Vitamin D Absorption, Plasma Concentration and Urinary Excretion of 25-Hydroxyvitamin D in Nephrotic Syndrome (43181)

G. KHAMISEH, N. D. VAZIRI, F. OVEISI, M. R. AHMADNIA, AND L. AHMADNIA
Department of Medicine, Division of Nephrology, University of California, Irvine, Irvine, California 92717

Abstract. **Nephrotic syndrome (NS) is commonly associated with vitamin D deficiency. Urinary losses of the protein-bound intermediary metabolite of this vitamin is thought to contribute to the deficiency state. The role of possible changes, if any, of vitamin D absorption has not been investigated previously in NS. We determined intestinal absorption of vitamin D_3 as well as plasma concentration and urinary excretion of 25-hydroxyvitamin D_3 in rats with puromycin aminonucleoside-induced NS. *In vivo* recirculating perfusion technique was employed at 100 and 600 n*M* perfusate concentrations. The results were compared with those obtained in animals receiving placebo injections provided with either free access to food (normal controls) or those pair-fed with their NS counterparts (pair-fed group). The NS group showed heavy proteinuria and hypoalbuminemia. In addition, the NS group exhibited marked urinary losses and significantly reduced plasma concentration of 25-hydroxyvitamin D. The rate of vitamin D_3 absorption (given as nmol/100 cm/min) at 100 n*M* perfusate concentration in the NS group (0.161 ± 0.029 was not significantly different from those obtained in the pair-fed group (0.202 ± 0.058) and the normal control group (0.143 ± 0.053). Likewise, no significant difference was found in the rates of vitamin D absorption at 600 n*M* concentration among the NS (1.073 ± 0.383), pair-fed (0.955 ± 0.229), and normal control (0.756 ± 0.314) groups. Accordingly, intestinal absorption of vitamin D appears to be unaffected by the presence of experimental NS and as such the associated vitamin D deficiency can be managed by enteral supplementation.** [P.S.E.B.M. 1991, Vol 196]

Nephrotic syndrome (NS) is commonly associated with hypocalcemia and vitamin D deficiency. The latter has been attributed to losses of 25-hydroxyvitamin D along with its binding protein in the urine (1). However, the possible effect of NS on vitamin D absorption has not been investigated previously. This is of particular interest since vitamin D absorption has been shown to be impaired in another type of nephropathy, namely, renal insufficiency (2). The present study was undertaken to determine the effect, if any, of experimental NS on intestinal absorption of vitamin D in rats.

Materials and Methods

Male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 280–300 g were randomized into three groups. Animals placed in the NS group received two intraperitoneal injections of puromycin aminonucleoside (130 mg/kg on Day 1 and 50 mg/kg on Day 16) and were monitored through Days 28–29. Animals assigned to the normal control group were given placebo injections (5% dextrose in water) at the same frequency and interval. Animals in both the NS and normal control groups were allowed free access to Purina Rat Chow and water. In an attempt to control for altered food intake, a group of rats pair-fed (PF) with their NS counterparts was included. Pair feeding was accomplished by limiting the amount of food available to each PF animal to that consumed by its NS counterpart during the preceding 24 hr. Animals were housed in individual metabolic cages. Twenty-four-hour urine collections were obtained for measurements of protein, creatinine, and creatinine clearance on a weekly basis throughout the study period. Arterial blood pressure was measured using a rat tail sphygmomanometer (Harvard Apparatus, South Natick, MA). Hematocrit, serum, and urinary concentrations of creatinine and proteins were determined using standard laboratory techniques.

Received February 5, 1990. [P.S.E.B.M. 1991, Vol 196]
Accepted October 17, 1990.

0037-9727/91/1962-0210$2.00/0

Materials. ^{3}H-Cholecalciferol (vitamin D3) with specific activity of 26 mCi/mg (Amersham Inc., Arlington Heights, IL) was used as a tracer compound. ^{14}C-Inulin with specific activity of 2 mCi/mg (New England Nuclear Inc., Boston, MA) was used as an unabsorbable marker. Unlabeled cholecalciferol (Sigma Co., St. Louis, MO) and sodium taurocholate (Calbiochem, La Jolla, CA) were used in the preparation of the perfusate.

A micellar solution was prepared from Krebs phosphate buffer (pH = 6.5) containing sodium taurocholate (10 nmol/liter), trace amounts of ^{14}C-inulin, ^{3}H-vitamin D_3, and unlabeled vitamin D_3 (100 nmol/liter or 600 nmol/liter) using sonication at 60 W for 5 min. The stability of the micellar solutions used was confirmed by demonstration of optical clarity after 24 hr at room temperature. All vitamin D_3 supplies and preparation were kept under nitrogen and away from light to prevent denaturation.

***In Vivo* Perfusion.** Animals were placed under general anesthesia by intraperitoneal injection of 65 mg of pentobarbital/kg. The abdomen was then opened through a midline incision. An inflow polyethylene catheter was inserted into the proximal end of the jejunum approximately 5 cm distal to the ligament of Treitz. An outflow L-shaped glass cannula was introduced 15 cm distal to the inflow catheter. Encircling ligatures, passed between parallel end vessels, were used to secure the cannulas without obstructing blood and lymphatic circulations. A second 15-cm perfusion loop was created with the inflow catheter inserted approximately 2 cm distal to the outflow cannula of the first loop using steps identical to those described above. Each segment was then flushed with 30 ml of Krebs phosphate buffer to remove intestinal contents and flushed with air to minimize the residual fluids remaining in the lumen. The intestine was then placed inside the peritoneal cavity and the abdomen was closed. Each inflow catheter was connected to a reservoir containing 25 ml of the perfusate with either high (600 nmol/liter) or low (100 nmol/liter) vitamin D_3 concentrations. A totally occlusive roller pump (Buchler Instruments Inc., Fort Lee, NJ) was used to pump the perfusate from the reservoir into the perfused segment. The perfusate was then returned to the reservoir by gravity. The perfusate within the reservoir was continuously stirred using a magnetic stirrer. Perfusion with the two perfusates was alternated between the two segments in sequential experiments to correct for the possible effect of intestine site. Both segments were perfused at a rate of 1 ml/min for 60 min, and 200-μl samples were obtained in duplicate at 10-min intervals. The animal was kept in a restraining cage inside a chamber with a thermostatic control (model 74; Thermistemp, Yellow Springs, OH). A rectal probe and a forced air heating device was used to maintain the animal's body temperature at 37°C.

At the end of the perfusion, the perfused segment was flushed with 30 ml of Krebs phosphate buffer, and the animal was sacrificed by an overdose of ether. Each segment was resected, its wet length and weight recorded, and then allowed to dry at room temperature for 24 hr while suspended with a 10-g weigh attached to its most dependent portion. The dry weight and length of the segment were then recorded. Three pieces, each measuring 3 cm in length, were subsequently cut and further dried at 60°C in an oven overnight. The oven-dried tissue was then crushed, solubilized with the BTS-450 (Beckman, Fullerton, CA), and prepared for the measurement of radioactivity remaining in the tissue. All radioassays were performed by a double isotope counting method carried to a σ error of $\pm$ 0.05% using a Beckman LS9000 with an automatic quench calibration program at ambient temperature. The rate of vitamin D_3 absorption was calculated from disappearance of the labeled compound from the perfusate. The latter was determined using the following formula:

$$A_t = A_0 \frac{I_0}{D_0} \times \frac{D_t}{I_t}$$

A_t and A_0 represent vitamin D_3 concentrations while D_t and D_0 represent the concentrations of labeled vitamin D_3, and I_t and I_0 represent the concentrations of labeled inulin at times t and zero, respectively. Accordingly, the absorption results were corrected for fluid shifts as reflected by changes of the concentration of the unabsorbable marker, inulin, during the experiment.

25-Hydroxyvitamin D_3 Measurements in Plasma and Urine. Plasma concentration of 25-hydroxyvitamin D_3 was measured using competitive protein binding radioassay as described previously (3, 4). The coefficient of variation for this assay is 15%. In preparation for the final assay, urine samples were concentrated using a Centricon TM3 Microconcentrator (Amicon Inc., Danvers, MA) and a high-speed centrifugation at 6722g for 4 hr. The retentate, containing protein-bound 25-hydroxyvitamin D_3, was then reconstituted to one-eighth of the original volume using phosphate-buffered saline.

Statistical Analysis. Analysis of variance and Student's t test were used in evaluation of the data which are presented as mean $\pm$ SE. Probability values equal to or greater than 0.05 were considered to be statistically significant. The authors wish to point out that while the n of 8 was originally planned for all experiments, on a few occasions insufficient plasma or urine samples were available to carry out all intended biochemical analyses. In addition, a few experiments were aborted due to technical difficulties. Hence, smaller n values of 6 or 7 were obtained for those measurements.

Results

Body weights in the three groups were comparable at the onset of the study and were significantly lower in the NS and PF groups as compared with that of the

normal control group at the conclusion of the study period. As expected, the NS group showed marked proteinuria and hypoalbuminemia which were lacking in the PF and normal controls. However, no significant difference was found in the creatinine clearance among the three groups. The mean serum calcium concentration in the NS group was lower than those found in the PF and normal control groups. The difference, however, did not attain statistical significance. The NS group exhibited a significant reduction in hematocrit and a significant rise in arterial blood pressure (Table I). The NS animals showed a significant urinary excretion of 25-hydroxyvitamin D_3 (8.3 ± 2.9 ng/24 hr, n = 6) representing a clearance rate of 1.32 ± 0.37 ml of plasma/24 hr. No detectable 25-hydroxyvitamin D_3 was found in the urine of the control groups. Plasma concentration of 25-hydroxyvitamin D_3 was significantly reduced in nephrotic animals when compared with both normal controls and pair-fed animals (Table I). Interestingly, comparison between the pair-fed and normal control groups revealed a significantly higher value of plasma 25-hydroxyvitamin D_3 in the pair-fed animals. No significant difference was found in the rate of intestinal absorption of vitamin D_3 between animals with nephrotic syndrome and those found in the PF and normal control groups at the 100 nM concentration of vitamin D. Although the mean values for the rates of vitamin D_3 absorption at the high luminal concentration (600 nmol/liter) were slightly greater in the NS and PF groups compared with the normal controls, the difference did not attain statistical significance. These observations were true whether the absorption rates were calculated for unit of intestine length or dry weight (Table II).

Discussion

Among various other proteins, cholecalciferol-binding globulin is lost in the urine of patients with nephrotic syndrome. This can result in acquired vitamin D deficiency, depressed plasma concentration of 25-hydroxyvitamin D, which is the primary circulating metabolite of this vitamin, normal to reduced plasma total $1,25(OH)_2$ vitamin D_3, normal to elevated level of free $1,25(OH)_2$ vitamin D_3, and depressed $24,25(OH)_2$ vitamin D_3 (1,5). The acquired vitamin D deficiency can, in turn, lead to depressed calcium absorption, hypocalcemia, secondary hyperparathyroidism, osteitis fibrosa cystica, and osteomalacia (5–9). The degree of hypocalcemia observed in patients with this disorder is often disproportional to that expected from hypoalbuminemia and a diminished protein-bound calcium level. These alterations of vitamin D metabolism with the resultant changes of calcium and phosphorus homeostasis and abnormal parathyroid hormone and bone metabolism can set the stage for an exaggerated renal osteodystrophy in nephrotic patients destined to reach end stage renal disease.

The present study revealed a marked reduction in

Table I. Body Weight, Hematocrit, Systolic Blood Pressure, Creatinine Clearance, 24-Hr Urinary Protein Excretion, Serum Albumin and Plasma 25-Hydroxyvitamin D_3 Levels in the Nephrotic, PF, and Normal Control Groups

	Nephrotic group	PF group	Normal control group
Initial weight (g)	313.83 ± 12.42 (n = 6)	300.60 ± 27.30 (n = 7)	322.57 ± 20.91 (n = 7)
Final weight (g)	303.60 ± 13.84[a] (n = 6)	318.40 ± 37.60[b] (n = 7)	389.40 ± 34.90 (n = 7)
Hematocrit (%)	36.33 ± 6.09[c,d] (n = 6)	44.79 ± 2.93 (n = 7)	45.79 ± 4.92 (n = 7)
Blood pressure (mm/Hg)	205.00 ± 40.40[a,e] (n = 6)	141.40 ± 44.10[b] (n = 7)	98.57 ± 21.16 (n = 7)
Creatinine clearance (ml/min)	1.00 ± 0.04 (n = 5)	0.91 ± 0.25 (n = 6)	0.95 ± 0.52 (n = 4)
Proteinuria (mg/24 hr)	155.80 ± 35.50[a,d] (n = 6)	4.38 ± 3.47 (n = 6)	4.55 ± 1.06 (n = 5)
Serum albumin (g/dl)	2.70 ± 0.35[a,e] (n = 6)	3.56 ± 0.46 (n = 7)	3.95 ± 0.51 (n = 7)
Serum calcium (mg/dl)	8.70 ± 0.45 (n = 6)	9.90 ± 1.30 (n = 6)	9.00 ± 0.37 (n = 6)
Plasma 25-hydroxyvitamin D_3 (ng/ml)	7.00 ± 0.85[a,d]	32.10 ± 2.23[f]	24.10 ± 1.40

[a] $P < 0.001$ nephrotic group versus normal control group.
[b] $P < 0.01$ PF versus normal control group.
[c] $P < 0.01$ nephrotic group versus normal control group.
[d] $P < 0.001$ nephrotic group versus PF group.
[e] $P < 0.05$ nephrotic group versus PF group.
[f] $P < 0.05$ PF versus normal control group.

Table II. Rate of Intestinal Absorption of Vitamin D_3 at 100 and 600 n*M* Concentrations in the Nephrotic, PF, and Normal Control Groups[a]

	Nephrotic group	PF group	Normal control group
Low concentration (100 n*M*)			
n*M*/100 cm/min	0.161 ± 0.029 (n = 8)	0.202 ± 0.058 (n = 8)	0.143 ± 0.053 (n = 8)
n*M*/g dry wt/min	0.112 ± 0.023 (n = 8)	0.137 ± 0.047 (n = 8)	0.087 ± 0.040 (n = 8)
High concentration (600 n*M*)			
n*M*/100 cm/min	1.073 ± 0.383 (n = 8)	0.955 ± 0.229 (n = 6)	0.756 ± 0.314 (n = 6)
n*M*/g dry wt/min	0.727 ± 0.178 (n = 8)	0.727 ± 0.238 (n = 6)	0.531 ± 0.258 (n = 6)

[a] Absorption rates are given per length as well as dry weight of the perfused segments. No significant differences were found in comparison of the data among the study groups.

plasma concentrations and a substantial urinary loss of 25-hydroxyvitamin D_3 in nephrotic animals, confirming the results of earlier studies (5). The observed reduction in plasma 25-hydroxyvitamin D_3 concentration was associated with a normal rate of vitamin D absorption in the NS animals. Occurrence of decreased plasma 25-hydroxyvitamin D_3 concentration in the NS group, but not in the pair-fed group despite comparable food intake combined with the results of the *in vivo* absorption studies, tends to exclude any dietary or digestive origin for the observed vitamin D deficiency in this model. In fact, a trend toward slightly higher rates of absorption was evident in both NS and PF animals at a high luminal concentration of vitamin D. Instead, significant urinary excretion of the vitamin D metabolite demonstrated by others and confirmed by the present investigation must be the principal culprit. From these observations it can be concluded that vitamin D_3 absorption is unaffected by experimental nephrotic syndrome and the associated vitamin D deficiency state in rats. Therefore, the vitamin D deficiency state associated with experimental and perhaps clinical nephrosis is not caused by impaired intestinal transport of this important vitamin and that enteral supplementation should be effective in preventing the deficiency state.

It was of interest to note that despite a restricted food intake, the plasma concentration of 25-hydroxyvitamin D in the pair-fed animals was significantly higher than that of the normal animals receiving liberal food intake. This observation is in agreement with the results obtained by Lorentzon and Danielsson (10) who showed that animals placed on vitamin D-deficient diets convert a greater percentage of administered labeled vitamin D to 25-hydroxyvitamin D and have a higher plasma level of this metabolite. In contrast, animals receiving normal or increased vitamin D intake store most of the administered vitamin D in the tissue in its native form.

1. Sato KA, Gray RW, Lemann J Jr. Urinary excretion of 25 hydroxy-vitamin D in health and nephrotic syndrome. J Lab Clin Med **99**:325, 1982.
2. Vaziri ND, Hollander D, Hung E, Vo M, Dadufalza L. Impaired intestinal absorption of vitamin D-3 in azotemic rats. Am J Clin Nutr **37**:403–406, 1983.
3. Bouillon R, Van Kerkhove P, De Moor P. Measurement of 25-hydroxyvitamin D_3 in serum. Clin Chem **22**:364–368, 1976.
4. Haddad JG, Chyu KJ. Competitive protein-binding radioassay for 25-hydroxycholecalciferol. J Clin Endocrinol **33**:992–995, 1971.
5. Chan YL, Mason RS, Parmentier M, Saudie E, Lissner D, Posen S. Vitamin D metabolism in nephrotic rats. Kidney Intl **24**:336–341, 1981.
6. Schmidt-Gayk H, Schmitt W, Grawunder C, Ritz E, Tschope W, Pietsch V, Andrassy K, Bouillon R. 25-Hydroxy-vitamin D levels in nephrotic syndrome. Lancet **2**:105, 1977.
7. Barragry JM, France MM, Carter ND, Anton JA, Beer M, Le Boucher BJ, Cohen RD. Vitamin D metabolism in nephrotic syndrome. Lancet **2**:629, 1977.
8. Lim P, Jacob E, Tock EPC, Pwee HS. Calcium and phosphorus metabolism in nephrotic syndrome. Q J Med **46**:327, 1977.
9. Goldstein DA, Oda Y, Kurokawa K, Massry SG. Blood levels of 25 hydroxy vitamin D in nephrotic syndrome. Ann Intern Med **87**:664, 1977.
10. Lorentzon R, Danielsson A. The effect of different vitamin D states on intestinal absorption of vitamin D_3 and its metabolites in rats. Acta Physiol Scand **123**:437–444, 1985.