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In Vivo Intestinal Calcium Absorption in Infant Rats: Influence of Methylprednisolone and Vitamin D[1] (4965)

M. K. YOUNOSZAI AND F. K. GHISHAN

University of Iowa, Pediatric Gastroenterology Laboratory, Iowa City, Iowa 52242

Although clinically adrenal glucocorticoids are used for their suppressing effect on calcium absorption from the small intestine, there is some conflict regarding their mode of action. Glucocorticoids can act either by suppressing a vitamin D-independent mechanism(s) for calcium transport or interfere with the metabolism of vitamin D (1). There is data to suggest that glucocorticoids do not interfere with metabolism of vitamin D (2–5). In the rat, experimental data to support the suppressive action of glucocorticoid on absorption of calcium from the small intestine come from *in vitro* studies of duodenum (3, 6, 7). Similar studies of the jejunum and ileum (3, 8) and measurements of calcium absorption using dietary balance did not show the suppressive effect of glucocorticoids (8). In suckling rats serum corticosterone levels are low (9) and intestinal absorption of calcium high (10). This situation provided an opportunity to investigate the effect of glucocorticoids on intestinal absorption of calcium *in vivo* when serum corticosterone levels are low.

Infants compared to adults seem to be more prone to develop toxicity from pharmacologic doses of vitamin D. In normal adolescent rats large doses of vitamin D (11) did not effect intestinal calcium absorption *in vivo*. The effect of large doses of vitamin D on intestinal absorption of infant rats has not been studied. The purpose of the present study was to investigate intestinal absorption of calcium *in vivo* in infant and adolescent rats treated with pharmacologic doses of methylprednisolone or vitamin D.

Materials and methods. Albino 15- to 18-day pregnant rats were obtained from Holtzman (Holtzman Company, Madison, WI). After birth the pups were randomized among the mothers and litter size maintained at seven to nine pups until the time of study. Intestinal absorption of calcium was measured in suckling (14- to 15-day-old), weanling (21- to 22-day-old) and adolescent (42-day-old) rats. All rats were fed a regular laboratory diet and allowed free access to water.

Four to six rats, at each age group, were injected intramuscularly with Solu-Medrol, (Methylprednisolone Sodium Succinate Upjohn, Kalamazoo, MI) 1.25 mg/100 g body weight twice daily, for 5 days prior to studies of intestinal calcium absorption. Control rats were injected similarly with the same volume of the solvent. Five to six other rats were fed Calciferol (Ergocalciferol, Vitamin D_2, Kremers-Urban Co, Milwaukee, Wisconsin), 5000 units/100 g body weight per day via an intragastric tube, for 3 days prior to the study. Control rats were fed a similar volume of the solvent. Rats were weighed daily during the treatment period.

At the time of the study, unfasted rats were anesthetized with intraperitoneal injection of a mixture of ethylurea–phenobarbital. After opening the abdominal cavity, the entire small intestine from the ligament of Treitz to the ileocecal junction was flushed with 20 ml of isotonic sodium chloride solution and then with 20 ml of air. A proximal cannula was inserted 1 cm aborad to the ligament of Treitz and a distal cannula 1 cm orad to the ileocecal junction. The cannulae were tied in place and the small intestine inserted back into the abdominal cavity. The cannulated jejunum + ileum was perfused *in situ* with solutions containing 3.0 μmoles of calcium chloride, tracer ^{45}Ca 100,000 dpm/ml ($^{45}CaCl_2$ New England Nuclear, Boston, MA), and sufficient sodium chloride to attain an osmotic pressure of 292–299 mOsmoles/kg of water. Body temperature of the rats was maintained at 36° to 37° during perfusion. All solutions contained Phenol Red, 20 μg/ml, as nonabsorbed indicator for volume change. The solutions were perfused at a constant rate of 0.16 ml/min. After the start of the perfusion a 45-min

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period was allowed for attainment of steady state conditions. Perfusates were then collected during the next two consecutive 30-min time intervals. At the end of the perfusion period, the perfused jejunum + ileum was stripped from the mesentry and its length and wet and dry weight determined. The dried samples were ashed at 560° for 24 hr. Phenol Red concentration was measured in the perfusion solutions and perfusates. The details of the procedures and methods used have been described previously (12). Calcium in the solutions and in the ashed intestinal samples was measured using a Perkin Elmer Atomic Absorption Spectrometer; ^{45}Ca radioactivity was measured in a Beckman LS 300 Liquid Scintillation Spectrometer.

Disappearance of calcium from the lumen was taken as net absorption, and disappearance of ^{45}Ca was taken as an index of lumen-to-mucosa flux of calcium. The calculations are shown below:

$$\text{Net calcium absorption, } \mu\text{moles/30 min.} = V_i\,(Ca_i\text{-}Ca_f PR_i/PR_f)$$

$$\text{Lumen to mucosa flux, } \mu\text{moles/30 min.} = V_i/\text{Sp. Act.}_i(^{45}Ca_i\text{-}^{45}Ca_f PR_i/PR_f)$$

where V is volume of solution perfused through the jejunum + ileum in 30 min or the pump rate; Ca is concentration of calcium in the solutions, as μmoles/ml; ^{45}Ca is radioactivity of ^{45}Ca in the solutions in dpm/ml; sp. act. is the specific activity of ^{45}Ca in the solutions as dpm per μmoles of calcium. The subscripts i and f refer to initial (i) values in the perfusion fluid before perfusion and to final (f) values in the perfusates collected, respectively.

The amount of calcium and ^{45}Ca absorbed from the intestinal lumen during each of the two 30-min periods of collection were steady and did not vary by more than 15% of each other. They were added to obtain amount absorbed per hr. Rate of absorption was expressed on the basis of length, wet weight and dry weight of the perfused jejunum + ileum and compared in rats at the different ages. At each age period, data for the two control groups were similar and were combined. The student's t test was used for statistical analysis of the differences between mean values in a treated group and the corresponding control group, and P values (13) of less than 0.05 were considered to be significant.

Results. Body weight and measurements of the perfused jejunum + ileum of the control rats and those treated with Solu-Medrol or Calciferol are shown in Table I. Gain in body weight over the treatment period and measurements of the perfused jejunum + ileum were not significantly different in the treated and corresponding control rats, except in the Solu-Medrol treated, 14- to 15-day-old group, where gain in body weight and weight/cm ratio of the perfused intestine were significantly lower than corresponding control values ($P < 0.05$).

Net absorption of calcium, its lumen-to-mucosa flux, and calcium concentration in the wall of the perfused jejunum + ileum at the end of the perfusion period are shown in Table II. Even though size of the jejunum + ileum varied in the rats at the different ages, total amount of calcium absorbed per hr was similar in all groups, except in the 14- to 15-day-old treated with Solu-Medrol, where total amount of calcium absorbed was less than half that in all other groups. Net absorption rates based on length or weight of the intestine, were similar in control and corresponding treated groups, except in the 14- to 15-day-old Solu-Medrol treated rats, where regardless of the basis of expression, absorption rates were significantly lower than corresponding control values ($P < 0.01$).

The lumen-to-mucosa (LM) flux of calcium, was estimated from rate of absorption of ^{45}Ca, since absorbed ^{45}Ca immediately dilutes in a large body pool of ^{40}Ca and only small amounts reenter the intestinal lumen. The mucosa-to-lumen (ML) flux of calcium can be calculated as the differences between LM flux and net absorption. The LM (Table II) and ML fluxes of calcium were similar in control and treated groups, except for the 14- to 15-day-old rats, where both fluxes were significantly lower than corresponding control values ($P < 0.01$).

Concentration of calcium in the wall of the perfused jejunum + ileum was higher in the suckling and weanling Solu-Medrol treated than in corresponding control rats ($P < 0.05$).

Discussion. The present studies are the first to show that treatment with a glucocorticoid suppressed the *in vivo* absorption of calcium in the small intestine. However, this treatment

TABLE I. BODY WEIGHT AND MEASUREMENTS OF THE JEJUNUM+ILEUM OF THE CONTROL AND TREATED RATS.[a]

Age	14–15 days			21–22 days			42 days	
Rats	Control	Solu-medrol[b]	Vitamin D[c]	Control	Solu-medrol[b]	Vitamin D[c]	Control	Solu-medrol[b]
Number studied	8	6	5	8	5	5	5	4
Body weight, g								
Prior to treatment	18±2	15±1	22±2	29±3	30±3	31±3	112±7	108±6
Change during treatment	6±2	2±1*	4±2	8±3	3±2	3±2	30±8	14±8
Measurements of jejunum+ileum								
Length, cm	37±2	38±1	39±0.9	52±1	51±2	50±2	86±4	81±2
Wet weight, g	0.58±0.08	0.47±0.03*	0.67±0.09	1.17±0.13	1.07±0.13	0.94±0.14	5.5±0.4	4.7±0.4
Wet weight, mg/cm	15.4±1.3	12.2±0.8*	17.1±1.5	22.3±2.1	20.8±1.8	18.6±2.1	64.4±2.6	58.3±4.2
Water content, mg/100 mg	82.8±0.5	83.6±0.6	80.9±0.6	82.1±0.4	82.9±0.6	82.4±0.6	84.2±0.8	84.2±0.5

[a] Numbers represent mean ±SE.
[b] Methylprednisolone, 1.25 mg/100 g body weight was injected intramuscularly, twice daily for 5 days prior to the studies.
[c] Calciferol (Vitamin D_2), 5000 IU/100 g body weight was administered once daily by intragastric tube for 3 days prior to the studies.
* Mean values in treated rats significantly different than mean value in corresponding control rats, $P < 0.05$.

TABLE II. CALCIUM ABSORPTION AND TISSUE CONCENTRATION IN THE JEJUNUM+ILEUM OF CONTROL AND TREATED RATS.[a]

Age	14–15 days			21–22 days			42 days	
Rats	Control	Solu-medrol[b]	Vitamin D[c]	Control	Solu-medrol[b]	Vitamin D[c]	Control	Solu-medrol[b]
Calcium absorption rate								
Total amount absorbed, μmoles/hr	2.5±0.4	0.91±0.17*	2.4±0.3	2.9±0.4	2.5±0.4	2.3±0.2	2.0±0.4	2.2±0.6
Net Absorption per hour:								
mμmoles/cm	63±2	24±4*	66±6	55±10	48±7	48±5	23±5	17±8
μmoles/g wet wt	4.4±0.5	1.7±0.4*	3.7±0.7	2.6±0.6	2.2±0.4	2.5±0.5	0.4±0.1	0.4±0.1
μmoles/g dry wt	22.9±3.6	12.6±2.5*	19.8±4.1	14.2±2.1	12.2±1.1	13.9±2.3	1.8±0.6	2.3±0.6
Lumen-to-mucosa flux per hr								
mμmoles/cm	153±21	46±7*	165±17	93±13	70±4	89±10	72±7	56±9
μmoles/g wet wt	9.5±0.8	3.8±0.4*	9.7±1.1	4.7±0.8	3.5±0.3	5.0±1.4	1.1±0.1	1.0±0.2
μmoles/g dry wt	53.2±5.1	22.9±2.9*	51.4±5.6	25.8±3.8	19.7±2.4	27.9±5.9	7.1±0.5	6.5±0.6
Calcium concentration in intestinal wall, μmoles/g wet wt	3.3±0.2	4.1±0.2*	2.9±.1	3.4±0.1	4.0±0.2*	3.6±0.1	3.8±0.2	3.4±0.4

[a, b, c, *] These letters and symbol have the same meaning as in Table I.

effect was present only in suckling, and as in previous studies (8), not demonstrable in the adolescent group. The mechanism(s) by which glucocorticoids suppress intestinal calcium absorption is unknown. There are data to suggest that glucocorticoids do not interfere with metabolism of vitamin D, hence the suppression of the vitamin D-dependent mechanism seems unlikely. In humans receiving chronic corticosteroid therapy serum levels of 25(OH)D were normal (2) suggesting the metabolism of vitamin D to 25(OH)D is not effected by the corticosteroid. The concentration of the most active metabolite of vitamin D, $1,25(OH)_2D$ in serum and intestinal mucosa were not diminished by administration of glucocorticoid (4). In fact, in glucocorticoid treated chicks, 1 α-hydroxylase activity was increased (5) and in rats, serum concentration of $1,25(OH)_2D$ was elevated

(6). The concentration of calcium binding protein in the intestinal mucosa thought to be important in absorption of calcium was also normal (3). These findings suggest that the glucocorticoid effect may involve a vitamin D-independent mechanism(s). Because in suckling rats, Solu-Medrol effected the bidirectional movement of calcium across the intestinal mucosa, a passive transport process may have been involved. In suckling rats serum corticosterone levels are reported to be low (9), below 3 μg/100 ml, and calcium absorption rate was high (23 μmoles/hr/g wet weight, Table II). At about the time of weaning a two fold increase in corticosterone level occurred and calcium absorption rates decreased to almost half (Table II). After 4 weeks of age corticosterone levels increased, to over 15 μg/100 ml and calcium absorption rates decreased to quite low levels. Thus in the rat, serum glucocorticoid levels appeared to have a reciprocal relationship to rate of absorption of calcium from the small intestine. In suckling rats administration of cortisone acetate and hydrocortisone cause alterations in biochemical properties of the intestinal mucosal membrane (9, 14). Thus the glucocorticoid effect could be due to such changes, which cause decreased permeability of the mucosal membrane to calcium.

It is possible that, in the small intestine of the rat, calcium is absorbed by vitamin D-dependent and vitamin D-independent processes. During suckling because serum corticosterone level is low (9), and $1,25(OH)_2D$ level is high (15) both mechanisms are more active than later in life when serum levels of corticosterone increase (9) and of $1,25(OH)_2D$ decrease (15). Administration of high doses of vitamin D to older rats did not increase 1,25(OH)D levels (16) and absorption of calcium was uneffected when measured *in vivo* (8, 11). Measured *in vitro* vitamin D enhanced calcium absorption only in duodenum (7, 8). In the present study intestinal absorption of calcium was not significantly different in controls and vitamin D treated suckling and weanling rats. Thus, it appears that oral intake of large doses of vitamin D for a 3-day period does not enhance net absorption of calcium from the small intestine of growing rats.

Summary. The influence pharmacological doses of methylprednisolone and vitamin D on *in vivo* absorption of calcium from the small intestine was studied in suckling (14- to 15-day-old), weanling (21- to 22-day-old) and adolescent (42-day-old) rats. Intramuscular injection of methylprednisolone (2.5 mg/100 g body weight daily for 5 days) suppressed absorption of calcium from the jejunum + ileum of the suckling rats only. Both lumen-to-mucosa and mucosa-to-lumen fluxes of calcium were lower in the treated than in the control suckling rats, indicating that the permeability of the mucosal membrane to calcium was decreased with injection of methylprednisolone. This suggested an effect on a passive process of calcium transport rather than on the vitamin D-dependent mechanism of calcium absorption. This effect is compatable with findings of other studies showing that, in glucocorticoid treated rats, the metabolism of vitamin D is not altered in a way to decrease absorption of calcium from the small intestine. Oral administration of vitamin D (5000 IU/100 g body weight daily for 3 days) to suckling and weanling rats, did not enhance the *in vivo* absorption of calcium in the small intestine, an observation similar to our previous *in vivo* study in adolescent rats.

1. Avioli, L. S., Birge, S. J., and Lae, S. W., J. Clin. Endocrinol. Metabol. **28,** 1341 (1968).
2. Hahn, T. J., Halstead, L. R., and Haddad, J. G., J. Lab. Clin. Med. **90,** 399 (1977).
3. Kimberg, D. V., Baerg, R. D., Gershon, E., and Graudusius, R. T., J. Clin. Invest. **50,** 1309 (1971).
4. Favus, M. J., Kimberg, D. V., Millar, G. N., and Gershon, E., J. Clin. Invest. **52,** 1328 (1973).
5. Spanos, E., and MacIntyre, I., *in* "Vitamin D Biochemical, Chemical and Clinical Aspects Related to Calcium Metabolism" (A. W. Norman, K. Schaefer, J. W. Coburn *et al.*, eds.), p. 191. Walter de Gruyter and Co., Berlin 30 (1977).
6. Lukert, B. P., Stanbury, S. W., and Mawer, E. B., Endocrinology **93,** 718 (1973).
7. Williams, G. A., Bowser, E. N., Henderson, W. J., and Uzgiries, V., Proc. Soc. Exp. Biol. Med. **106,** 644 (1961).
8. Williams, G. A., Bowser, E. N., Henderson, W. J., and Uzgiris, V., Proc. Soc. Exp. Biol. Med. **110,** 889 (1962).
9. Daniels, V. G., Hardy, R. N., Malinowska, K. W., and Nathanielsz, P. W., J. Physiol. **229,** 681 (1973).
10. Younoszai, M. K., and Ranshaw, J., Ped. Res. **11,** 414 (1977).

11. Younoszai, M. K., and Schedl, H. P. Proc. Soc. Exp. Biol. Med. **140,** 496 (1972).
12. Younoszai, M. K., and Schedl, H. P. Gastroenterology **62,** 565 (1972).
13. Documenta Geigy Scientific Tables (7th ed.), p. 32. Geigy, Basel, Switzerland (1970).
14. Forstner, G., and Galand, G., Can. J. Biochem. **54,** 224 (1976).
15. Pike, J. W., Toverud, S., Boass, A., McCain, T., and Haussler, M. R., *in* "Vitamin D Biochemical Chemical and Clinical Aspects Related to Calcium Metabolism" (A. W. Norman, K. Schaefer, J. W. Coburn *et al.*, eds), p. 187. Walter de Gruyter and Co., Berlin 30 (1977).
16. Hughes, M. R., Baylink, D. J., Gonnerman, W. A. et al, Endocrinology **100,** 799 (1977).

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