PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 161, 149-152 (1979)

Small Intestinal Calcium Absorption in the Rat with Experimental Diabetes (40508)

BARRY E. WALKER AND HAROLD P. SCHEDL

Gastroenterology Research Laboratories, Department of Medicine, University of Iowa College of Medicine, Iowa City, Iowa 52242

We previously studied downhill calcium absorption in the diabetic rat (luminal concentration 3.4 m*M*, serum ionized calcium, 1–1.1 m*M*): Duodenal absorption by diabetics was half that of controls whereas ileal absorption was normal (1). Although vitamin D intake and absorption of fat are normal in diabetic rats (1), we subsequently found vitamin D metabolism to be abnormal: serum concentrations of 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D) in diabetics are one-eighth the levels in controls (2). Since 1,25-$(OH)_2$D is the active metabolite of vitamin D that acts directly on the gut, the depression of 1,25-$(OH)_2$D concentration explains the decreased duodenal calcium absorption. Unexplained is the lack of effect of diabetes on ileal calcium absorption, since the ileum responds to vitamin D depletion and repletion (3). Since we had studied only downhill calcium absorption and had examined diabetics during the 5- to 10-day time span after injection (1), it was possible that we did not detect an effect of diabetes on ileal transport because of the luminal calcium concentration used or the time span after induction of diabetes selected for study (1). We therefore examined the effects of diabetes on duodenal and ileal calcium transport early (4 days) and late (11 days) after induction of diabetes and at luminal calcium concentraions below and above serum ionized calcium.

Materials and Methods. We used male Sprague Dawley derived rats (Simonsen Labs, Gilroy, CA) housed in air-conditioned quarters and fed Teklad diet (1.5% calcium; 1.3% phosphorus). Animals to be made diabetic received intraperitoneal streptozotocin freshly prepared in citrate buffer, pH 4.5. The initial dose was 100 mg/kg body wt and 24 hr later they received a second injection of 25 mg/kg. Controls received similar injections of buffer alone. Diabetes was assessed by loss of body weight, glycosuria and elevated fasting blood glucose. To measure absorption, rats were anesthetized with intraperitoneal Nembutal and perfused *in vivo* by recirculating 10 ml of solution at a pump rate of 2.0 ml/min for 2 hr. Absorption was studied following an overnight fast at 4 days and 11 days after injection of streptozotocin. The first 10 cm of duodenum and last 15–20 cm of ileum were perfused in each rat, and control and diabetic rats were always studied at the same time. Perfusates contained 0.8 m*M* calcium or 3.4 m*M* calcium, ^{45}Ca (12 μc/liter, initial specific activity 2000 μCi/mg, New England Nuclear Corp.), 165 m*M* NaCl, and 50 mg/l phenol red.

At the conclusion of perfusion blood was drawn from the inferior vena cava to measure glucose in serum (4). The duodenal and ileal segments were removed, measured for length, weighed, and the mucosa scraped from the underlying tissue. Total wet weight and total length of the small intestine were also obtained. Tissues were dried for 24 hr at 100° in a vacuum oven and reweighed to obtain dry weight. Calcium was measured by atomic absorption spectrometry in test solutions and luminal perfusates. ^{45}Ca was measured by liquid scintillation counting (5) and phenol red was estimated colorimetrically (6).

Calculations. Absorption is net movement of calcium from the lumen into the animal calculated by the equation

$$\text{Absorption} = \frac{V[^{40}Ca_i - (^{40}Ca_f)(PRR)]}{L \text{ or } W}$$

Lumen-to-plasma (LP) flux is unidirectional movement of calcium and is calculated by the equation (7)

$$\text{LP flux} = \frac{V[^{45}Ca_i - (^{45}Ca_f)(PRR)]}{[(SA_i + SA_f)/2]\ W}$$

The subscripts i and f refer to initial and final values respectively. ^{40}Ca is calcium concentration in μmoles per ml, and ^{45}Ca is concentration of radioactive calcium in cpm per

0037-9727/79/060149-04$01.00/0

ml. V is volume perfused in ml. PRR is ratio of initial to final phenol red concentration. L is segment length in cm and W is dry weight of mucosa in g. SA is specific activity of calcium in cpm per micromole.

Statistics. Data are given as mean values ± 1 SE. Statistical analysis between groups of controls and diabetics was by analysis of variance and Tukey's multiple comparison test (8). P values less than 0.05 are considered significant.

Results. Mean initial body weights did not differ among control and diabetic groups and ranged from 187 to 199 g. Control groups increased body wt by 7 g/day whereas diabetics maintained their initial body wt. Mean serum glucose (mg%) ranged from 100 to 117 in control groups. In diabetics mean serum glucose ranged from 240 to 334 and means of diabetics were greater than those of controls ($p < 0.05$). Mean serum calcium (mM) was the same in control (2.50) and 4-day diabetic groups (2.49), but was decreased ($p < 0.05$) in 11-day diabetics (2.22). Mean serum inorganic phosphorus (mg%) was also similar in control (10.8) and 4-day diabetic groups (11.9) but was increased ($p < 0.05$) in 11-day diabetic groups (13.8).

Small intestinal measurements for the various groups are shown in Table I. Total intestinal net weight was the same in controls and diabetics at 4 days, but was greater in diabetics at 11 days. Duodenal and ileal mucosal weight per unit length was the same in diabetics and controls 4 days after injection, but at 11 days, because of greater mucosal growth in diabetics, weight of mucosa in both duodenum and ileum had increased in diabetics as compared to controls.

Absorption data for calcium shown in Table 2 are expressed as μmole/hr per cm, and μmole/hr per g mucosal dry weight. Absorption per unit length defines absorptive function of the segment both before and after mucosal growth. Absorption per g dry weight mucosa defines absorptive specific activity of the mucosa. Duodenal net calcium absorption in diabetics was lower than in controls independently of the way in which absorption is expressed, concentration of calcium in the perfusate, and time after injection. Duodenal calcium absorption in diabetics at 11 days was lower than in diabetics at 4 days at both luminal calcium concentrations.

In contrast, ileal net calcium absorption (Table II) per cm segment and per g dry weight mucosa was similar in controls and diabetics at 4 and 11 days with 0.8 m*M* luminal calcium and at 4 days with 3.4 m*M* luminal calcium. With 3.4 m*M* luminal calcium at 11 days, mean absorption per cm was decreased in diabetics to about half the control value, but the decrease did not quite reach statistical significance. Absorption per g dry weight mucosa, however, was lower in diabetics than in controls. Lumen-to-plasma fluxes (Table II) also tended to be decreased in diabetics as compared with controls, but results were not as consistent as for net movements.

Discussion. These experiments confirm our previous observation of defective downhill duodenal calcium absorption in diabetes (1) as a result of depressed 1,25-$(OH)_2$D (2). In addition the studies demonstrate defective uphill duodenal calcium transport and document that the defect is progressive with duration of diabetes: duodenal specific aborption was 51–63% of controls at 4 days and 18–24% of controls at 11 days. Despite the increased mucosal growth in duodenum of diabetics at 11 days (68–84% above the 4-day

TABLE I. GROUPS OF CONTROL AND DIABETIC RATS STUDIED: SMALL INTESTINAL MEASUREMENTS. MEAN ± S.E.

Calcium concentration, m*M*	0.8 m*M*				3.4 m*M*			
Status	Control		Diabetic		Control		Diabetic	
Time, days	4 day	11 day	4 day	11 day	4 day	11 day	4 day	11 day
Number	8	7	8	7	7	7	7	7
Total intestine								
Length, cm	87.0 ± 1.9	94.0 ± 2.0	86.0 ± 1.4	108.0 ± 1.0″	88.0 ± 3.8	98.0 ± 1.0	85.0 ± 1.7	101.0 ± 3.0
Weight, g	5.4 ± 1.6	6.6 ± 0.2	3.9 ± 0.7	9.0 ± 0.4″	5.5 ± 0.5	5.8 ± 0.2	4.9 ± 0.3	8.7 ± 0.7″
Mucosal dry weight, mg/cm								
Duodenum	7.2 ± 0.3	8.2 ± 0.5	6.5 ± 0.5	10.9 ± 0.4″	6.5 ± 0.7	7.1 ± 0.8	6.4 ± 0.8	11.8 ± 0.6″
Ileum	6.4 ± 0.2	6.2 ± 0.1	5.6 ± 0.3	8.0 ± 0.4″	6.0 ± 0.5	7.1 ± 0.5	5.4 ± 0.2	8.6 ± 0.4″

″ Denotes significantly different from corresponding controls ($p < 0.05$).

TABLE II. DUODENAL AND ILEAL CALCIUM TRANSPORT IN CONTROL AND DIABETIC RATS AT 4 DAYS AND 11 DAYS AFTER INDUCTION OF DIABETES AT 0.8 AND 3.4 m*M* LUMINAL CALCIUM CONCENTRATIONS, MEAN ± S.E.

Concentration m*M*	0.8 m*M*				3.4 m*M*			
Time, days	4		11		4		11	
Status	Control	Diabetic	Control	Diabetic	Control	Diabetic	Control	Diabetic
Net absorption, μmol/hr								
Per cm segment length								
Duodenum	0.140 ± 0.009	0.082 ± 0.010[a]	0.120 ± 0.016	0.040 ± 0.014[a, b]	0.410 ± 0.028	0.250 ± 0.030[a]	0.460 ± 0.050	0.190 ± 0.030[a]
Ileum	0.020 ± 0.006	0.023 ± 0.004	0.013 ± 0.004	0.010 ± 0.005	0.060 ± 0.023	0.060 ± 0.015	0.076 ± 0.016	0.040 ± 0.010
Per g dry weight mucosa								
Duodenum	20.1 ± 1.8	12.6 ± 1.9[a]	19.7 ± 2.5	3.5 ± 1.0[a, b]	64.9 ± 7.4	33.4 ± 5.9[a]	63.9 ± 5.4	15.6 ± 2.4[a, b]
Ileum	4.2 ± 0.9	4.1 ± 1.4	1.5 ± 0.8	1.2 ± 0.6	9.6 ± 2.9	11.5 ± 2.7	10.1 ± 1.8	4.5 ± 1.3[a]
Lumen-to-plasma flux, μmole/hr								
Per cm segment length								
Duodenum	0.196 ± 0.007	0.145 ± 0.009[a]	0.177 ± 0.016	0.134 ± 0.012[a]	0.540 ± 0.024	0.412 ± 0.051	0.597 ± 0.040	0.465 ± 0.063
Ileum	0.088 ± 0.006	0.071 ± 0.006	0.084 ± 0.004	0.077 ± 0.017	0.183 ± 0.025	0.158 ± 0.014	0.204 ± 0.014	0.170 ± 0.010
Per g dry weight mucosa								
Duodenum	27.6 ± 1.6	22.2 ± 1.6[a]	22.8 ± 2.5	11.7 ± 0.8[a, b]	88.2 ± 10.6	56.1 ± 7.1[a]	88.7 ± 11.9	35.6 ± 5.9[a, b]
Ileum	12.6 ± 0.7	16.6 ± 2.3	12.8 ± 0.6	7.2 ± 1.4[a]	29.9 ± 4.3	26.4 ± 4.9	24.7 ± 3.9	17.2 ± 1.7

[a] Denotes significantly different from corresponding controls ($p < 0.05$).
[b] Denotes diabetics at 11 days significantly different from diabetics at 4 days ($p < 0.05$).

value as compared with a 9–14% increase in controls), absorption per cm segment remained lower in diabetics than controls. Thus, mucosal growth was ineffective in compensating for the absorptive defect. The present study demonstrates for the first time the development of defective ileal calcium absorption in diabetes at 11 days. This is consistent with vitamin D dependence of transport at both intestinal sites. Ileal calcium transport requires longer duration of diabetes for the defect to become evident, suggesting greater resistance of ileal transport to vitamin D depletion or maintenance of transport by lower concentrations of 1,25-$(OH)_2$D. The calcium transport response pattern of the ileum to diabetes resembles that of the large intestine: cecal and colonic calcium transport are not decreased until 11–14 days of diabetes (9). Thus, in experimental diabetes gut sites show individual time course patterns with respect to depression of calcium transport. These findings on ileal calcium transport are consistent with a role for this site in the small intestine in calcium homeostasis.

Summary. Both uphill and downhill transport of calcium per unit weight of mucosa is decreased in duodenum of diabetic rats at 4 days of diabetes and shows a further decrease at 11 days. Absorption per duodenal segment is decreased in diabetics compared to controls despite much greater mucosal growth in diabetics. Calcium transport is normal in ileum of diabetic rats at 4 days, and only downhill transport is depressed at 11 days. Since the decreased absorption is the consequence of depressed 1,25-dihydroxyvitamin D in the diabetic rat, the data suggest that the ileum is more resistant to vitamin D depletion than the duodenum.

Supported in part by Research Grant AM16841 and AM02534, National Institute of Arthritis, Metabolic and Digestive Diseases, National Institutes of Health, and Sterling Winthrop Ltd, England. One of us (B.E.W.) is Senior Fulbright-Hays Scholar and visiting Research Scientist from the University of Leeds, England.

1. Schneider, L. E., and Schedl, H. P., Amer. J. Physiol. **223,** 1319 (1972).
2. Schneider, L. E., Schedl, H. P., McCain, T., and Haussler, M. R., Science **196,** 1452 (1977).
3. Younoszai, M. K., Urban, E., and Schedl, H. P., Amer. J. Physiol. **225,** 287 (1973).
4. Somogyi, M., J. Biol. Chem. **195,** 19 (1952).
5. Younoszai, M. K., and Schedl, H. P., Gastroenterology **62,** 565 (1972).
6. Schedl, H. P., and Clifton, J. A., Gastroenterology **41,** 491 (1961).
7. Wasserman, R. H., Kallfelz, F. A., and Comar, C. L., Science **133,** 883 (1961).
8. Huntsberger, D. v., and Leaverton, P. E., "Statistical Inference in the Biomedical Sciences," Allyn and Bacon, Inc., Boston (1970).
9. Petith, M. M., and Schedl, H. P., Amer. J. Physiol. **235,** E699 (1978).

Received December 27, 1978. P.S.E.B.M. 1979, Vol. 161.