Short-term dietary phosphate restriction up-regulates ileal fibroblast growth factor 15 gene expression in mice

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Members of the fibroblast growth factor (FGF) 19 subfamily, including FGF23, FGF15/19, and FGF21, have a role as endocrine factors which influence the metabolism of inorganic phosphate (Pi) and vitamin D, bile acid, and energy. It has been reported that dietary Pi regulates circulating FGF23. In this study, the short-term effects of dietary Pi restriction on the expression of FGF19 subfamily members in mice were analyzed. An initial analysis confirmed plasma FGF23 levels positively correlated with the amount of dietary Pi. On the other hand, ileal Fgf15 gene expression, but not hepatic Fgf21 gene expression, was up-regulated by dietary Pi restriction. In addition, we observed the increase of plasma 1,25-dihydroxyvitamin D [1,25(OH)2D] levels by dietary Pi restriction, and the up-regulation of ileal Fgf15 mRNA expression by 1,25(OH)2D and vitamin D receptor (VDR). Importantly, dietary Pi restriction-induced Fgf15 gene expression was prevented in VDR-knockout mice. Furthermore, diurnal variations of plasma triglyceride concentrations and hepatic mRNA expression of the bile acid synthesis enzyme Cyp7a1 as one of Fgf15 negative target genes was influenced by dietary Pi restriction. These results suggest that dietary Pi restriction up-regulates ileal Fgf15 gene expression through 1,25(OH)2D and VDR, and may affect hepatic bile acid homeostasis.

Key Words: fibroblast growth factor 15, gene regulation analysis, inorganic phosphate, 1,25-dihydroxyvitamin D, mice

Inorganic phosphorus (Pi) plays a critical role in skeletal development, mineral metabolism, and diverse cellular functions involving intermediary metabolism and energy-transfer mechanisms. Serum Pi concentration is maintained through a complex interplay between intestinal absorption, exchange with intracellular and bone storage pools, and renal tubular reabsorption.1,2 Pi transport in the kidney and intestine is mediated by several sodium-dependent phosphate cotransporters (NaPi).3–5 Pi metabolism is regulated by many factors, such as parathyroid hormone (PTH), 1,25-dihydroxyvitamin D3 [1,25(OH)2D3], fibroblast growth factor (FGF) 23, insulin, thyroid hormone, and other factors.2 FGF23 was identified as a gene responsible for tumor-induced osteomalacia (TIO) and autosomal dominant hypophosphatemic rickets (ADHR).6,7 It has been shown that FGF23 suppresses the expression of type 2a and 2c sodium-phosphate cotransporters (NaPi-2a and NaPi-2c) in the brush border membrane (BBM) of proximal tubules which mediates physiological phosphate reabsorption. In addition, FGF23 reduces serum 1,25(OH)2D concentration by suppressing the expression of 25-

hydroxyvitamin D [25(OH)D]-1α-hydroxylase (CYP27b1) and also enhancing the expression of 25(OH)D-24-hydroxylase (CYP24a1).8–11 Circulating FGF23 is regulated by dietary Pi and 1,25(OH)2D.9,12 In fact, circulating FGF23 is decreased in vitamin D receptor (VDR) knockout (KO) mice.13–15 Dietary Pi deficiency stimulates renal 1,25(OH)2D synthesis and leads to an increase in Pi absorption in the small intestine. Intestinal absorption of Pi is mediated primarily via the type 2b sodium-phosphate cotransporter (NaPi-2b).16–18 Segawa et al. demonstrated an elevation of intestinal sodium-dependent Pi transport activity and BBM NaPi-2b protein content in mice fed a low-Pi diet.

Phylogenetic and sequence analyses have been used to group FGF15 (the mouse ortholog of human FGF19), FGF19, FGF21, and FGF23 from the other FGF family members, forming the FGF19 subfamily. FGF15 produced by distal intestine inhibits the expression of cholesterol 7α-hydroxylase Cyp7a1 in the liver where it functions as the key rate-limiting enzyme for the biosynthesis of bile acid through the Fgf receptor 4 (Fgfr4)/β Klotho complex.19,20 Additionally, Fgf15 gene expression is positively regulated by bile acids which bind to the farnesoid X receptor (FXR), thus indicating that regulation of Fgf15 gene expression is important in the maintenance of bile acid homeostasis.21–23 Furthermore, Fgf21 gene expression is regulated by free fatty acids (FFA) through peroxisome proliferator-activated receptor α (PPARα) and is associated with energy homeostasis.24–27 Previously, we have revealed the effect of Pi intake on circulating FGF23, PTH, and vascular endothelial function in humans and animals.28,29 In this study, we have focused on the effect of dietary Pi on the ileal Fgf15 and hepatic Fgf21 gene expression known as FGF19 subfamily members.

Materials and Methods

Animals. Eight week old C57BL/6J male mice (24–27 g) were purchased from Japan SLC (Shizuoka, Japan). Mice were maintained on 12 h light-12 h dark cycles (lights on from 8:00 to 20:00) with free access to distilled water and food. Zeitgeber time (ZT), the standardized notation for the time during an entrained circadian cycle, was used in this study. ZT0 is coincides with the onset of light, while ZT12 coincides with the onset of darkness. An egg white-based AIN-93 experimental diet formula-
tion, without casein, was fed to the mice. From this base diet, five diets containing 0.6% calcium plus 0.02%, 0.1%, 0.2%, 0.6%, or 1.2% Pi were prepared (Table 1). Groups of mice received one of the five diets for 1 to 5-day. Mice received 1.25(OH)\textsubscript{2}D\textsuperscript{3} (Solvay Pharmaceuticals, Marietta, GA) (0.5 μg/kg body weight) i.p. and were sacrificed 6-h after treatment and subsequently compared with saline-treated controls. The 7 to 8 week old male VDR KO mice used in the study were generated by heterozygous crosses; VDR genotypes were determined by analyzing the DNA obtained from each mouse.\textsuperscript{(21)} Study mice were mainly sacrificed between ZT5 and ZT7. The mice were anesthetized using diethyl ether and killed by exsanguinations. Protocols were approved by the Guidelines for Animal Experimentation of the Tokushima University School of Medicine.

**Plasma parameters.** Plasma concentrations of Pi, total cholesterol (TC), and total triglycerides (TG) were determined using the Phospho C-test Wako, T-cholesterol E-test Wako and Triglyceride E-test Wako kits, respectively (Wako Pure Chemical Tokyo, Japan). Plasma 1,25(OH)\textsubscript{2}D was measured with a RIA kit (Shibayagi, March 2014). Plasma Pi is significantly decreased in mice fed a 0.02% Pi diet, and the concentration of plasma Pi is significantly decreased in the mice fed 0.6% Pi compared with those fed 1.2% Pi diet (Fig. 1C). Conversely, hepatic FGF23 mRNA was decreased in the mice fed 0.6% Pi compared with those fed 1.2% Pi. However, the change in Fgf21 mRNA expression was independent of the amount of dietary Pi (Fig. 1D).

**Statistical analysis.** Data are expressed as means ± SEM. The Student’s unpaired t test and 1-way ANOVA was performed. Differences between experimental group means were analyzed using either the Tukey-Kramer or Fisher’s protected least significant difference (PLSD) post hoc tests. p<0.05 was considered significant.

**Results**

**Effects of dietary Pi on the expressions of FGF19 subfamily.** Firstly, to examine the effect of dietary Pi on Fgf15 and Fgf21 gene expression, mice were fed diets with different Pi contents. As reported previously,\textsuperscript{(12)} plasma Pi is significantly decreased in mice fed a 0.02% Pi diet, and the concentration of plasma FGF23 was decreased following restriction of dietary Pi (Fig. 1A and B). Quantitative RT-PCR analysis showed that Fgf15 mRNA expression in the ileum was significantly increased in the mice fed the 0.1% Pi diet compared with groups fed the 0.6% or 1.2% Pi diet (Fig. 1C). Conversely, hepatic Fgf21 mRNA was decreased in the mice fed 0.6% Pi compared with those fed 1.2% Pi. However, the change in Fgf21 mRNA expression was independent of the amount of dietary Pi (Fig. 1D). Secondly, the

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**Table 1. Composition of experimental diets**

| Ingredient (g) | 0.02% | 0.10% | 0.20% | 0.60% | 1.20% |
|---------------|-------|-------|-------|-------|-------|
| Egg-white     | 20.0  | 20.0  | 20.0  | 20.0  | 20.0  |
| L-Cystein     | 0.3   | 0.3   | 0.3   | 0.3   | 0.3   |
| Cornstarch    | 39.7  | 39.7  | 39.7  | 39.7  | 39.7  |
| α-Cornstarch  | 13.2  | 13.2  | 13.2  | 13.2  | 13.2  |
| Sugar         | 10.44 | 10.00 | 9.56  | 7.80  | 5.16  |
| Soybean Oil   | 7.0   | 7.0   | 7.0   | 7.0   | 7.0   |
| Cellulose     | 5.0   | 5.0   | 5.0   | 5.0   | 5.0   |
| Vitamin mix   | 1.0   | 1.0   | 1.0   | 1.0   | 1.0   |
| Choline bitartrate | 0.25 | 0.25  | 0.25  | 0.25  | 0.25  |
| Tert-butylhydroquinone | 0.0014 | 0.0014 | 0.0014 | 0.0014 | 0.0014 |
| CaCO\textsubscript{3} | 1.4894 | 1.4894 | 1.4894 | 1.4894 | 1.4894 |
| KClPO\textsubscript{4} | 0    | 0.4394 | 0.8789 | 2.6366 | 5.2731 |
| Mineral mix changed | 1.5645 | 1.5645 | 1.5645 | 1.5645 | 1.5645 |

**Table 2. Oligonucleotides used for real-time PCR**

| Gene name          | Forward Sequence (5' to 3') | Reverse Sequence (5' to 3') | Gene Accession No. |
|--------------------|-----------------------------|-----------------------------|-------------------|
| Mouse Fgf15        | CCAGAGAACACGTCCAAGGAC       | TCCATGCTGTCATCTCCAAG        | NM008003          |
| Mouse Fgf21        | CTACCAAGCATACCCCATC        | GCCTACCACTGTTCATCC          | NM020013          |
| Mouse Cyp7a1       | GAGCCCTGTAAGCAATGAAAGA      | GCTGTCCGATATTCAGAAGGA       | NM007824          |
| Mouse β-actin      | AGCAAGATCGTCTGGTGATAC       | GGCCGATGTGCTGCTGATAC        | NM007397          |
| Mouse Cyclophilin  | GGAGATGCGACAGGAGGAA         | GCCCCGTAGCTGCTCCAG          | NM011149          |

**Western blot analysis.** Fresh ileal mucosa was homogenized in Lysis buffer (10 mM Hepes-KOH, pH 7.9, 1.5 mM MgCl\textsubscript{2}, 10 mM KCl) with Protease Inhibitor Cocktail (SIGMA-ALDRICH, St. Louis, Missouri) and 1 mM DDT. This suspension was centrifuged for 20 min at 10,000 x g and the cytoplasmic fraction protein was collected from the supernatant fraction. Protein samples were heated at 95°C for 5 min in sample buffer in the presence of 5% 2-mercaptoethanol and subjected to SDS-PAGE. The separated proteins were transferred by electrophoresis on to polyvinylidene difluoride membrane (Immobilon-P, Millipore, Billerica, MA). The membranes were treated with diluted anti-FGF15 antibody (1:200) (Santa Cruz Biotechnology, CA). Mouse anti-β-actin monoclonal antibody (SIGMA-ALDRICH) was used as an internal control. HRP-labeled anti-IgG (BIO-RAD Hercules, CA) was utilized as a secondary antibody, and signals were detected using the ECL Prime system (GE Healthcare, Buckinghamshire, UK).

**Statistical analysis.** Data are expressed as means ± SEM. The Student’s unpaired t test and 1-way ANOVA was performed. Differences between experimental group means were analyzed using either the Tukey-Kramer or Fisher’s protected least significant difference (PLSD) post hoc tests. p<0.05 was considered significant.
The effect of dietary Pi restriction on ileal Fgf15 mRNA expression in VDR KO mice. To understand the role of VDR in the regulation of Fgf15 gene expression by dietary Pi, WT and VDR KO mice were fed the Pi-restricted (0.02% Pi) or Pi-sufficient diet (1.2% Pi) for 5-day. As a result, dietary Pi flux significantly changed the plasma Pi concentrations in both WT and VDR KO mice. A previous report showed that plasma Pi concentrations were lower in VDR KO mice.\(^{16}\) The plasma Pi concentrations of VDR KO mice were observed to be lower than those of WT mice fed the Pi-restricted diet; however, there was no difference between WT and VDR KO mice in the group fed the Pi-sufficient diet (Fig. 4A). In WT mice, Fgf15 mRNA expression was increased 20-fold in the Pi-restricted group relative to Pi-sufficient group, whereas there was no significance difference between both groups of VDR KO mice, despite the 3-fold increase in the Pi-restricted vs Pi-sufficient group (Fig. 4B).

The effect of dietary Pi restriction on ileal Fgf15 gene expression in VDR KO mice. To understand the role of VDR in the regulation of Fgf15 gene expression by dietary Pi, WT and VDR KO mice were fed the Pi-restricted (0.02% Pi) or Pi-sufficient diet (1.2% Pi) for 5-day. As a result, dietary Pi flux significantly changed the plasma Pi concentrations in both WT and VDR KO mice. A previous report showed that plasma Pi concentrations were lower in VDR KO mice.\(^{16}\) The plasma Pi concentrations of VDR KO mice were observed to be lower than those of WT mice fed the Pi-restricted diet; however, there was no difference between WT and VDR KO mice in the group fed the Pi-sufficient diet (Fig. 4A). In WT mice, Fgf15 mRNA expression was increased 20-fold in the Pi-restricted group relative to Pi-sufficient group, whereas there was no significance difference between both groups of VDR KO mice, despite the 3-fold increase in the Pi-restricted vs Pi-sufficient group (Fig. 4B).

The effect of dietary Pi restriction on diurnal variations of plasma lipid parameters, ileal Fgf15, and hepatic Cyp7a1 gene expression. It has been reported that the concentration of blood TG and gene expression of Cyp7a1, which is negatively regulated by Fgf15, exhibits diurnal variations.\(^{23–25}\) Therefore, our aim was to elucidate the effect of dietary Pi on the diurnal variations of plasma lipid parameters and Fgf15 and Cyp7a1 gene expression. Plasma Pi concentrations were significantly decreased in the Pi-restricted group compared with the Pi-sufficient group at all times (data not shown). Fig. 5A showed that the plasma TG concentrations exhibited diurnal variation and significantly increased 1.7-fold at ZT5 and increased 2.7-fold at ZT13 in the Pi-restricted group relative to the Pi-sufficient group. In contrast, there was no effect of dietary Pi on plasma cholesterol throughout
the day (Fig. 5B). Fgf15 mRNA expression in the ileum decreased between ZT9 and ZT13, and Fgf15 expression was higher in the Pi-restricted than in the Pi sufficient group throughout the day (Fig. 5C). Contrary to Fgf15, the expression pattern of the Cyp7a1 gene was increased between ZT9 and ZT13. Interestingly, a Pi-restricted diet increased Cyp7a1 mRNA levels at ZT9 and decreased them at ZT13 (Fig. 5D), showing that the effect of dietary Pi on Cyp7a1 expression varied according to time of day. These results suggest that dietary Pi regulates hepatic Cyp7a1 expression through Fgf15-dependent and -independent pathways, and may affect on the diurnal variations of plasma TG levels.

**Discussion**

This study examined the effect of dietary Pi on FGF19 subfamily gene expression in mice. While Pi restriction decreased plasma FGF23 concentrations, it increased Fgf15 mRNA expression in the ileum. Hepatic Fgf21 mRNA concentrations were increased in mice fed a diet containing 1.2% Pi relative to those fed the 0.6% Pi diet (Fig. 1). Elevated concentrations of FGF21 have been observed, not only during fasting, but also in obese individuals, people with type 2 diabetes, and in peritoneal dialysis patients. In addition, FGF21 was expressed not only in the liver but also in white adipose tissue (WAT) and the pancreas, suggesting that FGF21 expression might be associated with the state of nutritional metabolism. To assess the effect of dietary Pi on Fgf21 gene expression requires an examination of the blood concentration and the expression in WAT and the pancreas. We considered the possibility that dietary Pi restriction induced Fgf15 mRNA expression, and Fig. 2B shows that FGF15 protein concentrations are also increased by a Pi-restricted diet. It has been reported that the mechanism of Fgf23 gene expression includes not only transcriptional regulation, but also post-translational regulation via glycosylation and protease degradation, for example. However, the glycosylation and degradation of FGF15/19 and FGF21 are not yet fully elucidated. It is proposed that Fgf15 gene expression is positively regulated by dietary Pi restriction.

Previous reports show that a dietary Pi restriction is associated with decreased circulating FGF23 and elevated 1,25(OH)\(_2\)D\(_3\) concentrations. Through the VDR, 1,25(OH)\(_2\)D\(_3\) induced Fgf23 mRNA expression in bone and increased FGF23 concentrations in the blood, so we investigated whether 1,25(OH)\(_2\)D\(_3\) and VDR regulate Fgf15 gene expression. Fig. 3B shows that Fgf15 mRNA expression in the ileum is increased by the administration of

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**Fig. 2.** The effects of a Pi-restricted diet on the ileal Fgf15 mRNA and protein expression. Groups of 8-week-old C57BL/6 male mice were fed 0.02% Pi-restricted (0.02% Pi) or 1.2% Pi-sufficient (1.2% Pi) diets and then sacrificed at 1, 2, 3 and 5-day. (A) Fgf15 mRNA expression in the ileum. Total mRNA was prepared from the ileum and liver of each mouse, and gene expression was quantified by quantitative RT-PCR. Results were normalized to β-actin mRNA expression. The data represent the mean ± SEM (n = 4–6). *p < 0.05 vs 1.2% Pi-sufficient group. (B) FGF15 protein expression in the ileum. 0.02% Pi-restricted (0.02% Pi) or 1.2% Pi-sufficient (1.2% Pi) diets were fed to mice for 5-day. Cytoplasmic extracts were prepared from the lower ileum of each mouse. Protein (50 μg) was loaded per lane for Western blot analyses and probed with FGF15 antibody. β-actin was used as an internal control.

**Fig. 3.** The up-regulation of ileal Fgf15 mRNA expression by 1,25(OH)\(_2\)D\(_3\) and VDR. (A) Plasma 1,25(OH)\(_2\)D\(_3\) concentration. Groups of 8-week-old C57BL/6J male mice were fed 0.02% Pi-restricted (0.02% Pi) or 1.2% Pi-sufficient (1.2% Pi) diets and sacrificed at 5-day (n = 5). (B) Fgf15 expression in the ileum 6-h after treatment with 1,25(OH)\(_2\)D\(_3\). Groups of 8-week-old male C57BL/6J mice received saline i.p. (control) or 1,25(OH)\(_2\)D\(_3\) i.p. (0.5 μg/kg body weight) (n = 4–6). (C) Expression of Fgf15 in the ileum of WT and VDR KO male mice (n = 2). Total mRNA was prepared from the ileum of each mouse, and gene expression was measured by quantitative RT-PCR using β-actin as the internal control. The data represent the mean ± SEM. *p < 0.05 vs control.
Means without a common letter are significantly different.

Gene expression was measured by quantitative RT-PCR using Total mRNA was prepared from the ileum and liver of each mouse, and Pi-restricted (0.02% Pi) or 1.2% Pi-sufficient (1.2% Pi) diets for 5-day. The internal control. The data represent the mean ± SEM (n = 4-9). Means without a common letter are significantly different. p < 0.05.

1,25(OH)_2D_3 in addition, Fgf15 mRNA expression is decreased in VDR KO mice compared with WT mice. It is well known that 1,25(OH)_2D_3/VDR is regulated by transcriptional gene expression through the vitamin D response element (VDRE) in the promoter of target genes. Indeed, we observed that 1,25(OH)_2D_3 stimulates mouse Fgf15 gene promoter activity in several cells overexpressing VDR and RXR (data not shown). More importantly, the results of this study using VDR KO mice revealed that a Pi-restricted diet regulates Fgf15 gene expression through the VDR (Fig. 4B). In the past, it has been reported that some genes associated with bile acid metabolism are regulated by 1,25(OH)_2D_3 and VDR in vitro and in vivo. VDR has dual functions, as an endocrine receptor for 1,25(OH)_2D_3 and as a metabolic sensor for secondary bile acids such as lithocholic acid. It is hypothesized that 1,25(OH)_2D_3 suppresses bile acid synthesis thereby averting competition with bile acid. Both vitamin D and bile acid are the metabolic products of cholesterol, and interestingly, CYP27a1 that identified as vitamin D 25-hydroxylase has a role as a bile acid synthesis enzyme. There is a strong connection between vitamin D and bile acid metabolism, and it is suggested that dietary Pi regulates the vitamin D and bile acid metabolism through the FGF23 and FGF15.

FGF15 was shown to bind and activate the Fgfr4/β-klotho complex, leading to the down regulation of Cyp7a1 expression, and inhibiting synthesis of bile acid from cholesterol. Bile acids facilitate intestinal absorption and transport of lipids, and it has been reported that Fgfr4 KO and Fxr KO mice exhibit elevated Cyp7a1 gene expression in their liver and high concentrations of blood TG and cholesterol. A recent study has reported that hepatic Cyp7a1 gene expression and blood lipids parameters exhibit diurnal variations. These diurnal variations are caused by the regulation of some clock genes. As shown in Fig. 5, we observed that plasma TG concentration show diurnal variation, and this variation is affected by dietary Pi. Although dietary Pi restriction increased Fgf15 gene expression throughout the day, it also increased Cyp7a1 expression at ZT9 and decreased it at ZT13. This result suggested that Cyp7a1 is regulated by dietary Pi through an Fgf15-dependent and -independent pathway. Our recent study demonstrated that 12 days of restriction of dietary Pi increased high cholesterol diet-induced hepatic lipid accumulation and decreased hepatic Cyp7a1 mRNA expression. Therefore, we suggest that elevation of Fgf15 expression induced by dietary Pi restriction may inhibit hepatic Cyp7a1 gene expression and accelerate the development of high cholesterol diet-induced fatty liver.

In the chronic kidney disease (CKD) patient, hyperphosphatemia and dyslipidemia might be risk factors for the development of CKD and the pathogenesis of cardiovascular disease. A Pi-restricted diet may be a useful treatment for a CKD patient to ameliorate their hyperphosphatemia and to reduce their risk of CKD. This study strongly suggests that dietary Pi is involved in lipid metabolism, and proper control of dietary Pi in CKD might contribute to amelioration of lipid metabolism abnormalities. Future investigations of the effect of a Pi-restricted diet on bile acid and lipid metabolism in the CKD patient are warranted.

In summary, we revealed that dietary Pi restriction increased ileal Fgf15 gene expression through 1,25(OH)_2D_3 and VDR in mice. Furthermore, it was shown that dietary Pi affects diurnal variations in plasma TG concentrations and hepatic Cyp7a1 gene expression.

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Conflict of Interest

No potential conflicts of interest were disclosed.
Fig. 5. The effects of a Pi-restricted diet on diurnal variations of plasma lipid concentrations and ileal Fgf15 and hepatic Cyp7a1 mRNA expression. Groups of 8-week-old C57BL/6J male mice were fed 0.02% Pi-restricted (0.02% Pi) or 1.2% Pi-sufficient (1.2% Pi) diets for 5-day and sacrificed at ZT 1, 5, 9, 13, 17 and 21. (A) Plasma triglyceride concentrations. (B) Plasma cholesterol concentrations. (C) Fgf15 mRNA expression in the ileum. (D) Cyp7a1 mRNA expression in liver. Total mRNA was prepared from the ileum and liver of each mouse, and gene expression was measured by quantitative RT-PCR using β-actin or cyclophilin as the internal control. The data represent the mean ± SEM (n = 4–5). *p<0.05 vs 1.2% Pi-sufficient group.

References

1 Shaikh A, Berndt T, Kumar R. Regulation of phosphate homeostasis by the phosphatonin and other novel mediators. Pediatr Nephrol 2008; 23: 1203–1210.
2 Murer H, Forster I, Biber J. The sodium phosphate cotransporter family SLC34. Pflugers Arch 2004; 447: 763–767.
3 Murer H, Hernando N, Forster I, Biber J. Proximal tubular phosphate reabsorption: molecular mechanisms. Physiol Rev 2000; 80: 1373–1409.
4 Miyamoto K, Ito M, Takahashi M, Shelton CD. New aspect of renal phosphate reabsorption: the type IIc sodium-dependent phosphate transporter. Am J Physiol 2007; 293: F503–F515.
5 Laroche M, Boyer JF. Phosphate diabetes, tubular phosphate reabsorption and phosphatonins. Joint Bone Spine 2005; 72: 376–381.
6 Shimada T, Mizutani S, Muto T, et al. Cloning and characterization of FGF23 as a causative factor of tumor-induced osteomalacia. Proc Natl Acad Sci U S A 2001; 98: 6500–6505.
7 ADHR Consortium. Autosomal dominant hypophosphatemic rickets is associated with mutations in FGF23. Nat Genet 2000; 26: 345–348.
8 Takeda E, Yamamoto H, Nakashima K, Nishimura K, Sato T, Araki Y, Taketani Y. Inorganic phosphate homeostasis and the role of dietary phosphorus. J Cell Mol Med 2004; 8: 191–200.
9 Kuro-o M. Endocrine FGFs and Klothos: emerging concepts. Trends Endocrinol Metab 2008; 19: 239–245.
10 Shimada T, Hasegawa H, Yamazaki Y, et al. FGF-23 is a potent regulator of vitamin D metabolism and phosphate homeostasis. J Bone Miner Res 2004; 19: 429–435.
11 Nishimura K, Takahashi M, et al. The discovery of alpha-Klotho and FGF23 unveiled new insight into calcium and phosphate homeostasis. Cell Mol Life Sci 2008; 65: 3218–3230.
12 Perwad F, Azam N, Zhang MY, Yamashita T, Tenenhouse HS, Portale AA. Dietary and serum phosphorus regulate fibroblast growth factor 23 expression and 1,25-dihydroxyvitamin D metabolism in mice. Endocrinology 2005; 146: 3538–3546.
13 Shimada T, Yamazaki Y, Takahashi M, et al. Vitamin D receptor-independent FGF23 actions in regulating phosphate and vitamin D metabolism. Am J Physiol Renal Physiol 2005; 289: F1088–F1095.
14 Ito M, Sakai Y, Furumoto M, et al. Vitamin D and phosphate regulate fibroblast growth factor-23 expression in K-562 cells. Am J Physiol Renal Physiol 2005; 288: E1101–E1109.
15 Yu X, Sabbagh Y, Davis SI, Demay MB, White KE. Genetic dissection of phosphate- and vitamin D-mediated regulation of circulating Fgf23 concentrations. Bone 2005; 36: 978–977.
16 Segawa H, Kaneko I, Yamazaki S, et al. Intestinal Na-P(i) cotransporter
adaptation to dietary P(i) content in vitamin D receptor null mice. Am J Physiol Renal Physiol 2004; 287: F39–F47.
17 Fukumoto S. Actions and mode of actions of FGF19 subfamily members. Endocr J 2008; 55: 23–31.
18 Inagaki T, Choi M, Moschetta A, et al. Fibroblast growth factor 15 functions as an endoheptatic signal to regulate bile acid homeostasis. Cell Metab 2005; 2: 217–225.
19 Nishida Y, Taketani Y, Yamanaka-Okumura H, et al. Acute effect of oral phosphate loading on serum fibroblast growth factor 23 levels in healthy men. Kidney Int 2006; 70: 2141–2147.
20 Shuto E, Taketani Y, Tanaka R, et al. Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning. Nat Genet 1997; 16: 391–396.
21 Sommer S, Berndt T, Craig T, Kumar R. The phosphatonin and the regulation of phosphate transport and vitamin D metabolism. J Steroid Biochem Mol Biol 2007; 103: 497–503.
22 Pan X, Zhang Y, Wang L, Hussain MM. Diurnal regulation of MTP and plasma triglyceride by CLOCK is mediated by SHP. Cell Metab 2010; 12: 174–186.
23 Ma K, Xiao R, Tseng HT, Shan L, Fu L, Moore DD. Circadian dysregulation disrupts bile acid homeostasis. PLoS One 2009; 4: e6843.
24 Le Martelot G, Claudel T, Gatfield D, et al. REV-ERBalpha participates in circadian SREBP signaling and bile acid homeostasis. PLoS Biol 2009; 7: e1000181.
25 Beenen A, Mohammadi M. The FGF family: biology, pathophysiology and therapy. Nat Rev Drug Discov 2009; 8: 235–253.
26 Mraz M, Bartlova M, Lacinova Z, et al. Serum concentrations and tissue expression of a novel endocrine regulator fibroblast growth factor-21 in patients with type 2 diabetes and obesity. Clin Endocrinol (Oxf) 2009; 71: 369–375.
27 Han SH, Choi SH, Cho BJ, et al. Serum fibroblast growth factor-21 concentration is associated with residual renal function and insulin resistance in end-stage renal disease patients receiving long-term peritoneal dialysis. Metabolism 2010; 59: 1656–1662.
28 Zhang M, Xiong ZY, Zeng L, Wang YJ, Huang MJ, An ZM. Plasma fibroblast growth factor-21 and abdominal obesity. Sichuan Da Xue Xue Bao Yi Xue Ban 2010; 41: 487–489, 522.
29 Fon Tacer K, Bookout AL, Ding X, et al. Research resource: comprehensive expression atlas of the fibroblast growth factor system in adult mouse. Mol Endocrinol 2010; 24: 2050–2064.
30 Bhattacharyya N, Chong WH, Gafni RI, Collins MT. Fibroblast growth factor 23: state of the field and future directions. Trends Endocrinol Metab 2012; 23: 610–618.
31 Christakos S, Dhawan P, Liu Y, Peng X, Porta A. New insights into the mechanisms of vitamin D action. J Cell Biochem 2003; 88: 695–705.
32 Honjo Y, Sasaki S, Kobayashi Y, Misawa H, Nakamura H. 1,25-dihydroxyvitamin D3 and its receptor inhibit the chenodeoxycholic acid-dependent transactivation by farnesoid X receptor. J Endocrinol 2006; 188: 635–643.
33 Chen X, Chen F, Liu S, et al. Transactivation of rat apical sodium-dependent bile acid transporter and increased bile acid transport by 1alpha,25-dihydroxyvitamin D3 via the vitamin D receptor. Mol Pharmacol 2006; 69: 1913–1923.
34 Makishima M, Lu TT, Xie W, et al. Vitamin D receptor as an intestinal bile acid sensor. Science 2002; 296: 1313–1316.
35 Junutka PW, Thompson PD, Whitfield GK, et al. Molecular and functional comparison of 1,25-dihydroxyvitamin D3 and the novel vitamin D receptor ligand, lithocholic acid, in activating transcription of cytochrome P450 3A4. J Cell Biochem 2005; 94: 917–943.
36 Araya Z, Hosseinipour F, Bodin K, Wikvall K. Metabolism of 25-hydroxyvitamin D3 by microsomal and mitochondrial vitamin D3 25-hydroxylases (CYP2D25 and CYP27A1): a novel reaction by CYP27A1. Biochim Biophys Acta 2003; 1632: 40–47.
37 Russell DW. The enzymes, regulation, and genetics of bile acid synthesis. Annu Rev Biochem 2003; 72: 137–174.
38 Chiang JY. Bile acids: regulation of synthesis. J Lipid Res 2009; 50: 1955–1966.
39 Huang X, Yang C, Luo Y, Jin C, Wang F, McKeenan WL. FGFR4 prevents hyperlipidemia and insulin resistance but underlies high-fat diet induced fatty liver. Diabetes 2007; 56: 2501–2510.
40 Hartman HB, Lai K, Evans MJ. Loss of small heterodimer partner expression in the liver protects against dyslipidemia. J Lipid Res 2009; 50: 193–203.
41 Tanaka S, Yamamoto H, Nakahashi O, et al. Dietary phosphate restriction induces hepatic lipid accumulation through dysregulation of cholesterol metabolism in mice. Nutr Res 2013; 33: 586–593.
42 Taketani Y, Shuto E, Arai H, et al. Advantage of a low glycemic index and low phosphate diet on diabetic nephropathy and aging-related diseases. J Med Invest 2007; 54: 359–365.
43 Mulec H, Johnsen SA, Wklund O, Björek S. Cholesterol: a renal risk factor in diabetic nephropathy? Am J Kidney Dis 1993; 22: 196–201.
44 Dieter B, Radhakrishnan J, Avram MM, et al. Analysis of metabolic parameters as predictors of risk in the RENAAL study. Diabetes Care 2003; 26: 1402–1407.
45 Muntrer P, He J, Astor BC, Folsom AR, Corell J. Traditional and non-traditional risk factors predict coronary heart disease in chronic kidney disease: results from the atherosclerosis risk in communities study. J Am Soc Nephrol 2005; 16: 529–538.