The sodium phosphate cotransporter family and nicotinamide phosphoribosyltransferase contribute to the daily oscillation of plasma inorganic phosphate concentration

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Circulating inorganic phosphate exhibits a remarkable daily oscillation based on food intake. In humans and rodents, the daily oscillation in response to food intake may be coordinated to control the intestinal absorption, renal excretion, cellular shifts, and extracellular concentration of inorganic phosphate. However, mechanisms regulating the resulting oscillation are unknown. Here we investigated the roles of the sodium phosphate cotransporter SLC34 (Npt2) family and nicotinamide phosphoribosyltransferase (Nampt) in the daily oscillation of plasma inorganic phosphate levels. First, it is roughly linked to urinary inorganic phosphate excretion. Second, expression of renal Npt2a and Npt2c, and intestinal Npt2b proteins also exhibit a dynamic daily oscillation. Analyses of Npt2a, Npt2b, and Npt2c knockout mice revealed the importance of renal inorganic phosphate reabsorption and cellular inorganic phosphate shifts in the daily oscillation. Third, experiments in which nicotinamide and a specific Nampt inhibitor (FK866) were administered in the active and rest phases revealed that the Nampt/NAD^+ system is involved in renal inorganic phosphate excretion. Additionally, for cellular shifts, liver-specific Nampt deletion disturbed the daily oscillation of plasma phosphate during the rest but not the active phase. In systemic Nampt$^{+/-}$ mice, NAD levels were significantly reduced in the liver, kidney, and intestine, and the daily oscillation (active and rest phases) of the plasma phosphate concentration was attenuated. Thus, the Nampt/NAD^+ system for Npt2 regulation and cellular shifts to tissues such as the liver play an important role in generating daily oscillation of plasma inorganic phosphate levels.

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Hyperphosphatemia is linked to vascular calcification with chronic kidney disease (CKD), and is an independent risk factor for cardiovascular mortality in hemodialysis patients.[1–3] Serum inorganic phosphate (Pi), even within the normal range, is associated with cardiovascular events, cardiovascular mortality, and all-cause mortality, and exhibits a daily oscillation in both healthy individuals and patients with CKD.[4–9] Observational studies assessing the relationship between dietary intervention and serum Pi levels are confounded by the lack of standardization regarding the time of day that serum Pi was assessed.[10] Serum Pi levels exhibit a well-described daily oscillation in normal and CKD patients.[11] Pi peaks between 02:00 and 04:00 (rest phase), and the lowest levels are detected between 08:00 and 10:00 (active phase).[11–14] Most epidemiologic studies have demonstrated that the fasting morning serum Pi concentration is linked to cardiovascular events and mortality. The factors regulating this link, however, are not known.[15,16]

Plasma Pi concentrations and renal Pi excretion display significant daily oscillations in animals[17–19] as well as in humans.[12,13,20–23] The daily oscillation of plasma Pi levels in nocturnal rodents (rats) is roughly inverse to that in humans.[14,17–20,24] In humans and rodents, plasma Pi levels are decreased during the active phase and increased in the resting phase.[14,17–19] On the other hand, in humans and rodents, changes in urinary Pi excretion levels are roughly the reverse of the changes in the plasma Pi concentrations.[14,17–20,24] Prolonged fasting abolishes the nocturnal peak in serum Pi,[25,26] indicating that dietary intake contributes to the daily changes in serum Pi. Changes in parathyroid hormone (PTH), growth hormone, $1,25(OH)_2D_3$, and fibroblast growth factor 23 (FGF23) cannot fully explain the daily oscillation of plasma Pi concentrations.[12,14,27–29]

Pi homeostasis is predominantly regulated by sodium-dependent Pi transporters of the solute carrier family SLC34, including Npt2a, Npt2b, and Npt2c. Npt2a and Npt2c are responsible for reabsorption of approximately 70% to 80% of the Pi filtered by the kidney.[3,30] Small intestinal Npt2b also has an important functional role.[3,31–33] Serum Pi is a function of Pi homeostasis as well as the balanced movement of Pi between intracellular and extracellular spaces.[34] Detailed mechanisms of the cellular Pi shift are unknown, but cellular energy metabolism (ATP and nicotinamide adenine dinucleotide $[NAD]^+$) may be involved in Pi utilization.[35] The role of the SLC34 family in the daily oscillation remains unknown.

In a previous study, we investigated a partial hepatectomy-induced hypophosphatemia model and found that the nicotinamide phosphoribosyltransferase (Nampt)/NAD^+ system is important for systematic regulation of Npt2a, Npt2b, and Npt2c transporters.[36] Nampt acts via enzymatic activity to synthesize nicotinamide mononucleotide and to maintain homeostasis of NAD, which plays a dual role in energy metabolism and biologic signaling.[37,38] We hypothesized that the Nampt/NAD^+ system controls the daily oscillation. Here we investigated the roles of Npt2 and Nampt in the daily oscillation of plasma Pi concentrations.

RESULTS

Daily oscillation of plasma Pi levels and urinary Pi excretion in wild-type mice

First, we investigated the daily oscillation of plasma Pi and urinary Pi excretion in wild-type (WT) mice. Plasma Pi levels were lower at 08:00 AM (Zeitgeber time [ZT], light/dark cycle ZT0, lights on; ZT12, lights off) and gradually increased, peaking at around ZT10 (Figure 1a). Thereafter, the plasma Pi concentrations gradually decreased from ZT10 to ZT18. Renal Pi excretion values were highest from ZT10 to ZT14 (Figure 1b). Fractional excretion of phosphate (FEPi, %) values was highest at ZT14 (Figure 1c). We used brush border membrane vesicle (BBMV) total protein as a loading control because actin appears to undergo some time-of-day variation (data not shown). Renal and intestinal BBMV (20 μg/lane) were analyzed by immunoblotting. SDS–PAGE analysis revealed almost the same protein levels in each lane. Npt2a protein levels in the BBMVs gradually decreased from ZT2 to ZT14 and then increased to ZT22 (Figure 1d). The pattern of Npt2c protein levels was not as prominent as that of Npt2a. Daily oscillations of Npt2b protein were similar to those of renal Npt2a (Figure 1e).

Renal and intestinal Na/Pi transport activities in the BBMVs were significantly reduced at ZT14 compared with ZT2 (Figure 1f). Plasma PTH and FGF23 levels did not change between ZT2 (rodent rest phase) and ZT14 (rodent active phase; Figure 1g and h). These findings revealed that renal Npt2a protein and intestinal Npt2b protein levels exhibit daily oscillations, like plasma and urinary Pi levels, independent of the plasma FGF23 and PTH levels.

Effects of fasting on renal Pi excretion

Next, we investigated the effect of food deprivation on Pi excretion and plasma Pi levels (Supplementary Figure S1). Animals were analyzed during food deprivation and compared with those fed *ad libitum* (Supplementary Figure S1A). We analyzed 2 groups (feeding group and food-deprived group) beginning at ZT14 (Supplementary Figure S1B). In the food-deprived group, urinary Pi excretion levels gradually increased. Plasma Pi levels were significantly higher compared with the feeding group in all periods (Supplementary Figure S1B). The Npt2a protein levels were markedly decreased in the food-deprived mice (Supplementary Figure S1C). These findings suggest that the daily oscillation of plasma Pi concentrations depends on food intake, which is consistent with previous findings.[39]

Roles of Npt2 in the daily oscillations of plasma and urinary Pi levels

To investigate whether renal Npt2 proteins are involved in the daily oscillation of the plasma Pi concentration, we analyzed the daily oscillations of Npt2a$^{-/-}$, Npt2a$^{-/-}$/Npt2c$^{-/-}$, and intestine-specific Npt2b deletion mice (Npt2b$^{flox/flox}$-vCre) (Figure 2). Food intake behavior did not differ between groups (Figure 2c). Npt2a$^{-/-}$ mice have hypophosphatemia and hyperphosphaturia. During the diurnal phase (ZT2−ZT10), the increase in the plasma Pi concentration observed in Npt2a$^{+/+}$mice was not observed in Npt2a$^{-/-}$ mice (Figure 2a). During the active phase (ZT14−ZT22), however, the reduced plasma Pi concentration in Npt2a$^{+/+}$ mice was also observed in Npt2a$^{-/-}$ mice (Figure 2a). In contrast, during the active phase, renal Pi excretion levels were significantly increased in Npt2a$^{-/-}$ mice and Npt2a$^{+/+}$ mice (Figure 2b). Npt2a protein levels in Npt2a$^{+/+}$ mice were markedly decreased at ZT14 compared with ZT2 (Figure 2d). Npt2c protein levels were highest at ZT2 and ZT14 in Npt2a$^{-/-}$ mice. Unlike Npt2a$^{+/+}$ mice, Npt2a$^{-/-}$ mice showed no increase in the plasma Pi concentration during the rest phase (ZT2−ZT10), whereas the plasma Pi concentration was reduced during the active phase.

We further investigated the role of intestinal Npt2b in the daily oscillation of plasma Pi concentrations using Npt2b$^{flox/flox}$-vCre mice (intestine-specific Npt2b deletion mice). Npt2b$^{flox/flox}$-vCre mice had normal plasma Pi levels, but decreased renal Pi excretion, as reported previously.[33] Our established Npt2b$^{flox/flox}$-vCre mice, however, had lower plasma Pi levels than vCre$^+$ (control) mice at only 8 weeks. Increased plasma Pi concentrations were observed in intestinal vCre$^+$ mice and intestinal Npt2b$^{flox/flox}$-vCre mice during ZT2 to ZT10 (Figure 2e). Furthermore, plasma Pi concentrations were reduced during ZT14 to ZT22 in vCre$^+$ mice and Npt2b$^{flox/flox}$-vCre mice (Figure 2e). Urinary Pi excretion was suppressed in Npt2b$^{flox/flox}$-vCre mice compared with vCre$^+$ mice (Figure 2f). The daily oscillation pattern of plasma and urine Pi in Npt2b$^{flox/flox}$-vCre mice was similar to that in intestinal Npt2b vCre$^+$ mice.

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