Intestinal Na-Pₐ cotransporter adaptation to dietary Pₐ content in vitamin D receptor null mice

Hiroko Segawa, Ichiro Kaneko, Setsuko Yamanaka, Mikiko Ito, Masashi Kuwahata, Yoshio Inoue, Shigeki Kato, and Ken-ichi Miyamoto. Intestinal Na-Pₐ cotransporter adaptation to dietary Pₐ content in vitamin D receptor null mice. Am J Physiol Renal Physiol 287: F39–F47, 2004. First published March 2, 2004; 10.1152/ajpregal.00375.2003.—Recent studies suggest that vitamin D may play a role in intestinal Na⁺-dependent phosphate transport adaptation to variable levels of dietary Pₐ. Therefore, the goal of the current study was to assess Na⁺-dependent Pₐ cotransport activity in transgenic mice to determine whether vitamin D is a mediator of this process. Intestinal brush-border membrane (BBM), Na⁺-dependent Pₐ cotransport activity was significantly decreased in vitamin D receptor (VDR) null [VDR (−/−)] mice compared with wild-type (VDR +/+) mice. While intestinal Na-Pₐ cotransporter (type IIb) mRNA levels were similar in VDR (−/−) and VDR (+/+) mice, type IIb Na-Pₐ cotransporter protein expression was markedly suppressed in VDR (−/−) mice compared with VDR (+/+) mice. Furthermore, Na-Pₐ cotransport activity in renal BBM was similar in VDR (−/−) and VDR (+/+) mice, but type IIa Na-Pₐ cotransporter protein expression was decreased in VDR (−/−) mice. After administration of a low-Pₐ diet, type IIb protein expression was significantly increased in VDR (+/+) and VDR (−/−) mice, and type IIb protein expression was present in the intestinal BBM of VDR (−/−) mice. These data demonstrate that intestinal Na-Pₐ cotransport adaptation to a low-Pₐ diet occurs independently of vitamin D.

MATERIALS AND METHODS

Animals and diet. VDR null mutant mice were generated by gene targeting as described previously (25, 56). The locus targeted for the disruption of the VDR gene included exon 2, and the mutant locus contained the neomycin-resistant gene.

VDR genotypes were determined by analyzing DNA obtained from each mouse at ~10 days after birth. Genomic DNA was extracted from tail clippings and amplified by PCR using primers specific for VDR (+/+) exon 2 or for the neomycin-resistant gene. Novel primers with the sequences of 5'-GATGTTGTTATCTGGTTGTC-3' and 5'-CGAGGCAGCGGATCTGATA-3' were prepared for VDR (+/+) exon 2 and for the neomycin-resistant gene. Novel primers with the sequences of 5'-TTGGCTCTTTCGTTGCTTG-3' and 5'-CGATACCGTAAAGCAGG-3' were prepared for detection of the neomycin-resistant gene (Fig. 1).

Mice [VDR (+/+) and VDR (−/−)] were weaned at 3 wk of age and given free access to water and a control diet containing 0.5% Pₐ and 0.5% Ca for 6 days (30, 31). On day 7, mice were assigned to one of two groups: the control group, which was fed a diet containing 0.5% Pₐ, 0.5% Ca, and 20% lactose; and the low-Pₐ group, which was fed a diet containing 0.25% Pₐ, 0.5% Ca, and 20% lactose (30, 31). After 4 wk of test diet administration, mice were anesthetized with intraperitoneal pentobarbital sodium, and the tissues were rapidly removed.

Northern blot analysis. Poly(A)+ RNA (3 μg/lane) isolated from mouse intestine or kidney was separated on a 1% agarose gel in the presence of 2.2 M formaldehyde and blotted onto a Hybond-N membrane (Amerham Pharmacia Biotech) as described previously (37, 44). The specific probes for each phosphate transporter subtype were labeled with [32P]dCTP using the Megaprimer DNA Labeling System (Amerham Pharmacia Biotech). Hybridization proceeded for 3 h at 65°C in Rapid-hyb buffer (Amerham Pharmacia Biotech). The final stringent wash of the membrane was performed with 0.1% standard sodium phosphate-EDTA, 0.1% SDS at 65°C, and the blot was autoradiographed using a FujiBio bioimaging analyzer (BAS-1500, FujiFilm, Tokyo, Japan).

Preparation of BBMVs and transport assay. BBMVs were prepared from mouse kidney or intestine by the Ca²⁺ precipitation method. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

INTESTINAL ABSORPTION of Pₐ has been characterized in several mammalian and avian species (2, 3, 5, 7–12, 16–18, 21–23, 32, 36, 38, 39, 41). Studies conducted with isolated intestinal brush-border membrane vesicles (BBMVs) have demonstrated that the transepithelial uptake of Pₐ occurs primarily in the proximal small intestine secondary to passive diffusion across the intestinal brush border and Na⁺-dependent, carrier-mediated uptake (8, 10, 16). Intestinal absorption of Pₐ is mediated primarily via the type IIb sodium-phosphate cotransporter, whereas renal Pₐ reabsorption is mediated by the type IIa and IIc cotransporters located in the apical membrane of the proximal tubule (14, 20, 35, 37).

Dietary Pₐ deficiency stimulates vitamin D synthesis (40, 48) and leads to an increase in active Pₐ absorption in the small intestine (5, 8, 10, 22, 36, 41). Extensive clinical and experimental studies support the existence of an active, 1,25(OH)₂D₃-responsive Pₐ transport mechanism in the intestine (9, 11–13, 17, 18, 21–24, 28, 32, 38, 39, 51, 54). Vitamin D₃, a steroid hormone, plays a central role in modulating phosphate homeostasis and Pₐ uptake by the small intestine. The active form of vitamin D₃ is 1,25(OH)₂D₃, which binds the vitamin D receptor (VDR) and induces changes in gene expression. Hattenhauer et al. (19) demonstrated that stimulation of intestinal Na-Pₐ cotransport by a low-Pₐ diet or vitamin D₃ is mediated via an increase in type IIb transporter expression in BBMVs. The goal of the present study was to determine whether vitamin D action is an essential factor for adaptation of intestinal Na-Pₐ cotransport to dietary Pₐ deficiency.
method as described previously (37, 44, 45). Levels of leucine aminopeptidase, Na\(^+\)-K\(^+\)-ATPase, and cytochrome c oxidase were measured to assess the purity of the membranes. The uptake of \(^{32}\)P-into BBMVs was measured by the rapid filtration technique. Next, 10 \(\mu\)L of vesicle suspension were added to 90 \(\mu\)L of vesicle suspension that was composed of (in mM) 100 NaCl, 100 mannitol, 20 Tris, and 0.1 KH\(_2\)PO\(_4\), and the preparation was incubated at 20\(^\circ\)C for 5 min in the presence of 5% 2-mercaptoethanol and subjected to SDS-PAGE (37, 44). The separated proteins were transferred by electrophoresis to Hybond-P polyvinylidene difluoride transfer membranes (Amerham Pharmacia Biotech). The membranes were treated with diluted anti-type IIb Na-P\(_i\) cotransporter antisera (1:2,000), affinity-purified anti-type IIa (1:4,000), or type IIc (1:1,000) Na-P\(_i\) cotransporter antibody. Type IIb Na-P\(_i\) cotransporter polyclonal antibody was generated in rabbits against a COOH-terminal peptide corresponding to amino acid residues 682-697 (QVEVLSMKAL-AJP-Renal Physiol VOL 287 JULY 2004). Levels of leucine aminopeptidase, Na\(^+\)-K\(^+\)-ATPase, and cytochrome c oxidase were measured to assess the purity of the membranes. The uptake of \(^{32}\)P-into BBMVs was measured by the rapid filtration technique. Next, 10 \(\mu\)L of vesicle suspension were added to 90 \(\mu\)L of incubation solution that was composed of (in mM) 100 NaCl, 100 mannitol, 20 Tris, and 0.1 KH\(_2\)PO\(_4\), and the preparation was incubated at 20\(^\circ\)C. Na\(^+\)-dependent \(\text{Pi}\) uptake was measured as described previously (44, 45). Transport was terminated by rapid dilution with ice-cold saline. The reaction mixture was immediately transferred to a premoistened filter (0.45 \(\mu\)m) and maintained under vacuum.

**Immunoblotting.** Protein samples were heated at 95\(^\circ\)C for 5 min in sample buffer in the presence of 5% 2-mercaptoethanol and subjected to SDS-PAGE (37, 44). The separated proteins were transferred by electrophoresis to Hybond-P polyvinylidene difluoride transfer membranes (Amerham Pharmacia Biotech). The membranes were treated with diluted anti-type IIb Na-P\(_i\) cotransporter antisera (1:2,000), affinity-purified anti-type IIa (1:4,000), or type IIc (1:1,000) Na-P\(_i\) cotransporter antibody. Type IIb Na-P\(_i\) cotransporter polyclonal antibody was generated in rabbits against a COOH-terminal peptide corresponding to amino acid residues 682-697 (QVEVLSMKAL-AJP-Renal Physiol). Levels of leucine aminopeptidase, Na\(^+\)-K\(^+\)-ATPase, and cytochrome c oxidase were measured to assess the purity of the membranes. The uptake of \(^{32}\)P-into BBMVs was measured by the rapid filtration technique. Next, 10 \(\mu\)L of vesicle suspension were added to 90 \(\mu\)L of incubation solution that was composed of (in mM) 100 NaCl, 100 mannitol, 20 Tris, and 0.1 KH\(_2\)PO\(_4\), and the preparation was incubated at 20\(^\circ\)C. Na\(^+\)-dependent \(\text{Pi}\) uptake was measured as described previously (44, 45). Transport was terminated by rapid dilution with ice-cold saline. The reaction mixture was immediately transferred to a premoistened filter (0.45 \(\mu\)m) and maintained under vacuum.

**Immunohistochemistry.** Mice were anesthetized with pentobarbital sodium (50 mg/kg body wt) and perfused via the left ventricle with PBS followed by paraformaldehyde-lysine-periodate (PLP). Tissues were postfixed with 10 and 20% sucrose at 4\(^\circ\)C and embedded in OCT compound (Miles, Elkhart, IN). Frozen sections (5 \(\mu\)m) were thaw-mounted onto silane-coated slides and air dried. For immunofluorescence, serial sections were incubated with rabbit anti-type IIb Na-P\(_i\) cotransporter antisera (1:200), rabbit anti-type IIa (1:4,000), guinea pig anti-type IIc (1:200), or rabbit anti-PepT1 antibody (1,000:1) overnight at 4\(^\circ\)C. Thereafter, they were treated with Alexa Fluor 568 anti-rabbit IgG (Molecular Probes) or Alexa Fluor 488 anti-guinea pig IgG (Molecular Probes) as the secondary antibody for 60 min (37).

**RESULTS**

**Serum Ca, P, PTH, and vitamin D in VDR (\(-/-\)) mice.** Serum Ca, P, PTH, and vitamin D in VDR (\(+/+\)) and (\(-/-\)) mice that were fed a control P\(_i\) diet (0.5% P\(_i\)) are shown in Fig. 2. Serum Ca and P\(_i\) levels were significantly decreased in VDR (\(-/-\)) mice compared with VDR (\(+/+\)) mice (Fig. 2, A and B). In contrast, plasma PTH and 1\(\alpha\),25-(OH)\(_2\)D\(_3\) levels were markedly increased in VDR (\(-/-\)) mice compared with VDR (\(+/+\)) mice (Fig. 2, C and D). These observations are consistent with previous reports (25, 30, 31, 56).
Na–P\textsubscript{i} cotransport activity in VDR (−/−) mice. Intestinal BBM Na–P\textsubscript{i} cotransport activity was assessed in VDR (+/+) and VDR (−/−) mice fed a control P\textsubscript{i} diet. P\textsubscript{i} uptake was linear up to 30 s, with a slow increase persisting to 5 min (data not shown). In the VDR (−/−) mice, intestinal Na–P\textsubscript{i} cotransport activity was reduced to 60% of that seen in wild-type (+/+) mice (Fig. 3A). In contrast, renal BBM Na–P\textsubscript{i} cotransport activity did not differ between VDR (+/+) and VDR (−/−) mice (Fig. 3B).

Expression of type II Na–P\textsubscript{i} cotransporter mRNA in VDR (−/−) mice. Type II Na–P\textsubscript{i} cotransporter mRNA levels were determined in VDR (−/−) or VDR (+/+) mice fed a control P\textsubscript{i} diet (Fig. 4). Intestinal type IIb Na–P\textsubscript{i} cotransporter mRNA did not differ in a comparison of VDR (−/−) mice and wild-type VDR (+/+) mice (Fig. 4, A and B). In contrast, calbindin D\textsubscript{9k} mRNA levels were significantly lower in VDR (−/−) mice compared with VDR (+/+) mice (Fig. 4, A and C). Furthermore, renal type IIa or type IIc mRNA levels did not differ in a comparison of VDR (−/−) and VDR (+/+) mice (Fig. 4, D–F).

Expression of the type II Na–P\textsubscript{i} transporter proteins in VDR (−/−) mice. Type IIb Na–P\textsubscript{i} cotransporter protein was detected as a 108-kDa band on Western blotting. Intestinal BBMV type IIb protein expression was significantly decreased in VDR (−/−) mice compared with VDR (+/+) mice (Fig. 5, A and B).

Furthermore, type IIa protein expression was slightly but significantly decreased in VDR (−/−) mice compared with VDR (+/+) mice (Fig. 5, C and D). In contrast, there was no difference in type IIc protein expression in a comparison of VDR (−/−) and VDR (+/+) mice (Fig. 5, E and F). The amount of P\textsubscript{i} excretion was not different in a comparison of VDR (−/−) mice and VDR (+/+) mice [VDR (+/+); FE\textsubscript{PI} 11.8 ± 1.9 vs. VDR (−/−); FE\textsubscript{PI} 11.1 ± 0.2].

Immunohistochemical analysis of the type II Na–P\textsubscript{i} cotransporters in VDR (−/−) mice. To confirm the reduction in type IIb or type IIa Na–P\textsubscript{i} cotransporter expression in VDR (−/−) mice, immunohistochemical analysis was performed (Fig. 6). The type IIb Na–P\textsubscript{i} cotransporter-immunoreactive signals were clearly present at the apical membrane of enterocytes from VDR (+/+) mice (Fig. 6A), whereas enterocytes from VDR (−/−) mice showed no immunoreactivity (Fig. 6B). In contrast, oligopeptide transporter PepT1-immunoreactive signals showed a similar distribution in a comparison of VDR (−/−) and VDR (+/+) mice (Fig. 6, C and D).

Type IIa Na–P\textsubscript{i} cotransporter-immunoreactive signals were slightly reduced at the apical membrane of the superficial nephrons in VDR (−/−) mice compared with VDR (+/+) mice (Fig. 6, E–H). In contrast, type IIc immunoreactivity showed a similar distribution in a comparison of VDR (+/+) and VDR (−/−) mice (data not shown).

Effect of low-P\textsubscript{i} diet on phosphate transport activity and type IIb Na–P\textsubscript{i} cotransporter expression. VDR (−/−) mice fed a low-P\textsubscript{i} diet displayed an intestinal sodium-dependent phos-
Phase cotransporter activity and intestinal type IIb Na-P\textsubscript{i} cotransporter mRNA level that were significantly increased compared with those fed the control P\textsubscript{i} diet (Fig. 7, A and B). Type IIb transporter protein expression was also increased in VDR (-/-) mice fed a low-P\textsubscript{i} diet (Fig. 7C). Furthermore, type IIb-immunoreactive signals were observed at the apical membrane of enterocytes from VDR (-/-) mice fed a low-P\textsubscript{i} diet (Fig. 7D). Similar observations were detected in VDR (+/+) mice fed a low-P\textsubscript{i} diet.

Calbindin D\textsubscript{9k} mRNA levels were significantly decreased in VDR (+/+) and VDR (-/-) mice that were fed a low-P\textsubscript{i} diet compared with those animals fed a control P\textsubscript{i} diet (Fig. 8). These data suggest that the effect of a low-P\textsubscript{i} diet was specific to the Na-P\textsubscript{i} cotransport system and had no effect on transcellular Ca\textsuperscript{2+} transport system.

Effect of a low-P\textsubscript{i} diet on serum Ca, P\textsubscript{i}, PTH, and vitamin D in VDR (-/-) mice fed a low-P\textsubscript{i} diet. Serum Ca levels were significantly increased in VDR (-/-) mice fed a low-P\textsubscript{i} diet compared with those fed a control P\textsubscript{i} diet (7.7 ± 0.3 vs. 8.2 ± 0.2 mg/dl) (Fig. 9A). In contrast, serum levels of P\textsubscript{i} were lower in VDR (-/-) mice fed a low-P\textsubscript{i} diet compared with VDR (-/-) mice fed a control P\textsubscript{i} diet (3.5 ± 0.3 vs. 5.5 ± 0.5 mg/dl) (Fig. 9B). Serum PTH levels were significantly decreased in VDR (-/-) mice fed a low-P\textsubscript{i} diet compared with those fed a control P\textsubscript{i} diet (Fig. 9C). This may be due to the elevation of serum calcium levels in VDR (-/-) mice fed the low-P\textsubscript{i} diet. In VDR (+/+) mice fed a low-P\textsubscript{i} diet, serum 1α,25(OH)\textsubscript{2}D\textsubscript{3} levels were significantly increased compared with those mice fed a control P\textsubscript{i} diet. In contrast, there were no differences in serum 1α,25(OH)\textsubscript{2}D\textsubscript{3} levels between VDR (-/-) mice fed a low- and control P\textsubscript{i} diet (Fig. 9D). Furthermore, FE\textsubscript{Pi} was significantly decreased in VDR (-/-) mice fed a low-P\textsubscript{i} diet compared with those mice fed a control P\textsubscript{i} diet (control P\textsubscript{i} vs. low-P\textsubscript{i}, 11.2 ± 1.5 vs. 2.4 ± 0.2).

DISCUSSION

Regulation of intestinal P\textsubscript{i} absorption by dietary P\textsubscript{i} content has been extensively studied using isolated BBM and cell cultures (4, 8, 35, 40). Specifically, low-P\textsubscript{i} diets result in increased intestinal Na-P\textsubscript{i} cotransport activity, rapid decreases in plasma P\textsubscript{i}, activation of renal 1,25-hydroxylase, and an increase in vitamin D\textsubscript{3} levels (29, 40). Several studies suggest that adaptation of small intestinal Na-P\textsubscript{i} cotransport to a low-P\textsubscript{i} diet is mediated by vitamin D\textsubscript{3} by demonstrating that changes in apical Na\textsuperscript{+}-dependent P\textsubscript{i} cotransport rates, but not in the apparent K\textsubscript{m} value for P\textsubscript{i}, respond to different levels of dietary P\textsubscript{i}, content (5, 17, 24, 32, 36, 41). Similar observations were reported in the renal P\textsubscript{i} reabsorption system (35). While the precise mechanisms remain unclear, adaptation in the renal Na-P\textsubscript{i} cotransporter system may involve PTH, vitamin D, growth hormone, thyroid hormone, calcitonin, or other agents (33). Taken in concert, the above findings suggest that vitamin D\textsubscript{3} may also play a role in the adaptation of the intestinal Na-P\textsubscript{i} system to dietary P\textsubscript{i}. However, the present study demonstrated that adaptation to a low-P\textsubscript{i} diet occurred even in VDR (-/-) mice, demonstrating that vitamin D action is not necessary for this phenomenon. A low-P\textsubscript{i} diet stimulated expression of type IIb transporter protein in enterocytes and type IIa and type IIc transporter proteins in renal cells from VDR (-/-) mice. We previously demonstrated that upregulation of renal type IIa Na-P\textsubscript{i} cotransporter by a low-P\textsubscript{i} diet (26) was mediated via increased expression of transcription factor E3 (TFE3) (34). This transcription factor promotes expression of the type IIa transporter protein gene via phosphate-response elements in its promoter sequence. Furthermore, Moz et al. (34) described a posttranscriptional modification of the type IIa gene product by a low-P\textsubscript{i} diet; renal proteins from rats fed a low-P\textsubscript{i} were able to stabilize type IIa mRNA in vitro.
Similar mechanisms may mediate upregulation of intestinal type IIb Na-Pi cotransporter by a low-Pi diet. Hattenhauer et al. (19) and Xu et al. (53) demonstrated that stimulation of intestinal Na-Pi cotransport by 1,25(OH)2D3 can be explained by an increased number of type IIb Na-Pi cotransporter proteins without an increased rate of transcription of the type IIb gene. The present study also demonstrated that stimulation of intestinal P1 absorption by 1,25(OH)2D3 may be not mediated by increases in type IIb transporter gene expression.

Furthermore, Hattenhauer et al. (19) demonstrated that dietary P1 restriction increased type IIb protein but not type IIb mRNA. However, the present study indicates that dietary P1 restriction increased intestinal type IIb mRNA in VDR (+/+ ) and VDR (-/-) mice. Although we do not have a clear explanation, this difference might be due to the content of P1 in the diets or the period of P1 restriction. In any case, vitamin D may not be involved in the upregulation of the type IIb mRNA by dietary P1 restriction.

Another potential mediator of dietary P1 adaptation is fibroblast growth factor 23 (FGF23) (42, 49, 50). We previously reported that injection of FGF23 DNA into rats blunted adaptation of renal Na-P1 cotransport to dietary P1 (44). Other studies have demonstrated that FGF23 administration resulted in decreased intestinal Na-Pi cotransport activity (43) and that administration of FGF23 suppressed expression of renal type II Na-Pi cotransporters (type IIa and type IIc) (44). A recent study reported that a low-P1 diet resulted in decreased plasma FGF23, whereas a high-P1 diet resulted in increased plasma FGF23 (55). Measurements of serum FGF23 are needed to clarify the mediators of dietary P1 adaptation.

VDR (-/-) mice exhibit features similar to those of patients with hereditary vitamin D-resistant rickets, which results from genetic mutations in the VDR gene (25, 30, 31, 56). VDR

(-/-) mice display retarded growth, hypocalcaemia, hypophosphatemia, and severely impaired bone mineralization (25, 56). However, Masuyama et al. (30, 31) demonstrated that administration of a low-Pi VDR (-/-) "rescue diet" (low-Pi diet; 0.25% P_i) resulted in normalization of serum calcium and phosphate concentration and improved bone mineralization and turnover in VDR (-/-) mice. In the present study, a low-Pi diet resulted in attenuation of hypocalcaemia but had no effect on phosphate levels in VDR (-/-) mice. An increase in intestinal calcium transport appeared to occur via acceleration of passive absorption of calcium transport rather than by vitamin D-dependent absorption, as calbindin D_9k mRNA levels remained unchanged. Improvements in plasma P_i were likely mediated via upregulation of the type IIb Na-P_i cotransporter in VDR (-/-) mice.

In addition, the present data showed that a low-Pi diet (0.25% P_i) stimulates intestinal BBM phosphate transport and type IIb protein synthesis in VDR (-/-) mice comparable to that in VDR (+/+). To determine the degree of P_i restriction necessary to stimulate intestinal Na-P_i cotransport activity in VDR (+/-) mice, groups of mice were fed a diet containing 0.02, 0.25, or 0.6% (control) P_i (data not shown). Severe (0.02% P_i) restriction induced a 2.0-fold increase in intestinal Na-P_i cotransport activity compared with the moderately P_i-restrictive diet (0.25% P_i). In addition, serum 1,25(OH)_{2}D_3 levels with the 0.02% P_i diet were significantly increased compared with the 0.25% P_i diet (336 ± 32 vs. 108 ± 45 pg/ml). These data suggest that changes in serum 1,25(OH)_{2}D_3 concentration per se are sufficient to determine the activity of intestinal Na-P_i cotransport in VDR (+/-) mice (52). In contrast, both severe (0.02%) and moderate (0.2%) P_i restriction induced 4.2- and 4.4-fold increases, respectively, in intestinal Na-P_i cotransport in VDR (-/-) mice compared with the control diet (0.6% P_i) (data not shown). The question
remains as to the underlying mechanisms for the regulation of Na-P$_i$ cotransport by the degree of the P$_i$ restriction in VDR (−/−) mice. One possible explanation is that P$_i$ demand may be increased in VDR (−/−) mice compared with wild-type animals, because of impaired bone mineralization and reduced bone turnover (4, 27, 42). However, the role of bone mineralization and turnover in controlling intestinal Na-P$_i$ cotransport by P$_i$ restriction is not yet established.

The present study also showed that the PTH levels in VDR (−/−) animals are much higher than in controls, but urinary FE$_{P_i}$ is unchanged. Forte et al. (15) demonstrated that the blunted phosphaturic response to PTH observed in vitamin D-deficient animals is associated with the reduced responsiveness of renal cortical adenylate cyclase to the hormone. Furthermore, in a previous study, we demonstrated that renal type IIa transporter expression was decreased in the deep cortex of vitamin D-deficient rats, and administration of 1,25-dihydroxyvitamin D$_3$ in these animals resulted in normalization of type IIa transporter expression (46). The present study demonstrated that type IIa transporter mRNA levels were similar in a comparison of VDR (−/−) and VDR (+/+) mice. In addition, immunohistochemical analysis indicated that the reduction of type IIa immunoreactive signals was in the superficial cortex rather than the deep cortex. The discrepancy between these studies may be due to a species-dependent difference in vitamin D metabolism (1, 6, 7).

In conclusion, the present study demonstrated an elevation of intestinal P$_i$ transport activity and type IIb protein content in mice fed a low-P$_i$ diet. Furthermore, this phenomenon was independent of vitamin D$_3$.

---

![Fig. 8](image_url)

**Fig. 8.** Effect of a low-P$_i$ diet on calbindin D$_{9k}$ (CaBP-9k) mRNA levels in VDR (−/−) mice. Top: Northern blot analysis of calbindin D$_{9k}$ was performed. Bottom: relative intensity was calculated using GAPDH as the internal control. Values are means ± SE (n = 6). *P < 0.01.

---

**Fig. 9.** Effect of a low-P$_i$ diet on the serum levels of Ca (A), phosphate (B), PTH (C), and 1α,25(OH)$_2$D$_3$ (D) in VDR (−/−) mice 4 wk after administration of test diet, as described in the MATERIALS AND METHODS. Values are means ± SE (n = 6–8). *P < 0.05, **P < 0.005, ***P < 0.0001.

---

ACKNOWLEDGMENTS

We thank Eri Kawakami and Junya Furutani for technical support.

GRANTS

This work was supported by Grants 15790430 (to H. Segawa) and 11557202 (to K. Miyamoto) from the Ministry of Education, Science, Sports and Culture of Japan and the Human Nutritional Science on Stress Control 21st Century Center of Excellence Program.

REFERENCES

26. Danisi G and Murer H.
25. Feild JA, Zhang L, Brun KA, Brooks DP, and Edwards RM.
24. Hattenhauer O, Traebert M, Murer H, and Biber J.
23. Fuchs R and Peterlik M.
22. Cross HS and Peterlik M.
21. Jungbluth H and Binswanger U.
20. Hattenhauer O, Traebert M, Forster I, Murer H, and Biber J.
19. Tenenhouse HS and Sabbagh Y.


