that uremia inhibits the "appropriate" response to hyperglycemia. However, this interpretation is based upon the unsupported assumption that plasma glucose concentration is the major determinant of insulin secretion, whereas recent experimental data[34,35] suggests that administered glucose load may be a more important determinant of insulin secretion. Therefore, since uremia is not associated with a decrease in the rate of delivery of insulin into plasma, we believe that an effect of

metabolic acidosis on insulin secretion cannot be primarily responsible for the glucose intolerance of uremia. Thus, we are led to conclude that some characteristic of uremia other than acidosis is the major cause of hyperglycemia in patients with renal failure.

The conclusion that metabolic acidosis is not primarily responsible for glucose intolerance in uremia should not obscure the fact that it did produce a statistically significant degree of insulin resistance. As such, it is possible that a decrease in pH of patients with diabetic ketoacidosis could account for the insulin resistance commonly seen in such patients. However, upon closer inspection of the data, one might question the degree to which this is true. For example, dogs with a mean pH of 7.21 have a mean plasma glucose rise of only 37 mg./100 ml., and, if we exclude dog Kr (a rise of 116 mg./100 ml.), the remaining five dogs had an average increase of only 24 mg./100 ml. Even the group of dogs with severe acidosis (mean pH = 7.05) had an average rise in plasma glucose of only 69 mg./100 ml. If we exclude dog Co (rise of 147 mg./100 ml.), the mean increase of the remaining five dogs drops to 53 mg./100 ml. Thus, although acidosis unequivocally increases insulin resistance, the effect seems to be (quantitatively) relatively modest.

However, before discarding the possibility that acidosis plays a major role in the insulin resistance of the patient with diabetic ketoacidosis, it must be pointed out that acidosis in such individuals is due to the accumulation of organic acids. Since there is no a priori reason to dismiss the possibility that acidosis produced by different means might not result in variable effects on carbohydrate metabolism, we plan to investigate the effect of various forms of experimental acidosis on glucose tolerance in future studies.

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