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Effects of Alloxan Diabetes on Magnesium Metabolism in the Rat[1] (38373)

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Metabolic stresses of uncontrolled diabetes cause adaptations in intestinal absorption. For example, in the alloxan diabetic rat, hexose and amino acid absorption are increased (1–3), but duodenal calcium transport is decreased (4). The decrease in calcium absorption is associated with virtual absence of a duodenal calcium binding protein specific for the rat (5). Because calcium and magnesium metabolism are closely related, *e.g.*, show mutual competition for transport, we examined the effects of diabetes on magnesium balance in the rat and report our findings here.

Materials and Methods. We used male albino rats of the Carworth strain weighing 170–200 g. Rats to be made diabetic were injected intraperitoneally with alloxan, 210 mg/kg of a freshly prepared 100 mg/ml solution. Weight-matched controls received distilled water. Decreased growth rate, hyperglycemia, polyuria, and glucosuria were criteria for diabetes. Magnesium and calcium balance (4) were measured in control and diabetic animals housed individually in stainless steel metabolism cages and fed *ad lib* with Wayne Lab-Blox (Allied Mills). Food consumption was measured daily, but no attempt at equilization was made, because we wanted to carry out our studies under conditions in which this parameter was spontaneously regulated by the animal conditions, *i.e.*, normal growth or diabetic hyperphagia. The food compartment projected from the side of the metabolism cage and was of a size that did not permit the rat to turn once he had entered. This design minimized food scattering. During the first few days after injection food intake of alloxan-treated animals was about half that of controls, but by the fifth day post injection, controls and diabetics consumed about the same amount of food. Thereafter, food intake was greater in diabetics. Data are from specimens collected from the fifth to the 11th day following injection. Body weights, water consumption, and 24-hr urine volumes were measured daily. Feces were collected and pooled for the 6 day period beginning the morning of the fifth day and terminating the morning of the 11th day after injection. Each 24-hr urine and 6-day stool was analyzed for magnesium and calcium by atomic absorption spectrometry. The diet was ashed in a muffle furnace at 500°, dissolved in concentrated hydrochloric acid, and analyzed for magnesium and calcium by atomic absorption spectrometry. Magnesium content was 0.23% and calcium content was 1.2%. Since fat content of diet influences absorption of divalent cations, the diet was analyzed for fat (6), and contained 4.5% fat. This content of fat would be expected to have minimal influence on calcium and magnesium absorption. Urinary glucose was measured daily (Tes-Tape, Lilly). On the 11th day, animals were sacrificed, and blood was drawn from the vena cava for blood glucose (7) and plasma magnesium and calcium determination as described above. The animals were opened by a midline abdominal incision, and the proximal 10 cm and distal 20 cm of small intestine, corresponding to the duodenum and ileum, respectively, were removed and rinsed, and segment length and weight were obtained. Each segment was then slit open, the mucosa was scraped from the underlying tissue with a glass slide, and mucosal wet weight was obtained.

Results and Discussion. During the 6-day study period, control rats grew at a rate of 5–7 g/day. Diabetics failed to grow and showed greater than 2% glycosuria and postprandial blood glucose of 400–700 mg% in comparison

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with 100–150% in controls. Mean plasma magnesium (0.94 ± 0.03 m*M*) in diabetics was significantly lower than in controls (1.11 ± 0.03 m*M*).

Fecal excretion. Magnesium. Because of hyperphagia, magnesium intake in diabetics (18.0 mmole) was 60% greater than in controls (11.1 mmole) (Table I). Fecal magnesium excretion was 82% greater in diabetics (14.8 mmole) than in controls (8.1 mmole). Therefore, fecal magnesium excretion in diabetics increased proportionately more than intake, and net magnesium absorption (intake minus fecal excretion) was the same in diabetics (3.2 mmole) and controls (3.0), despite greater intake in diabetics.

Previous studies in normal animals at magnesium intakes similar to those in our control and diabetic groups demonstrated increased intake to be associated with increased net magnesium absorption (8). In the present study, net magnesium absorption in diabetics did not increase with increased intake. Percent absorption (intake minus fecal excretion/intake) of magnesium is lower in diabetics (18%) than controls (27%), while fecal magnesium excretion as percent of intake is greater in diabetics (82.2%) than controls (73.0%). These findings suggest depression of gastrointestinal magnesium transport in the alloxan diabetic rat.

Diabetic animals demonstrating these effects on magnesium absorption showed depression of body growth and augmentation of intestinal growth in comparison with controls. The relation of both of these variables to magnesium balance must be considered. Segment wet weight per unit length was 12% greater in duodenum and 20% greater in ileum in diabetics (Table II). Mucosal weight was increased even more: 31% greater in duodenum and 28% greater in ileum in diabetics. Hence, even though the diabetic animal fails to grow, the mucosal growth in diabetics shows that effects on absorption cannot be attributed to mucosal atrophy. Since magnesium is absorbed by both duodenum and ileum (9), and diabetics show the same net magnesium absorption as controls despite greater mucosal mass, absorptive activity per unit of mucosa is decreased in diabetics. Total intestinal absorptive function for magnesium as measured by balance is not decreased: net absorption is the

TABLE I. Magnesium and Calcium Balance in Control and Diabetic Rats.

	Mean ± SE[a]			
	Magnesium		Calcium	
	Control	Diabetic	Control	Diabetic
Intake, mmole/rat	11.1 ± 0.2	18.0 ± 1.0[b]	34.1 ± 0.5	54.1 ± 3.2[b]
Excretion, mmole/rat				
Fecal	8.1 ± 0.1	14.8 ± 0.9[b]	30.4 ± 0.6	55.6 ± 2.2[b]
Urinary	2.1 ± 0.1	3.7 ± 0.2[b]	0.2 ± 0.02	1.8 ± 0.2[b]
Absorption (intake-fecal excretion)				
Net, mmole/rat	3.0 ± 0.2	3.2 ± 0.8	3.7 ± 0.7	−1.5 ± 0.8[b]
Net, mmole/100 g body wt	1.1 ± 0.1	1.6 ± 0.4	1.4 ± 0.2	−0.8 ± 0.4[b]
Percent	27 ± 3	18 ± 3[b]	11 ± 1	−3 ± 2[b]
Percent/100 g body wt	10 ± 1	9 ± 1	4 ± 0	−2 ±1[b]
Excretion as percent of intake				
Fecal	73.0± 1.3	82.2 ± 2.8[b]	89.1 ± 2.0	102.7 ± 3.0[b]
Urinary	18.9 ± 0.9	20.5 ± 0.7	0.6 ± 0.1	3.3 ± 0.3[b]
Total	91.9 ± 1.5	102.7 ± 2.7[b]	89.7 ± 2.5	106.0 ± 3.5[b]
Net balance				
Percent	8.1 ± 1.5	−2.7 ± 2.7[b]	10.3 ± 2.5	−6.0 ± 3.5[b]
mmole/rat	0.9 ± 0.3	−0.5 ± 0.4[b]	3.5 ± 0.8	−3.3 ± 3.0[b]

[a] All data refer to the 6-day balance period.

[b] Significantly different from control, $P < 0.05$.

TABLE II. Weights of Intestinal Segments and Mucosa.

	Mean ± SE			
	Segment wet wt, g/cm		Mucosa wet wt, g/cm	
	Duodenum	Ileum	Duodenum	Ileum
Control	0.0818 ± 0.017	0.0649 ± 0.0023	0.0579 ± 0.0013	0.0442 ± 0.0022
Diabetic	0.1089[a] ± 0.0015	0.0813[a] ± 0.0023	0.0841[a] ± 0.0012	0.0612[a] ± 0.0018

[a] Significantly greater than control, $P < 0.01$.

same in controls and diabetics (Table I) because of increased intake and intestinal mass in diabetics.

In addition to being expressed in terms of intestinal function, magnesium balance should also be considered in terms of total body metabolism, *i.e.*, per 100 g body weight. Diabetics do not grow in body weight. Hence, when expressed in terms of body weight, net magnesium absorption (mmole/100 g body weight) is increased in diabetics (1.6 mmole) as compared with controls (1.1 mmole). Hence, assuming metabolic requirements of controls and diabetics for magnesium can be compared per 100 g body weight, net magnesium absorption would be increased in diabetics. This should not be considered a true increase since it is the consequence of greater intake of magnesium and a larger small intestine in a smaller diabetic animal.

Calcium. Although calcium intake was 60% greater in diabetics (54.1 mmole) than controls (34.1 mmole), diabetics showed net calcium secretion of 1.5 mmole and controls showed net absorption of 3.7 mmole. Previous studies in normal animals demonstrated that increased calcium intake is associated with moderate but not proportional increases in absorption (10). Therefore, effects of diabetes on percentage fecal calcium excretion are striking: 102.7% of intake in diabetics as compared with 89.1% of intake in controls.

Urinary excretion. Magnesium. Mean urinary magnesium excretion in diabetics (3.7 mmole) was slightly, but not significantly, greater than mean net absorption (3.2 mmole). In contrast, urinary magnesium excretion in controls (2.1 mmole) was only 70% of net absorption (3.0 mmole). Therefore, despite the failure of magnesium absorption to increase in parallel with intake, urinary magnesium rose in proportion to intake, and percent excretion was the same in diabetics (20.5% of intake) and controls (18.9% of intake). In normal animals increased magnesium intake is associated with both increased absorption and urinary excretion (8). Increased urinary magnesium excretion in diabetics, even in the absence of increased net absorption (Table I) is explained in part by their osmotic diuresis from glycosuria. Excretion of both magnesium and calcium is increased by osmotic diuresis (11). In diabetic animals in our study, correlation coefficients within rats between urine volume and excretion were significant ($P < 0.05$) for magnesium ($r = 0.799$) and calcium ($r = 0.722$) (12).

Calcium. Although urinary calcium excretion is only a small fraction of intake in both controls (0.6%) and diabetics (3.3%), nine times as much calcium is excreted in urine in diabetics as in controls (Table I). This effect is probably too great to be explained simply by osmotic diuresis.

These studies show that effects of diabetes on magnesium and calcium transport are similar but differ in degree. Diabetic animals are in negative calcium balance as a result of effects on alimentary function *per se*: fecal excretion is 102.7% of intake. This contrasts with effects of diabetes on intestinal magnesium transport: fecal excretion is only 82.2% of intake (Table I). Diabetic animals are in negative magnesium balance because of their failure to increase net absorption while urinary magnesium excretion increased in parallel with intake. Hence, on the basis of fecal and urinary excretion, diabetic animals show a mild defect in magnesium transport in comparison with calcium transport.

The only previous study of magnesium metabolism in experimental diabetes appears to be an examination of magnesium balance and

^{28}Mg metabolism in the alloxan diabetic rabbit (13). Five days after alloxan injection diabetic rabbits had lost 4% of their initial body weight, and exchangeable magnesium had decreased by 23%. Animals had the same positive magnesium balance before and after alloxan injection, as well as the same magnesium intake and urinary excretion of magnesium and ^{28}Mg. These studies in rabbits were at an earlier time interval after alloxan injection than our study in rats, and diabetes did not appear to be as severe: rabbits were not hyperphagic and showed only a threefold increase in urine volume. In our study, mean weight of diabetic rats was 23% lower than controls, mean urine volume was 13-fold greater and mean food intake was 65% greater in diabetics. The present paper appears to be the first demonstration of altered magnesium balance in experimental diabetes, since studies in the rabbit showed no effects "detectable...by external balance..." (13). Human diabetics show abnormal magnesium metabolism. Urinary magnesium excretion by acidotic diabetics is increased (14). Serum magnesium is decreased in diabetics requiring more than 20 units of insulin per day (15), and diabetes was the most common condition associated with hypomagnesemia in routine clinical screening (16). However, ^{28}Mg metabolism was normal in three of four diabetics (17).

Summary. This study examines effects on magnesium and calcium balance consequent to uncontrolled alloxan diabetes in the rat. Intake and fecal and urinary excretion of both magnesium and calcium were greater in diabetics than controls. Net magnesium absorption was the same in controls and diabetics, but percent absorption was lower in diabetics (18%) than in controls (27%) because of their greater intake. For calcium, although controls absorbed 11% of intake, diabetics excreted 103% of intake in stool. Controls were in positive and diabetics in negative balance for both magnesium and calcium. Negative magnesium balance in diabetics resulted from failure to increase net absorption while urinary excretion increased in parallel with increased intake. These studies demonstrate a mild defect in intestinal magnesium transport in comparison with the gross defect in calcium transport in diabetes.

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