# Effect of Parathyroid Hormone on Intestinal Mucosal Sodium Dependent Phosphorus Transporter

Wang Guo, Li-Yan Wang, Xue Han, Wen-Hu Liu

Department of Nephrology, Capital Medical University Affiliated Beijing Friendship Hospital, Beijing, China

**Keywords.** sodium-dependent phosphorus transporter, parathyroid hormone, intestinal mucosa, secondary hyperparathyroidism, hyperphosphatemia

Original Paper

**Introduction.** Hyperphosphatemia is an important symptom of chronic kidney disease-mineral bone disorder (CKD-MBD). Various oral phosphate binders have been used, but have not been very effective, especially for severe secondary hyperparathyroidism (SHPT) in patients undergoing maintenance dialysis. Maintenance dialysis patients with severe SHPT can develop hypophosphatemia for several months after parathyroidectomy without elevated alkaline phosphatase. Based on these clinical phenomena, we hypothesized that high levels of parathyroid hormone (PTH) might inhibit intestinal phosphorus absorption which mediated by sodium-dependent phosphorus transporters.

**Methods.** Forty BALB/c mice were divided into four groups. Mice in group 1 were given an intravenous injection of normal saline as the control group. Mice in groups 2, 3, and 4 were given PTH(1-34) in doses of 40 µg/100 g, 200 µg/100 g, and 400 µg/100 g body weight intravenously, respectively. All mice were euthanized 8 hours after the injection. The mRNA and protein expression of sodium-dependent phosphorus transporter NPT-2b and Pit-1 on the membrane of the intestinal epithelial cells was detected by real-time polymerase chain reaction (PCR) and western blot analysis, respectively.

**Results.** In group 4, intestinal epithelial NPT-2b and Pit-1 protein expression was significantly decreased, whereas in groups 2 and 3, no significant changes were found.

**Conclusion.** A high PTH level decreases the protein expression of NPT-2b and Pit-1 in the intestinal mucosa.

IJKD 2021;15:48-55
www.ijkd.org

## INTRODUCTION

With the increased prevalence of chronic kidney disease (CKD), hyperphosphatemia has been found to play an important role in CKD mineral and bone disorders.[1] It is generally recognized that as the glomerular filtration rate decreases, kidney phosphorus discharge ability is decreased. Moreover, when the amount of phosphorus uptake from the intestinal exceeds the amount of phosphorus excreted from the kidneys, hyperphosphatemia occurs. In patients with CKD 5D stage, and especially in patients with severe SHPT undergoing dialysis, hyperphosphatemia is more severe and difficult to control. Even with various new oral phosphate-binding drugs, hyperphosphatemia in dialysis patients is still a worldwide clinical problem. Previous studies have revealed that in addition to obtaining phosphorus from bone metabolism, absorbing dietary phosphorus through the intestine can increase blood phosphorus also. Thus, current strategies for treating hyperphosphatemia in CKD

patients include dietary phosphorus restriction and oral phosphorus binders. But this treatment is not very effective, especially in patients with serious SHPT. On the other hand, clinical data show that parathyroidectomy in patients with serious SHPT may lead to a period of hypophosphatemia 3 to 6 months after parathyroidectomy, which cannot be fully explained by hungry bone syndrome, because hypophosphatemia in these patients is not accompanied by an increase in alkaline phosphatase level.[2] These conditions suggest that phosphorus absorption in the intestinal tract may be inhibited in patients with severe SHPT, and hyperphosphatemia in these patients is primarily caused by excessive release of phosphorus from the bone into the blood due to high PTH. After the parathyroidectomy, with the decrease of PTH level, although the release of phosphorus from bone tissue is reduced, the absorption of phosphorus in the intestinal tract is still at a low level, which leads to the postoperative phenomenon of hypophosphatemia. In the process of intestinal phosphorus absorption, more than 50% of phosphorus transport is carried out by sodium-dependent phosphorus transporters including NPT-2b and Pit-1.[3] We hypothesized that in patients with severe SHPT, excessive PTH concentration inhibits the expression of intestinal NPT-2b and Pit-1, thereby decreasing the intestinal absorption of phosphorus in these patients. To test this hypothesis, we designed the current study to determine the effect of different doses of PTH on intestinal sodium-dependent phosphorus transporters in mice.

## MATERIALS AND METHODS

### Animals and Experimental Design

Eight-week old BALB/c male mice were obtained from the Academy of Military Medical Sciences (AMMS). The mice were housed in cages in a climate-controlled room with a 12-hour light/dark cycle at a temperature of 25°C. The mice were allowed free access to standard food and water. After 1 week of acclimation, the mice were randomly assigned to different groups with 10 mice in each group as follows: group 1 (control) received saline injections through the tail vein; group 2 received PTH(1-34) (MB10333, Dalian Meilun Biotechnology Co, LTD) of 40 µg / 100 g body weight injections; group 3 received PTH(1-34) of 200 µg / 100 g body weight injections; group 4 received PTH(1-34) of 400 µg / 100 g body weight injections. The mice in all groups were euthanized under isoflurane anesthesia 8 hours after injections. The segment of the small intestine was removed and washed with 0.9% saline, and the segment length was recorded. Five-centimeter-long segments of the duodenum (first 5 cm from the pylorus) and jejunum (5 cm from the ligament of Treitz or ileum (last 5 cm) were cannulated and flushed with warm 0.9% saline.

### Western Blotting

Polyclonal antibodies for NPT-2b were raised in rabbit against a 16 amino-acid-peptide near the center of the NPT-2b (Abcam Cambridge, UK). Mouse monoclonal antibody was raised against a synthetic peptide corresponding to amino acids 30-146 of Pit1 (Abcam Cambridge, UK). Mouse monoclonal antibodies raised against synthetic peptide corresponding to the N-terminal of human β-actin (ACTB) conjugated to KLH were used as a loading control (TA-09, ZSGB-Bio, China). For western blot analysis, 30 µg protein samples were used. The electrophoresis conditions were as follows: concentrated gel constant pressure 90 V, for approximately 20 minutes; the gel was separated at a constant pressure of 120 V, and the electrophoresis stopping time was determined by the prestained protein marker. The proteins were transferred to nitrocellulose membranes by semidry electrophoretic blotting for 60 minutes at a constant current of 1 mA / $cm^{-2}$. Non-specific protein-binding sites were blocked with 5% SA-tris-buffered saline tween (TBST) for 1 hour at room temperature. The membranes were incubated with NPT-2b (1: 1000), Pit1 (1: 50), and β-actin antibodies (1: 1000) for 16 hours at 4°C. The filters were then washed (3 × 10 min) with TBST and incubated with goat anti-rabbit immunoglobulin G (IgG), rabbit anti-goat IgG, and goat anti-mouse IgG conjugated to horseradish peroxidase for 40 minutes at room temperature and finally washed again with TBST. Bound antibodies were detected by an enhanced chemiluminescence system and visualized and quantified using a Fluor-S MultiImager System. The ratio of NPT-2b to β-actin was established for each sample and expressed in arbitrary units (a.u.).

### Real-Time Polymerase Chain Reaction (PCR)

Quantitative PCR was performed using ABI 7500 (Applied Biosystems, USA). The reaction

mixture comprised 10 µL SYBR Master Mix (2x) Universal and ROX Reference Dye II with specific primers. We determined mRNA levels using the following primers: NPT-2b [ACTGGCTCTCTGTGTTCGTG (forward) and TTATCAGTCGAGGGGACGGT (reverse)], Pit-1 [GAGAAGGTGGGAGCAAACGA (forward) and TCAGCCATCCGCATGATCTC (reverse)], and β-actin [CCACCATGTACCCAGGCATT (forward) and CGGACTCATCGTACTCCTGC (reverse)]. The PCR reaction mixtures were pre-incubated at 95°C for 10 minutes, amplified at 95°C for 10 seconds, and 59°C for 60 seconds. Analyses of mRNA expression data were conducted using the $2^{-\Delta\Delta CT}$ methods as described previously.

## Statistical Analysis

Data were analyzed for statistical significance using unpaired two-tailed Student *t* tests and two-way ANOVA test. The data are presented as means ± standard error of the mean (SE). All analyses were performed using Graphpad software with $P < .05$ denoting statistical significance.

## RESULTS

The first 5 cm of the small intestine was taken to represent the duodenum. The jejunum was taken 5 cm distal from the ligament of Treitz, and the ileum represented the final 5 cm of the small intestine.

We examine the protein levels of NPT-2b in the tissue isolated from the distinct regions of the small intestine by western blot analysis. The ratio of the protein to β-actin enabled to access the regional distribution of the two phosphate transporters. Although quantitative examination showed that NPT-2b was most expressed in the ileum and least expressed in the duodenum, the difference was not statistically significant (Figure 1-A). Quantification of NPT-2b mRNA expression in the intestine mucosa using real-time PCR demonstrated that the highest levels of NPT-2b mRNA were detected in the jejunum, followed by the ileum and duodenum (Figure 1-B).

Mouse monoclonal antibody against a synthetic peptide corresponding to amino acids 30-146 of Pit-1 was used to examine the protein abundance of Pit-1 in the tissue isolated from the three segments of the small intestine. Different from NPT-2b, Pit-1 protein is most expressed in the jejunum and least expressed in the duodenum (Figure 2-A). In addition, Pit-1 mRNA expression levels were highest in the ileum, followed by the jejunum and duodenum (Figure 2-B).

## Effect of PTH on NPT-2b Expression

After injection PTH of 40 µg / 100 g body weight, there was no significant change in the NPT-2b protein and mRNA expression levels in duodenum, jejunum and ileum epithelial cells. After the administration of 200 µg / 100 g body weight PTH, NPT-2b protein expression in the duodenum was unchanged, but that in the jejunum and ileum was lower than that in the control group; *P* values were < .05 and < .01, respectively (Figure 3 and Figure 4-A). Although the expression of NPT-2b protein in the jejunum and ileum mucosal epithelial cells was significantly

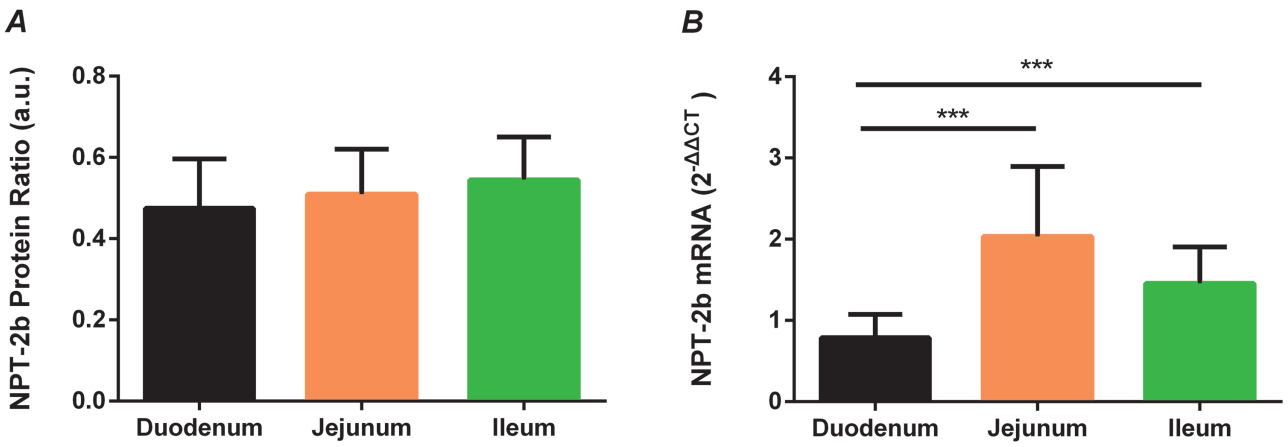


**Figure 1.** NPT-2b protein and mRNA expressed in different small intestine segments in mouse. (A) NPT-2b protein was found in mouse duodenum, jejunum, and ileum mucosa. There was no significant difference in protein abundance among the three segments of the small intestine. (B) NPT-2b mRNA was most expressed in the jejunum and least expressed in the duodenum (***$P < .001$, Unpaired two-tailed Student t test).

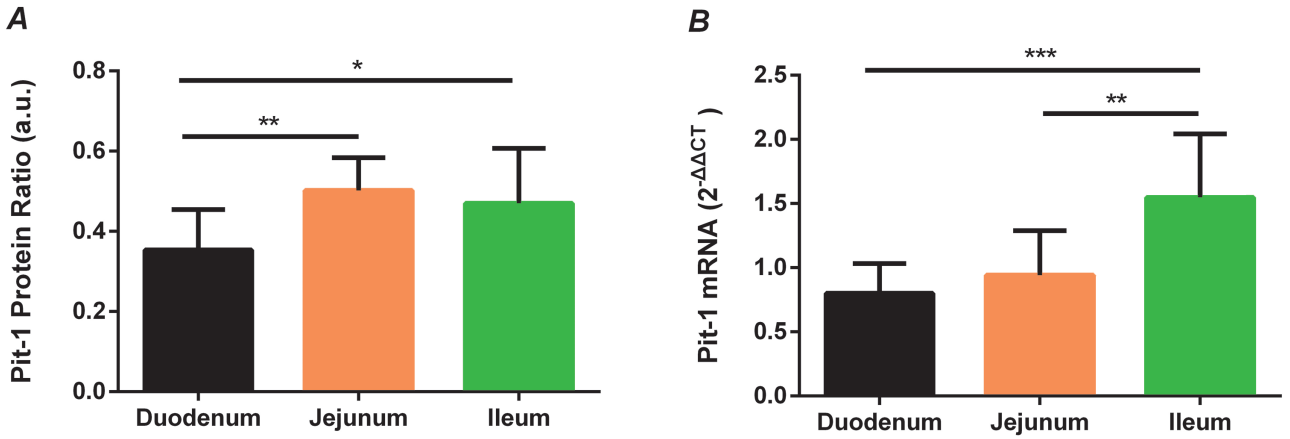


**Figure 2.** Pit-1 protein and mRNA expressed in mouse duodenum, jejunum, and ileum. (A) Pit-1 protein expressed in the three segments of the small intestine in the mouse. The abundance of the protein is the highest in the jejunum, followed by the ileum. (B) Pit-1 mRNA expression in ileum mucosa was significantly higher than that in the duodenum and ileum (*$P$ < 0.05, **$P$ < 0.01, ***$P$ < .001; Unpaired two-tailed Student's t test).

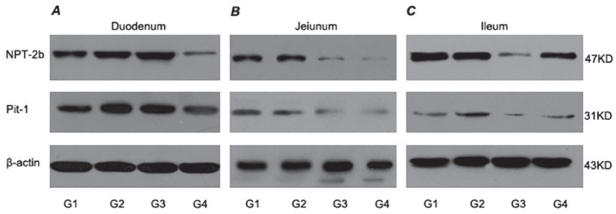


**Figure 3.** Western blot analysis of NPT-2b and Pit-1 protein in distinct regions of the mouse small intestine 8 hours after intravenous injection of different doses of PTH(1-34). (A) Detection of NPT-2b and Pit-1 protein in mouse duodenum. (B) Analysis of NPT-2b and Pit-1 protein in mouse jejunum. (C) Detection of NPT-2b and Pit-1 protein in mouse ileum. G1: group 1 is the control group. G2: group 2 received 40 µg / 100 g body weight PTH. G3: group 3 received 200 µg / 100 g body weight PTH intravenous injection. G4: group 4 received 400 µg / 100 g body weight PTH intravenous injection.

decreased after intravenous injection of 200 µg / 100 g body weight PTH, there was no significant difference in NPT-2b mRNA expression in all three small intestine segments by quantitative detection of RT-PCR (Figure 4-B). After the administration of high-dose PTH (400 µg / 100 g body weight), NPT-2b protein expression in the duodenal, jejunum, and ileum mucosal cells was significantly decreased, with $P$ values of < .01, < .05, and < .01; respectively. In line with the protein expression measured by western blot, after intravenous injection PTH of 400 µg / 100 g body weight, the NPT-2b mRNA in the three intestinal segments showed a significant decrease compared with that of the control group, with $P$ values of < .001, < .05, and < .001; respectively (Figure 4-B).

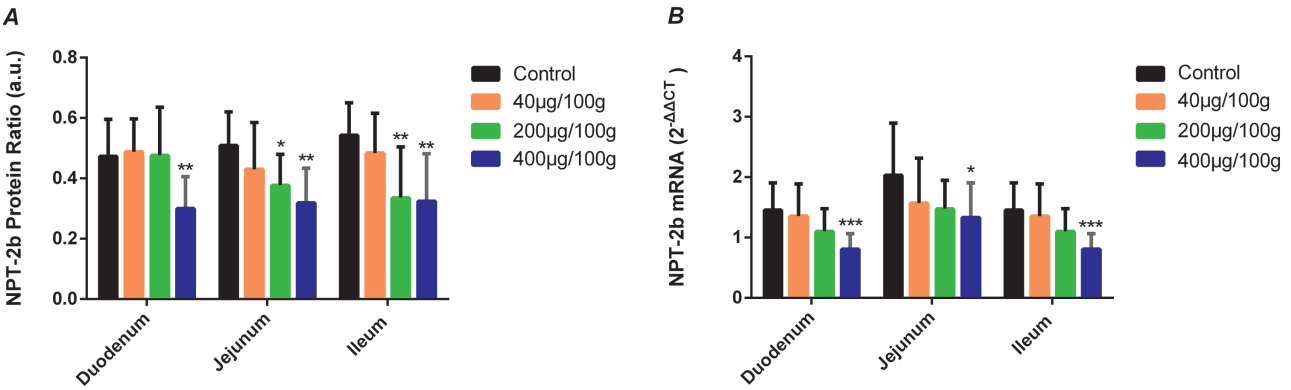


**Figure 4.** Quantification of NPT-2b protein expression relative to β-actin and quantification RT-PCR of NPT-2b mRNA using the $2^{-\Delta\Delta CT}$ method in distinct regions of the mouse small intestine 8 hours after intravenous injection of different doses of PTH(1-34). (A) In the three segments of the mouse small intestine, NPT-2b protein abundance was significantly decreased by intravenous injections of 400 µg / 100 g body weight PTH, and also decreased in the jejunum and ileum after 200 µg / 100 g body weight PTH injection. (B) NPT-2b mRNA was downregulated in all three regions of mouse intestine only after 400 µg / 100 g body weight PTH injection compared with the control group (*$P$ < .05, **$P$ < .01, ***$P$ < .001; Unpaired two-tailed Student's t test).

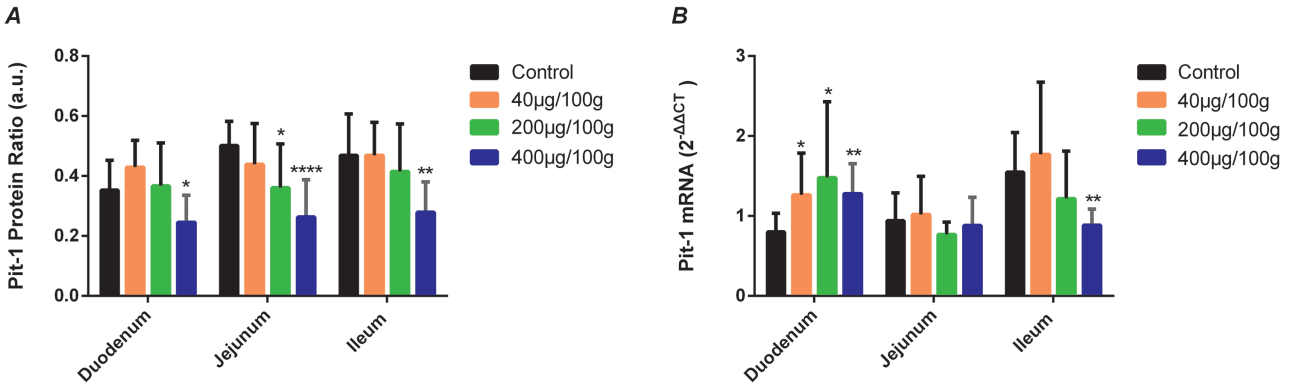


**Figure 5.** Quantification of Pit-1 protein expression relative to β-actin and quantification RT-PCR of Pit-1 mRNA using the $2^{-\Delta\Delta CT}$ method in distinct regions of the mouse small intestine 8 hours after intravenous injection of different doses of PTH(1-34). (A) In the three segments of the mouse small intestine, Pit-1 protein abundance was significantly decreased by intravenously injected 400μg/100g body weight PTH, and also decreased in the jejunum only after 200 μg / 100 g body weight PTH injection. (B) Pit-1 mRNA was up regulated in the mouse duodenum and down regulated in the ileum after 400 μg / 100 g body weight PTH injection compared with the control group (**P* < .05, ***P* < .01, *****P* < .0001; Unpaired two-tailed Student's t test).

### Effect of PTH on Pit-1 Expression

High-dose PTH (400 μg / 100 g body weight) significantly decreased the abundance of Pit-1 protein in all regions of the mouse small intestine, similar to NPT-2b (Figure 3 and Figure 5-A). 40 μg / 100 g body weight PTH could not affect the expression of Pit-1 mRNA; 200 μg / 100 g body weight PTH decreased the expression of Pit-1 mRNA in the jejunum. The expression of Pit-1 mRNA was decreased only in ileum mucosal cells after injection of 400 μg / 100 g body weight PTH. No significant changes were observed in the jejunum, but the expression of Pit-1 mRNA was up regulated in the duodenum (Figure 5-B).

## DISCUSSION

Along the small intestine dietary phosphate is absorbed, on entering the circulation, phosphate in plasma is constantly exchanged with bones and soft tissues. Phosphate in plasma is filtered in the glomerulus freely and then reabsorbed along the renal tubule. Previous studies on the absorption of phosphate in the intestinal tract have focused on sodium-dependent transfer of phosphate across the intestinal mucosal cell brush border membrane (BBM). The mechanism of transcellular phosphorus transport is poorly understood.

Sodium-dependent phosphate cotransporters from the *SLC34* and *SLC20* families of solute carriers mediate the uptake of phosphate in the intestinal tract.[4-6] NPT-2b/*SLC34A2* and Pit-1/*SLC20A1* are expressed in the intestinal epithelial cells and promoting intestinal absorption of phosphate. NPT-2b as the first molecule involved in the active transport of phosphate in the intestinal tract was identified in 1998, the sodium-dependent phosphate cotransporter encoded by the *SLC34A2* gene.[7] The gene in humans, mice, and rats contains 13 exons, with the initiator ATG in the first (rats) or second (humans and mice) exons and the terminator codon in exon 13. NPT-2b mediates a sodium-dependent transport of phosphate with a substrate stoichiometry of 3 $Na^{+:}$ 1 $HP_4^{2}$.[8] Previous studies indicate that under normal dietary conditions, NPT-2b play a moderate role in overall intestinal phosphate absorption, but a major role of in the active intestinal transport of phosphate. Sabbagh *et al.* showed that approximately half of the total intestinal phosphate absorption may mediated by this transporter when maximally expressed.[9] As we know, administration of low levels of phosphate diets results in the increased expression of NPT-2b (and increased intestinal phosphate transport), but we do not know the requirement for transcriptional regulation clearly.[10-5] On the other hand, $1,25(OH)_2$ vitamin $D_3$ can increase protein abundance of NPT-2b in the intestinal tract and increase intestinal transport of phosphate by a mechanism that does not always clearly correlates with transcriptional activation[(11,16-22)]. These effects on the expression of intestinal NPT-2b protein are all possible mechanisms by which the body regulates phosphorous homeostasis, but the effect of another important substance affecting phosphorous homeostasis, PTH, on intestinal NPT-2b has not been reported.

In the present study, NPT-2b protein and mRNA expression levels are different in different segments of the small intestine in mice. Similar to previous studies, [12,21] we find that NPT-2b protein and mRNA expression levels are highest in the ileum of mice. Our data demonstrate that NPT-2b protein and mRNA are also found in the duodenal mucosa of mice, which is inconsistent with previous research results. Data collected from several animal models show that the amount of phosphate entering the intestinal BBM via the sodium-dependent transporter decreases with age.[23-5] Mice aged 10 to 12 weeks were used in previous studies, whereas in our study we used 8-week-old mice; this difference may explain why NPT-2b is highly expressed in both duodenum and jejunum in the present data. In previous studies, PTH(1-34) injected intravenously (10 μg / 100 g body weight) was often used to simulate the effect of normal PTH concentration on target organs in mice or rats. [26,27] To simulate PTH levels in patients with severe SHPT that exceed the normal upper limit by 30 to 40 times, we used PTH(1-34) doses of 40 μg / 100 g, 200 μg / 100 g, and 400 μg / 100 g body weight as stimulus doses. Our data show that the conventional doses of PTH do not affect NPT-2b protein expression on the three segments of intestinal mucosa, but medium-dose PTH stimulation can decrease NPT-2b protein abundance in the jejunum and ileum mucosa. High-dose PTH stimulation can decrease the NPT-2b protein expression in all three segments of intestinal mucosa. At the same time RT-PCR test results show that high-dose PTH can downregulate NPT-2b mRNA expression in all the intestinal mucosa. These findings suggest that for severe hyperparathyroidism, excessive PTH may down regulate the expression of NPT-2b in the intestinal mucosa of mice through a transcriptional regulation mechanism. This effect may reduce the total amount of phosphorus absorbed from the intestines of the patients with SHPT, making them less effective at using intestinal phosphorus binders than patients without SHPT.

Pit-1 encoded by the *SLC20A1* genes was first identified as retroviral receptors for the gibbon ape leukemia virus (Glvr-1),[28] and its role as a high-affinity sodium-dependent phosphate cotransporter was soon reported.[29] Pit-1 transports stoichiometry of 2 $Na^+$ per monovalent phosphate.[30] Because of Pit-1 mRNA expressed ubiquitously,[31] it was initially thought that the transporter had a housekeeping role, mediating the constitutive import of phosphate from the interstitial fluid into cells. The distribution of Pit-1 in the intestinal mucosa plays a role in the absorption of intestinal phosphorus, similar to NPT-2b.

Some recent research demonstrates that Pit-1 is expressed in BBM purified from rat duodenum and jejunum, whereas no expression was observed in the ileum.[14,15] Some unpublished data show Pit-1 mRNA is expressed in mice duodenum, jejunum, and ileum[3]. Our data suggested that Pit-1 protein and mRNA are expressed in the duodenum, jejunum, and ileum of mice, among which the abundance of Pit-1 protein and mRNA in the ileum was the highest and in the duodenum was the lowest.

For the stimulation of different doses of PTH, the changes in Pit-1 protein abundance in the intestinal mucosa were similar to those of NPT-2b. There was no change in the Pit-1 protein abundance of the intestinal mucosa for low-dose PTH. Pit-1 protein expression in the jejunum and ileum was down regulated by intravenous injection of high-dose PTH. Interestingly, with high doses of PTH stimulation, only the Pit-1 mRNA expression in ileum mucosa decreased; the mRNA expression in the jejunum did not change, but the Pit-1 mRNA expression in the duodenum increased. These data suggest that high concentrations of PTH also decrease the expression of Pit-1 protein in the small intestine, but may not be regulated by the transcriptional mechanism.

## CONCLUSION

In summary, the data of the present study show that high concentrations of PTH can down regulate the expression of NPT-2b and Pit-1 proteins in the small intestine mucosa of mice. The change in NPT-2b protein expression is mediated by the transcriptional mechanism, whereas the change in Pit-1 protein abundance is not achieved by the transcriptional mechanism. These findings suggest that in patients with severe SHPT, proteins that mediate phosphorous absorption are decreased in the intestine, which may impair the efficacy of oral phosphorous binding agents in these patients.

## ACKNOWLEDGMENTS

The Beijing Municipal Administration of

Hospitals Clinical Medicine Development of Special Funding Support supports this work (code: ZYLX201824).

## DISCLOSURE

All the authors have declared no competing interest.

## REFERENCES


1. Hruska KA, Mathew S, Lund R, Qiu P, Pratt R. Hyperphosphatemia of chronic kidney disease. Kidney Int. 2008;74(2):148-57.
2. Ho LY, Wong PN, Sin HK, et al. Risk factors and clinical course of hungry bone syndrome after total parathyroidectomy in dialysis patients with secondary hyperparathyroidism. BMC Nephrology. 2017;18(1):12-22.
3. Hernando N, Wagner CA. Mechanisms and Regulation of Intestinal Phosphate Absorption. Compr Physiol. 2018;8(3):1065-90.
4. Biber J, Hernando N, Forster I. Phosphate transporters and their function. Annu Rev Physiol. 2013;75:535-50.
5. Forster IC, Hernando N, Biber J, Murer H. Phosphate transporters of the SLC20 and SLC34 families. Mol Aspects Med. 2013;34(2-3):386-95.
6. Wagner CA, Hernando N, Forster IC, Biber J. The SLC34 family of sodium-dependent phosphate transporters. Pflugers Arch. 2014;466(1):139-53.
7. Hilfiker H, Hattenhauer O, Traebert M, Forster IC, Murer H, Biber J. Characterization of a murine type II sodium-phosphate cotransporter expressed in mammalian small intestine. Proc Natl Acad Sci U S A. 1998;95(24):14564-69.
8. Forster IC, Loo DD,Eskandari S. Stoichiometry and Na+ binding cooperativity of rat and flounder renal type II Na+-Pi cotransporters. Am J Physiol. 1999;276(4):F644-9.
9. Sabbagh Y, O'Brien SP, Song WP, et al. Intestinal npt2b plays a major role in phosphate absorption and homeostasis. J Am Soc Nephrol. 2009;20(11):2348-58.
10. Capuano P, Radanovic T, Wagner CA, et al. Intestinal and renal adaptation to a low-Pi diet of type II NaPi cotransporters in vitamin D receptor- and 1αOHase-deficient mice. American Journal of Physiology-Cell Physiology. 2005;288(2):C429-34.
11. Hattenhauer O, Traebert M, Murer H, Biber J. Regulation of small intestinal Na-P(i) type IIb cotransporter by dietary phosphate intake. Am J Physiol. 1999;277(4):G756-62.
12. Radanovic T, Wagner CA, Murer H, Biber J. Regulation of Intestinal Phosphate Transport I. Segmental expression and adaptation to low-Pi diet of the type IIb Na+-Pi cotransporter in mouse small intestine. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2005; 288(3):G496-500.
13. Segawa H, Kaneko I, Yamanaka S, et al. Intestinal Na-Pi cotransporter adaptation to dietary Pi content in vitamin D receptor null mice. American Journal of Physiology-Renal Physiology. 2004;287(1):F39-47.
14. Candeal E, Caldas YA, Guillén N, Levi M, Sorribas V. Intestinal phosphate absorption is mediated by multiple transport systems in rats. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2017;312(4):G355-66.
15. Giral H, Caldas Y, Sutherland E, et al. Regulation of rat intestinal Na-dependent phosphate transporters by dietary phosphate. American Journal of Physiology-Renal Physiology. 2009;297(5):F1466-75.
16. Danisi G, Bonjour JP, Straub RW. Regulation of Na-dependent phosphate influx across the mucosal border of duodenum by 1,25-dihydroxycholecalciferol. Pflugers Arch. 1980;388(3):227-32.
17. Harrison HE, Harrison HC. Intestinal transport of phosphate: action of vitamin D, calcium, and potassium. Am J Physiol. 1961;201:1007-12.
18. Hildmann B, Storelli C, Danisi G, Murer H. Regulation of Na+-Pi cotransport by 1,25-dihydroxyvitamin D3 in rabbit duodenal brush-border membrane. Am J Physiol. 1982;242(5): G533-9.
19. Taylor AN. In vitro phosphate transport in chick ileum: effect of cholecalciferol, calcium, sodium and metabolic inhibitors. J Nutr. 1974;104(4):489-94.
20. Walling MW. Intestinal Ca and phosphate transport: differential responses to vitamin D3 metabolites. Am J Physiol. 1977;233(6):E488-94.
21. Marks J, Srai SK, Biber J, Murer H, Unwin RJ, Debnam SE. Intestinal phosphate absorption and the effect of vitamin D: a comparison of rats with mice. Exp Physiol. 2006;91(3):531-7.
22. Katai K, Miyamoto KI, Kishida S, et al. Regulation of intestinal Na+-dependent phosphate co-transporters by a low-phosphate diet and 1,25-dihydroxyvitamin D3. Biochem J. 1999;343:705-12.
23. Arima K, Hines ER, Kiela PR, Drees JB, Collins JF, Ghishan FK. Glucocorticoid regulation and glycosylation of mouse intestinal type IIb Na-P(i) cotransporter during ontogeny. Am J Physiol Gastrointest Liver Physiol. 2002;283(2):G426-34.
24. Borowitz SM, Granrud GS. Ontogeny of intestinal phosphate absorption in rabbits. Am J Physiol. 1992; 262(5 Pt 1):G847-53.
25. Xu H, Bai LQ, Collins JF, Ghishan FK. Age-dependent regulation of rat intestinal type IIb sodium-phosphate cotransporter by 1,25-(OH)(2) vitamin D(3). Am J Physiol Cell Physiol. 2002;282(3): C487-93.
26. Bacic D, LeHir M, Biber J, Kaissling B, Murer H, Wagner CA. The renal Na+/phosphate cotransporter NaPi-IIa is internalized via the receptor-mediated endocytic route in response to parathyroid hormone. Kidney Int, 2006;69(3):495-503.
27. Li X, Liu H, Qin L, Tamasi J, et al. Determination of Dual Effects of Parathyroid Hormone on Skeletal Gene Expression in Vivo by Microarray and Network Analysis. Journal of Biological Chemistry, 2007;282(45):33086-97.
28. Miller DG, Edwards RH, Miller AD. Cloning of the cellular receptor for amphotropic murine retroviruses reveals homology to that for gibbon ape leukemia virus. Proc Natl Acad Sci U S A. 1994;91(1):78-82.
29. Kavanaugh MP, Miller DG, Zhang WB, et al. Cell-surface receptors for gibbon ape leukemia virus and amphotropic

murine retrovirus are inducible sodium-dependent phosphate symporters. Proc Natl Acad Sci U S A. 1994;91(15):7071-5.

30. Ravera S, Virkki LV, Murer H, Forster IC. Deciphering PiT transport kinetics and substrate specificity using electrophysiology and flux measurements. Am J Physiol Cell Physiol. 2007;293(2):C606-20.
31. Nishimura M, Naito S. Tissue-specific mRNA expression profiles of human solute carrier transporter superfamilies. Drug Metab Pharmacokinet. 2008;23(1):22-44.

Correspondence to:
Wen-hu Liu, MD
doctorate, Department of Nephrology, Capital Medical University Affiliated Beijing Friendship Hospital, Beijing, China
Tel: 0086 010 6313 8579
Fax: 0086 010 6313 9744
E-mail: wenhuliu@mail.ccmu.edu.cn

Received August 2020
Revised September 2020
Accepted November 2020