Published in final edited form as:
Kidney Int. 2008 May ; 73(10): 1141–1150. doi:10.1038/ki.2008.33.

Estrogen downregulates the proximal tubule type IIa sodium phosphate cotransporter causing phosphate wasting and hypophosphatemia

S Faroqui[1], **M Levi**[2], **M Soleimani**[1,3], and **H Amlal**[1]

[1] Department of Medicine, University of Cincinnati, Cincinnati, Ohio, USA

[2] Department of Medicine, Division of Renal Diseases and Hypertension, University of Colorado Health Sciences Center, Denver, Colorado, USA

[3] Department of Medicine, University of Cincinnati and Veterans Affair Medical Center, Cincinnati, Ohio, USA

Abstract

Estrogen treatment causes significant hypophosphatemia in patients. To determine the mechanisms responsible for this effect, we injected ovariectomized rats with either 17β-estradiol or vehicle for three days. Significant renal phosphate wasting and hypophosphatemia occurred in estrogen-treated rats despite a decrease in their food intake. The mRNA and protein levels of the renal proximal tubule sodium phosphate cotransporter (NaPi-IIa) were significantly decreased in estradiol-treated ad-libitum or pair-fed groups. Estrogen did not affect NaPi-III or NaPi-IIc expression. In ovariectomized and parathyroidectomized rats, 17β-estradiol caused a significant decrease in NaPi-IIa mRNA and protein expression compared to vehicle. Estrogen receptor alpha isoform blocker significantly blunted the anorexic effect of 17β-estradiol but did not affect the downregulation of NaPi-IIa. Our studies show that renal phosphate wasting and hypophosphatemia induced by estrogen are secondary to downregulation of NaPi-IIa in the proximal tubule. These effects are independent of food intake or parathyroid hormone levels and likely not mediated through the activation of estrogen receptor alpha subtype.

Keywords

phosphorus; PTH; sex steroids; calcium

Inorganic phosphorus (Pi) is essential for various important functions, including synthesis of cellular energy (ATP), intracellular signaling, protein synthesis, as well as bone matrix and phospholipid synthesis. Published reports indicate that Pi homeostasis is maintained and controlled efficiently in response to changes in Pi intake or physiological conditions. Intestinal absorption and renal excretion or reabsorption are the major regulatory steps in Pi homeostasis.[1–3] In both kidney and small intestinal epithelium, Pi absorption is mediated via the type II Na^+ -dependent phosphate cotransporter from SLC34 family,[4] which includes IIa, IIb, and IIc variants. The intestinal absorption of Pi is mediated predominantly through type IIb (NaPi-IIb),[5] whereas in the kidney, both NaPi-IIa and NaPi-IIc are expressed in the brush-border membrane of proximal tubule cells and mediate the reabsorption of filtered Pi from the tubular filtrate.[6–8] The increased urinary Pi excretion in NaPi-IIa-knockout mice suggests that NaPi-

Correspondence: H Amlal, Division of Nephrology and Hypertension, University of Cincinnati, 231 Albert Sabin Way, MSB 259G, Cincinnati, Ohio 45267-0585, USA. E-mail: hassane.amlal@uc.edu.

IIa is the limiting barrier for Pi reabsorption in the renal proximal tubule.[9,10] NaPi-IIa is regulated by several factors, including glucocorticoids,[11] epidermal growth factors,[12,13] parathyroid hormone (PTH),[14–16] vitamin D3,[17] thyroid hormone,[18,19] dopamine,[20] metabolic acidosis,[21] dietary phosphate,[10,22] and phosphatonins.[23] Phosphatonins belong to a new class of phosphate-regulating factors, which include fibroblast growth factor-23 (FGF-23) and secreted frizzled-related protein 4. These factors were reported to be associated with hypophosphatemic diseases.[23] In the small intestine, NaPi-IIb is also expressed in apical membranes of the intestinal villi and is regulated by the same above factors, mostly dietary phosphate,[24,25] epidermal growth factor,[26] vitamin D3,[25] glucocorticoid,[27] and estrogen (EST).[28]

Pi and calcium are the two major minerals that play an important role in bone matrix and bone mineralization. Several pathophysiologic states such as metabolic acidosis are associated with enhanced urinary Pi and calcium wasting and bone loss.[29–31] Sex steroids depletion, mainly ESTs, has been suggested as a major factor in bone minerals loss in postmenopausal osteoporosis.[32,33] However, the effect of sex steroids on renal Pi handling is poorly understood. Clinical studies have shown that women treated with ESTs exhibited hypophosphatemia;[34–38] in some studies, a reduction in proximal tubular Pi reabsorption was observed.[35,36,38] Conversely, EST-depleted patients showed elevated plasma levels of Pi and increased rate of proximal tubule Pi reabsorption.[34] In rats, recent studies demonstrated that EST stimulated the expression of small intestine NaPi-IIb.[28] In the kidney, however, other studies reported that EST treatment of ovariectomized (OVX) rats caused a significant decrease in Pi uptake by the brush-border membrane vesicles of proximal tubules as compared to control animals.[39] These studies, however, did not examine the molecular basis and signaling pathways responsible for the reduction in Pi uptake by EST in the proximal tubule cells.

Thus, the aim of these studies was to examine the effects of female sex steroids on renal Pi handling, various Pi transporters, and systemic phosphate homeostasis. Toward this end, OVX rats, with and without parathyroidectomy, were treated with progesterone or EST in the absence or presence of EST receptor-α isoform (ERα) blocker. The results demonstrated that EST, but not progesterone, specifically downregulates the expression of NaPi-IIa and causes phosphaturia. This effect is independent of PTH or alteration in Pi intake, and is not mediated through ERα.

RESULTS

Blood electrolytes composition in vehicle- vs EST-treated rats

Blood chemistry data shown in Table 1 indicate that rats treated with EST for 3 days showed normal electrolytes composition, except for a significant hypophosphatemia ($P<0.05$), as compared to vehicle-injected rats.

Food intake, urinary Pi, and calcium excretion in response to EST

The results depicted in Figure 1 indicate a significant reduction in food intake, which occurred as early as 24 h after EST injection (from 21±0.9 g at baseline to 14±1.1 g after 24 h of EST, $P<0.001$, Figure 1a), and further decreased to a lower level after 3 days of EST treatment (6.6 ±0.96 g, $P<0.0001$, $n = 4$ rats in each group, Figure 1a). Food intake in vehicle-treated group remained unchanged for the duration of the treatment (20±0.43 g before and 19±1.0 g after 3 days of vehicle treatment, $P>0.05$, $n = 4$ rats, Figure 1a).

Despite the sharp reduction in food intake described above (Figure 1a), EST-treated rats exhibited a significant phosphaturia as shown by a sustained or even a slight increase in urinary Pi excretion after EST injection (from 0.88±0.13 mg/24 h at baseline to 1.36±0.14 mg/day after

24 h, $P<0.05$, Figure 1b and to 1.11±0.23 mg/24 h after 3 days of treatment, $P>0.05$, $n = 4$ rats in each time point, Figure 1b).

Unlike Pi excretion, urinary calcium excretion decreased sharply within the first 24 h of EST treatment (from 3.65±0.31 mg/24 h at baseline to 1.88±0.45 mg/24 h after 24 h of EST treatment, $P<0.02$, Figure 1c), and further decreased to an even lower level after 3 days of EST injection (0.14±0.05 mg/24 h, $P<.001$, $n = 4$ rats, Figure 1c). Urinary calcium excretion remained unchanged for the duration of the experiment in vehicle-injected animals (3.90±0.75 mg/24 h before and 3.98±0.58 mg/24 h after vehicle injections, $P>0.05$, $n = 4$ rats, Figure 1c). The decrease in the urinary calcium excretion, which parallels the reduction in food intake, indicates an increase in renal calcium conservation in response to EST treatment.

NaPi-IIa expression in response to EST treatment

The mRNA expression and protein abundance of NaPi-IIa were examined by northern hybridization and immunoblotting, respectively, using total RNA and membrane proteins harvested from renal cortex of vehicle- and EST-treated rats. The results depicted in Figure 2 indicate a significant decrease in NaPi-IIa mRNA expression in response to 3 days of EST injections (32±7 vs 100±23% in vehicle-treated animals, $P<0.04$, $n = 4$ rats in each group, Figure 2a and b). This decrease in NaPi-IIa mRNA correlates with a significant reduction in its protein abundance in EST-injected rats, as shown by a decrease in the expression levels of both 50 kDa band (50±6 vs 100±5% in vehicle group, $P<0.01$, $n = 4$ rats, Figure 2c (upper panel) and d) and 80–90 kDa band (45±8 vs 100±7% in vehicle group, $P<0.01$, $n = 4$ rats, Figure 2c (upper panel) and d) of NaPi-IIa protein. A parallel Coomassie blue-stained gel indicates the equity of the protein loading between different lanes (Figure 2c, lower panel).

Progesterone treatment of OVX rats did not alter the mRNA expression or protein abundance of NaPi-IIa compared to its vehicle group (data not shown).

Cortical NHE3 and AQP-1 expression in response to EST treatment

Using the same total membrane protein fractions used above for NaPi-IIa, we examined the protein abundance of cortical NHE3 and aquaporin-1 (AQP-1) in EST- vs vehicle-treated animals. The results shown in Figure 3 indicate that EST treatment did not alter the protein abundance of NHE3 (96±13 vs 100±8% in vehicle group, $P>0.05$, $n = 4$ rats in each group, Figure 3a and b) or that of AQP-1 (109±8 vs 100±8% in vehicle group, $P>0.04$, $n = 4$ rats in each group, Figure 3c and d) in the renal cortex.

Effects of EST treatment on Pi transporters in a pair-feeding protocol

To determine whether the downregulation of NaPi-IIa by EST is independent of changes in food intake, OVX rats were treated with EST for 3 days and compared to vehicle-injected rats that had access to the same amount of food consumed by EST rats (pair-feeding, PF) as shown in Figure 4. In this setting, EST reproducibly decreased food intake from 15±0.68 to 5.06±0.43 g/24 h during 3 days of treatment (Figure 4a). Vehicle-treated rats were subjected to daily food restriction as described in Materials and Methods. Food consumption was reduced from 15 ±0.46 to 5.2±0.32 g/24 h during 3 days of treatment (Figure 4a). Urinary Pi excretion correlated with decreased food intake in vehicle-treated rats (from 0.51±0.05 mg/24 h before to 0.09±0.04 mg/24 h after food restriction, $P<0.001$, $n = 4$ rats, Figure 4b), whereas it remained unchanged or even slightly higher in EST-injected rats (from 0.45±0.03 mg/24 h before to 0.61±0.05 mg/ 24 h after EST treatment, $n = 4$ rats, Figure 4b).

The results depicted in Figure 5 indicate a significant reduction in the mRNA expression of NaPi-IIa in response to EST treatment (56±4 vs 100±7% in vehicle-PF group, $P<0.003$, $n = 4$ rats in each group, Figure 5a). This correlates with a significant decrease in NaPi-IIa protein

abundance, as indicated by the immunoblot depicted in Figure 5c (57±17 vs 100±6% in vehicle-PF group, $P<0.05$, $n = 4$ rats in each group, Figure 5c and d). A parallel Coomassie blue-stained gel indicates the equity of the protein loading between different lanes (data not shown).

The expression of other transporters involved in Pi reabsorption in the proximal tubule was also examined under the above experimental conditions. The results shown in Figure 6 indicate that EST treatment did not alter the mRNA expression of NaPi-IIc, another apical Na^+ -dependent Pi transporter[7,8] (110±12 vs 100±6.9% in vehicle-PF group, $P>0.05$, $n = 4$ rats in each group, Figure 6), and did not alter the expression of type III Na^+ -dependent Pi transporter (PiT1), which is localized to the basolateral membrane of proximal tubule cells[8] (91±9 vs 100 ±5% in vehicle-PF group, $P>0.05$, $n = 4$ rats in each group, Figure 6).

Lastly, the effect of EST on the expression of klotho and Na^+/H^+ exchanger regulatory factor 1 (NHERF1) was examined by northern hybridization. The results indicate that EST treatment did not affect the mRNA expression of klotho (101±8 vs 100±5.9% in vehicle-PF group, $P>0.05$, $n = 4$ rats in each group, Figure 6b) or that of NHERF1 (115±14 vs 100±2.5% in vehicle-PF group, $P>0.05$, $n = 4$ rats in each group, Figure 6b).

Effects of EST on NaPi-IIa expression in OVX–PTX rats

To determine whether PTH plays any role in the down-regulation of NaPi-IIa by EST, we repeated these experiments in rats deficient in PTH. Toward this end, OVX and parathyroidectomized (PTX) rats were subjected to either vehicle or EST treatment for 3 days, as described in Materials and Methods, and used to examine the expression of NaPi-IIa in the renal cortex. The results shown in Figure 7 indicate the following: first, OVX–PTX rats consume significantly less food than OVX rats (6.94±0.16 g/24 h, Figure 7a, vs 21±0.9 g/24 h, Figure 1a, $P<0.001$, $n = 4$–5 rats in each group). Second, EST treatment of OVX–PTX rats surprisingly did not affect food intake (6.94±0.16 g/24 h before and 6.73±0.11 g/24 h after 3 days of EST treatment, $P>0.05$, $n = 5$ rats, Figure 7a) as compared to EST-treated OVX animals (Figure 1a). However, EST caused a significant increase in urinary Pi excretion as shown in Figure 7b. Lastly, EST treatment of OVX–PTX rats was associated with a significant reduction in NaPi-IIa mRNA expression levels as compared to vehicle treatment (from 100±12 to 53 ±10%, $P<0.02$, $n = 4$–5 rats in each group, Figure 7a and b). This effect correlated with a significant reduction in NaPi-IIa protein abundance in EST-treated animals (48±11 vs 100±5% in vehicle-injected rats, $P<0.03$, $n = 4$–5 rats in each group, Figure 7c (upper panel) and d). A parallel Coomassie blue-stained gel indicates the equity of the protein loading between different lanes (Figure 7c, lower panel).

Effect of EST in the presence of ERα inhibitor

Effect of EST on food intake in the presence of ERα blocker—EST acts on its target cells through different receptor subtypes, including ERα, ERβ, and GRP.[40–43] In the next experiments, we tested the role of ERα in EST-induced phosphaturia and NaPi-IIa downregulation. Accordingly, OVX rats were placed in metabolic cages and treated with EST, ICI182,780, ICI + EST, or their vehicle as described in Materials and Methods. The results depicted in Figure 8 indicate that EST reproducibly decreased food intake from 18±0.68 g/24 h at baseline to 5±0.85 g/24 h after 3 days of treatment ($P<0.001$, $n = 4$ rats, Figure 8a and b). This effect is partially, but significantly, blocked by the presence of ERα inhibitor, as food intake decreased from 17±0.68 g/24 h at baseline to 9±1.14 g/24 h after 3 days of ICI + EST treatment ($P<0.01$, $n = 6$ rats, Figure 8a and b). The treatment of the animals with ICI alone for 3 days did not significantly alter food intake (16±0.56 g/24 h before and 14±0.86 g/24 h after 3 days of ICI treatment, $P>0.05$, $n = 6$ rats, Figure 8a and b). The food intake in vehicle-injected rats remained unchanged for the duration of the experiment (17±1.1 g/24 h before and 16±0.89 g/24 h after 3 days of vehicle treatment, $P>0.05$, $n = 4$ rats, Figure 8b). It should be

noted that the results described above for ICI alone and ICI + EST are actually pooled results of two different doses of ICI (300 and 600 μg/rat/day), which showed similar effects on food intake (n = 3 OVX rats in each group).

Effect of EST on NaPi-IIa expression in the presence of ERα blocker—The expression of NaPi-IIa was examined in the renal cortex of rats treated with ICI182,780 alone, ICI + EST, or their vehicle by northern hybridization and immunoblotting. The results shown in Table 2 indicate that the mRNA expression level of NaPi-IIa is significantly decreased in ICI + EST rats as compared to vehicle (46±1.36 vs 100±5.5%, P<0.001, n = 3–4 rats in each group, Table 2). ICI treatment alone did not significantly alter the mRNA expression levels of NaPi-IIa (85±3.4 vs 100±5.5% in vehicle group, P>0.05, n = 3 rats in each group, Table 2). Similarly, the protein abundance of NaPi-IIa decreased significantly in ICI + EST rat as compared to vehicle (51±7 vs 100±6%, P<0.02, n = 3 rats in each group, Table 2), whereas it remained unchanged in ICI alone vs vehicle-treated rats (Table 2).

DISCUSSION

This is the first report demonstrating that EST can regulate phosphate homeostasis in Sprague Dawley rat, and is highly relevant to the postmenopausal women taking EST hormone to maintain their bone health. Increased bone turnover and loss of bone density are common in postmenopausal women. EST therapy reduces bone resorption and prevents bone loss and osteoporosis.[44] This process has been advanced to explain the decrease in circulating levels of calcium and inorganic phosphate (Pi) in postmenopausal women on EST replacement therapy.[36,37,45]

It is well accepted that the proximal tubular reabsorption of Pi is the major determinant of Pi homeostasis. Given the impressive downregulation of the Na^+ -dependent phosphate (Pi) transporter NaPi-IIa and the subsequent renal Pi wasting (Figure 1b) and hypophosphatemia (Table 1) in rats treated with EST, we suggest that the hypophosphatemic effect of EST replacement in postmenopausal women is predominantly mediated via its effect on the kidney.

Earlier studies showed that EST treatment of rats was associated with an inhibition of Pi uptake in brush-border membrane vesicles harvested from the renal cortex.[39] However, those studies did not examine the role of EST on the expression of the renal phosphate-dependent transporters and systemic phosphate homeostasis. Our results demonstrate that EST significantly downregulates the mRNA expression and protein abundance of NaPi-IIa (Figure 2), a major Na^+ -dependent Pi transporter exclusively expressed in the apical membrane of proximal tubule cells.[9,10] Unlike EST, progesterone treatment of OVX rats did not alter Pi metabolism and did not affect the expression of NaPi-IIa. This effect of EST is specific to NaPi-IIa, as the expression levels of other Pi-dependent (PiT1 and NaPi-IIc) or Pi-independent (NHE3 and AQP-1) transporters expressed in the proximal tubule were not altered in response to EST treatment (Figures 3 and 6).

In these studies, we did not examine the transport activity of NaPi-IIa in EST-treated rats; however, based on studies showing that Pi uptake is significantly inhibited in brush-border membrane vesicle of EST-injected arts,[39] and based on the fact that the genetic deletion of NaPi-IIa caused a significant and uncompensated phosphaturia,[9] one can conclude that impairment in NaPi-IIa transport activity should certainly correlate with the decrease in its expression to account for increased Pi excretion in EST-injected rats. Hence, EST-induced renal Pi wasting is likely secondary to the downregulation of NaPi-IIa in the proximal tubule.

EST is an important physiological regulator involved in the modulation of several behavioral and hormonal functions, indicating that the mechanisms by which EST regulates Pi metabolism

could be complex, and could involve direct and/or indirect effect(s). EST has been shown to induce anorexia and decrease appetite.[46–48] This effect is clearly reproduced in our studies (Figures 1a and 4a). However, it is unlikely that the downregulation of NaPi-IIa is secondary to the alteration in food intake, as the phosphaturic effect of EST persisted even when EST-treated rats were compared to pair-fed rats injected with vehicle (Figures 4 and 5). Further, an eventual role of PTH, which is a potent inhibitor of NaPi-IIa expression and activity,[14–16] is excluded in these studies. In fact, our studies performed in PTH-deficient (PTX) OVX rats exhibited a significant downregulation of NaPi-IIa (Figure 7). These findings are in support of the inhibition of Pi uptake in brush-border membrane vesicle previously reported in OVX-PTX rats.[39] Finally, NaPi-IIa expression is downregulated in metabolic acidosis;[21] however, EST-treated rats did not show any acid–base abnormality, as indicated by normal serum total CO_2 levels shown in Table 1.

In addition to the PTH, studies have demonstrated that dopamine is a potent phosphaturic hormone. These studies demonstrated that dopamine regulates renal Pi transport by an autocrine/paracrine mechanism.[20] The phosphaturic effect of dopamine is induced by both dopamine and D1-like receptor agonists, and inhibited by D1-like antagonists.[20] Furthermore, studies have shown that EST upregulates the expression and activity of D1A dopamine receptor,[49] and that the incubation of human neuroblastoma cells with EST caused a significant increase in dopamine levels, elicited by increased dopamine synthesis and decreased dopamine catabolism.[50] However, whether EST regulates dopamine activity and/or metabolism in the renal proximal tubule, and whether dopamine mediates the phosphaturic effects of EST remain to be demonstrated in future studies. Lastly, FGF-23 is a potent regulator of Pi metabolism by directly inhibiting Pi reabsorption in the proximal tubule.[51] Whether the effect of EST is mediated through the stimulation of FGF-23 remains to be determined.

Unlike other hormones, the effects of sex steroids on salt transport or other functions of proximal tubule cells have not been extensively studied. Several ligand-binding studies demonstrated the presence of EST receptor in both the cytosol and nucleus of rat kidney cells.[52,40] These receptors are expressed mainly in S1 and S2 segments of rat proximal tubule.[41] The majority of EST response is mediated through two different forms of EST receptor usually referred to as ERα and ERβ, each encoded by a separate gene. ERα and ERβ are ligand-regulated transcription factors that mediate genomic effects of different estrogenic molecules, including 17β-estradiol.[42] Interestingly, downregulation of NaPi-IIa and subsequent phosphaturia by EST are independent of ERα activation. This is clearly shown by our studies using ICI182,780, a potent inhibitor of ERα,[43,53–55] which significantly blunted the anorexic effect of EST (Figure 8), without preventing the downregulation of NaPi-IIa (Table 2). Whether the effect of EST on Pi transport is mediated through ERβ remains to be determined.

It should be noted that there is now strong evidence that EST can also exert a rapid and non-genomic effect on certain target tissues. These include activation of G protein-coupled receptors such as GPR 30[56–58] and mobilization of intracellular calcium,[57] stimulation of adenylate cyclase and cAMP production,[58] and activation of the mitogen-activated protein kinase signaling pathways.[56] It remains to be determined whether these pathways contribute to the phosphaturic effect of EST in rats.

Finally, we should emphasize that the hypophosphatemic and hyperphosphaturic effect of EST has been reported in clinical settings. Several studies have demonstrated that administration of EST to postmenopausal women is associated with hypophosphatemia which, in certain cases, is secondary to a reduction in Pi reabsorption in the proximal tubule.[35,36,38] A significant decrease in serum Pi levels was reported in male patients with metastatic prostate cancer treated with EST.[35] On the other hand, it was found that EST-deficient patients exhibited significant hyperphosphatemia secondary to increased proximal tubule Pi reabsorption.[34]

In conclusion, EST treatment of OVX rats caused significant hyperphosphaturia and hypophosphatemia, which were secondary to the downregulation of a major apical Pi-dependent transporter NaPi-IIa in the renal proximal tubule. The effect of EST on this transporter is not mediated through changes in food behavior or alterations in the circulating levels or activity of PTH, and is likely not mediated through ERα. It is highly plausible that the downregulation of NaPi-IIa in the proximal tubule is the molecular basis of hyperphosphaturia/hypophosphatemia observed in patients subjected to EST therapy. In treating postmenopausal women or patients with breast or prostate malignancy with EST-containing hormonal replacement regimens, health-care professionals should monitor and pay attention to the renal handling of phosphate very closely.

MATERIALS AND METHODS

Animal treatments

The experimental protocols were approved by Institutional Animal Care and Use Committee of University of Cincinnati. OVX Sprague Dawley rats (Harlan, Indianapolis, IN, USA) were housed two rats per cage with free access to rat chow and distilled water, and maintained in a temperature-controlled room regulated on a 12-h light/dark cycle for 1 week before and during the following treatments.

EST and progesterone treatment—OVX rats were placed in metabolic cages and allowed free access to food and water. After 3–4 days of adjustment to metabolic cages, rats were randomly divided into two groups and injected subcutaneously with either 17β-estradiol (15 μg/100 g body weight/day) or its vehicle (sesame oil). To determine the role of PTH in the observed effects of EST on Pi excretion, the above experiment was repeated in OVX and PTX rats (Harlan). In another set of studies, OVX rats were treated similarly in metabolic cages and injected subcutaneously with either progesterone (1.6 mg/100 g body weight/day) or its vehicle (sesame oil). The rats remained on food and water for an additional 3 days.

EST treatment in a PF protocol—As EST treatment of OVX rats is associated with a significant reduction in food intake (Figure 1), another set of OVX rats housed in metabolic cages were injected with either EST or its vehicle. However, the vehicle-treated rats were also subjected to daily food restriction, as they were given the same amount of food consumed by EST-treated rats determined during the first experiment (Figure 1a).

EST treatment in the presence of ERα inhibitor—In this experiment, another set of OVX rats were housed in metabolic cages and divided into four groups. The first group (EST) was injected with EST (15 μg/100 g body weight/day), the second group was injected with ERα blocker ICI182,780 or Fulvestrant alone,[43,53–55] the third group was injected with ICI 2 h before EST injection (ICI + EST), and the fourth group was injected with the vehicle (ethanol/propylene glycol, 1:4 v/v). To minimize the number of rats used, EST was also dissolved in ethanol/propylene glycol solution. It should be noted that two doses of ICI were used in these studies both in ICI alone and ICI + EST groups (that is, 300 and 600 μg/rat/day).

During each of the above studies, food and water intake and urine volume were measured daily. At the end of each experiment (3 days after the beginning of the treatment), the animals were killed and the kidneys were removed. Cortex was dissected, snap frozen in liquid nitrogen, and stored at −80 °C for total RNA and membrane protein isolation.

Blood composition and urine analyses

Serum electrolytes, creatinine, and blood urea nitrogen levels were measured using commercial services (LabOne of Ohio Inc., Cincinnati, OH, USA). Urinary excretion of Pi and calcium

was measured using phosphorus and calcium assay kits (Pointe Scientific, Canton, MI, USA; http://www.pointescientific.com).

Total RNA isolation and northern hybridization

Total RNA isolation—Total cellular RNA was extracted from renal cortex by the method of Chomczynski and Sacchi.[59] In brief, cortical tissues were homogenized in Tri Reagent (Molecular Research Center Inc., Cincinnati, OH, USA) at room temperature. Total RNA was extracted by phenol/chloroform and precipitated by isopropanol. Total RNA was quantitated by spectrophotometry and stored at −80 °C.

Northern hybridization—Total RNA samples (30 μg/lane) were fractionated on a 1.2% agarose–formaldehyde gel and transferred to Magna NT nylon membranes using 10 × sodium chloride–sodium phosphate–EDTA as a transfer buffer. Hybridization was performed according to Church and Gilbert[60] and as previously used in our laboratory.[55,61,62] Membranes were hybridized overnight with ^{32}P-labeled specific DNA probes (25 ng). The membranes were washed, blotted, and exposed to Phosphor-imager cassette at room temperature for 24–72 h, and read by Phosphor-imager (Molecular Dynamics, Sunnyvale, CA, USA). Specific probes for various Pi transporters, NHERF1 and klotho genes, were generated by reverse transcription-PCR using total RNA from cortex as a template for reverse transcription. PCR was performed using the following sets of specific primers: rat NaPi-IIa: sense, 5′-ACCTCTGTCACCAACAC CAT-3′; antisense, 5′-TGAAGAAGAAGTGGCAGAGG-3′; rat NaPi-IIc: sense, 5′-GAGGAACAGCTCAGCATCTCC-3′; antisense, 5′-CAGC GGTATTGTGCAGTCAGG-3′; rat PiT1: sense, 5′-CATCTCGGTGG GATGTGC-3′; antisense, 5′-TGTTGGTCTCCTCCTTCA-3′; rat klotho: sense, 5′-TAAGGTTCAAGTATGGAGAC-3′; antisense, 5′-GGGCGTTCACACTTATTTAT-3′; rat NHERF1: sense, 5′-CATCAG CAGGTGGTGAGCCG-3′; antisense, 5′-CCATCCAGGTGCTCCTG GGA-3′. The PCR products were then separated on 1.2% agarose gels containing ethidium bromide and visualized under an ultraviolet illuminator. The expected DNA fragment was verified by DNA sequencing using commercial services (DNA Core, University of Cincinnati, Cincinnati, OH, USA).

Membrane protein isolation and immunoblotting

Preparation of membrane fractions—A total cellular fraction containing plasma membrane and intracellular membrane proteins was prepared from cortex as previously described.[61,62] The total protein concentration was measured using BCA protein assay kit (Pierce, Rockford, IL, USA; http://www.piercenet.com), and membrane fractions were solubilized at 65 °C for 20 min in Laemmli buffer and stored at −20 °C.

Electrophoresis and immunoblotting—Semiquantitative immunoblotting experiments were carried out as previously described.[61,62] Briefly, the solubilized membrane proteins were size fractionated on 10% polyacrylamide minigels (Novex, San Diego, CA, USA) and transferred electrophoretically to a nitrocellulose membrane. The membrane was blocked with 5% milk proteins, and then probed with affinity-purified antibody.[61,62] The secondary antibody was a donkey anti-rabbit IgG conjugated to horseradish peroxidase (Pierce). The site of antigen–antibody complexation on the nitrocellulose membranes was visualized using chemiluminescence method (Super Signal Substrate; Pierce) and captured on light-sensitive imaging film (Kodak). The equity in protein loading in all blots was first verified by gel staining using the Coomassie brilliant blue R-250 (Bio-Rad, Hercules, CA, USA).

Materials

^{32}P-dCTP was purchased from New England Nuclear (Boston, MA, USA). Paper blotting nitrocellulose membranes used for northern hybridization were purchased from Midwest Scientific (St Louis, MO, USA) (http://www.midsci.com). ICI182,780 was purchased from Tocris (Ellisville, MO, USA) (http://www.tocris.com). 17β-Estradiol, progesterone, and all other chemicals were purchased from Sigma Chemical Co. (St Louis, MO, USA) (http://www.sigmaaldrich.com). High Prime DNA labeling kit was purchased from Roche Diagnostics GmbH (Mannheim, Germany). Rabbit polyclonal NaPi-IIa antiserum was generated and used as described before.[63,64] NHE3 and AQP-1 antibodies were purchased from Alpha Diagnostic International (San Antonio, TX, USA) (http://www.4adi.com).

Statistical analysis

Semiquantification of immunoblot and northern hybridization band densities was determined by densitometry using a scanner (ScanJet ADF; Hewlett Packard) and UN-SCAN-IT gel software (Silk Scientific Inc., Orem, UT, USA) and ImageQuaNT software (Molecular Dynamics), respectively. Data were expressed as % of control. Results were presented as means ±s.e. Statistical significance between control and experimental groups was determined by one-way analysis of variance or Student's unpaired *t*-test as needed. $P<0.05$ was considered significant.

Acknowledgments

These studies were supported by the University of Cincinnati Academic Development Fund (to H Amlal). We thank Ms Terry Fettig for her assistance in the preparation and careful editing of the manuscript.

References

1. Berndt, TJ.; Knox, FG. Renal regulation of phosphate excretion. In: Seldin, DW.; Giebisch, G., editors. The Kidney, Physiology and Pathophysiology. Raven; New York, NY: 1992. p. 2511-2532.
2. Danisi, G.; Murer, H. Inorganic phosphate absorption in small intestine*Handbook of Physiology. The Gastrointestinal System*. In: Field, M.; Frizzel, RA., editors. Intestinal Absorption and Secretion, section 6. Vol. IV. Am Physiol Soc; Bethesda, MD: 1991. p. 323-336.
3. Dennis, VW. Phosphate homeostasis*Handbook of Physiology*. In: Windhager, EE., editor. Renal Physiology, section 8. Vol. II. Am Physiol Soc; Bethesda, MD: 1992. p. 1785-1815.
4. Murer H, Forster I, Biber J. The sodium phosphate cotransporter family SLC34. Pflugers Arch 2004;447:763–767. [PubMed: 12750889]
5. Hilfiker H, Hattenhauer O, Traebert M, et al. Characterization of a murine type II sodium-phosphate cotransporter expressed in mammalian small intestine. Proc Natl Acad Sci USA 1998;95:14564–14569. [PubMed: 9826740]
6. Murer H, Hernando N, Forster I, et al. Proximal tubular phosphate reabsorption: molecular mechanisms. Physiol Rev 2000;80:1373–1409. [PubMed: 11015617]
7. Segawa H, Kaneko I, Takahashi A, et al. Growth-related renal type II Na/Pi cotransporter. J Biol Chem 2002;277:19665–19672. [PubMed: 11880379]
8. Forster IC, Hernando N, Biber J, et al. Proximal tubular handling of phosphate: a molecular perspective. Kidney Int 2006;70:1548–1559. [PubMed: 16955105]
9. Beck L, Karaplis AC, Amizuka N, et al. Targeted inactivation of Npt2 in mice leads to severe renal phosphate wasting, hypercalciuria, and skeletal abnormalities. Proc Natl Acad Sci USA 1998;95:5372–5377. [PubMed: 9560283]
10. Hoag HM, Martel J, Gauthier C, et al. Effects of Npt2 gene ablation and low-phosphate diet on renal Na(+)/phosphate cotransport and cotransporter gene expression. J Clin Invest 1999;104:679–686. [PubMed: 10491403]
11. Guner YS, Kiela PR, Xu H, et al. Differential regulation of renal sodium-phosphate transporter by glucocorticoids during rat ontogeny. Am J Physiol Cell Physiol 1999;277:C884–C890.

12. Arar M, Baum M, Biber J, et al. Epidermal growth factor inhibits Na-Pi cotransport and mRNA in OK cells. Am J Physiol Renal Fluid Electrolyte Physiol 1995;268:F309–F314.
13. Arar M, Zajicek HK, Elshihabi I, et al. Epidermal growth factor inhibits Na-P_i cotransport in weaned and suckling rats. Am J Physiol Renal Physiol 1999;276:F72–F78.
14. Jankowski M, Biber J, Murer H. PTH-induced internalization of a type IIa Na/P_i cotransporter in OK-cells. Pflugers Arch 1999;438:689–693. [PubMed: 10555567]
15. Murer H, Forster I, Hernando N, et al. Posttranscriptional regulation of the proximal tubule NaPi-II transporter in response to PTH and dietary P_i. Am J Physiol Renal Physiol 1999;277:F676–F684.
16. Pfister MF, Forgo J, Ziegler U, et al. cAMP-dependent and -independent downregulation of type II Na-Pi cotransporters by PTH. Am J Physiol Renal Physiol 1999;276:F720–F725.
17. Taketani Y, Segawa H, Chikamori M, et al. Regulation of type II renal Na^+-dependent inorganic phosphate transporters by 1,25-dihydroxyvitamin D_3. Identification of a vitamin D-responsive element in the human NAPi-3 gene. J Biol Chem 1998;273:14575–14581. [PubMed: 9603973]
18. Alcalde AI, Sarasa M, Raldua D, et al. Role of thyroid hormone in regulation of renal phosphate transport in young and aged rats. Endocrinology 1999;140:1544–1551. [PubMed: 10098486]
19. Sorribas V, Markovich D, Verri T, et al. Thyroid hormone stimulation of Na/Pi-cotransport in opossum kidney cells. Pflugers Arch 1995;431:266–271. [PubMed: 9026788]
20. Bacic D, Capuano P, Baum M, et al. Activation of dopamine D1-like receptors induces acute internalization of the renal Na^+/phosphate cotransporter NaPi-IIa in mouse kidney and OK cells. Am J Physiol Renal Physiol 2005;288:F740–F747. [PubMed: 15547113]
21. Ambühl PM, Zajicek HK, Wang H, et al. Regulation of renal phosphate transport by acute and chronic metabolic acidosis in the rat. Kidney Int 1998;53:1288–1298. [PubMed: 9573544]
22. Collins JF, Bulus N, Ghishan FK. Sodium-phosphate transporter adaptation to dietary phosphate deprivation in normal and hypophosphatemic mice. Am J Physiol Gastrointest Liver Physiol 1995;268:G917–G924.
23. Schiavi SC, Kumar R. The phosphatonin pathway: new insights in phosphate homeostasis. Kidney Int 2004;65:1–14. [PubMed: 14675031]
24. Hattenhauer O, Traebert M, Murer H, et al. Regulation of small intestinal Na-P_i type IIb cotransporter by dietary phosphate intake. Am J Physiol Gastrointest Liver Physiol 1999;277:G756–G762.
25. Katai K, Miyamoto K, Kishida S, et al. Regulation of intestinal Na^+-dependent phosphate co-transporters by a low-phosphate diet and 1,25-dihydroxyvitamin D_3. Biochem J 1999;343:705–712. [PubMed: 10527952]
26. Xu H, Collins JF, Bai L, et al. Regulation of the human sodium-phosphate cotransporter NaP_i-IIb gene promoter by epidermal growth factor. Am J Physiol Cell Physiol 2001;280:C628–C636. [PubMed: 11171583]
27. Arima K, Hines ER, Kiela PR, et al. Glucocorticoid regulation and glycosylation of mouse intestinal type IIb Na-P_i cotransporter during ontogeny. Am J Physiol Gastrointest Liver Physiol 2002;283:G426–G434. [PubMed: 12121891]
28. Xu H, Uno JK, Inouye M, et al. Regulation of intestinal NaPi-IIb cotransporter gene expression by estrogen. Am J Physiol Gastrointest Liver Physiol 2003;285:G1317–G1324. [PubMed: 12893629]
29. Eiam-ong S, Kurtzman NA. Metabolic acidosis and bone disease. Miner Electrolyte Metab 1994;20:72–80. [PubMed: 8202056]
30. Lemann J, Adams ND, Wilz DR, et al. Acid and mineral balances and bone in familial proximal renal tubular acidosis. Kidney Int 2000;58:1267–1277. [PubMed: 10972690]
31. Domrongkitchaiporn S, Pongskul C, Sirikulchayanonta V, et al. Bone histology and bone mineral density after correction of acidosis in distal renal tubular acidosis. Kidney Int 2002;62:2160–2166. [PubMed: 12427141]
32. Weitzmann MN, Pacifici R. Estrogen deficiency and bone loss: an inflammatory tale. J Clin Invest 2006;116:1186–1194. [PubMed: 16670759]
33. Pacifici R. Estrogen, cytokines, and pathogenesis of postmenopausal osteoporosis. J Bone Miner Res 1996;11:1043–1051. [PubMed: 8854239]
34. Adami S, Gatti D, Bertoldo F, et al. The effects of menopause and estrogen replacement therapy on the renal handling of calcium. Osteoporos Int 1992;2:180–185. [PubMed: 1611223]

35. Citrin DL, Elson P, Kies MS, et al. Decreased serum phosphate levels after high-dose estrogens in metastatic prostate cancer. Possible implications. Am J Med 1984;76:787–793. [PubMed: 6539069]
36. Stock JL, Coderre JA, Mallette LE. Effects of a short course of estrogen on mineral metabolism in postmenopausal women. J Clin Endocrinol Metab 1985;61:595–600. [PubMed: 2993339]
37. Castelo-Branco C, Martínez de Osaba MJ, Pons F, et al. The effect of hormone replacement therapy on postmenopausal bone loss. Eur J Obstet Gynecol Reprod Biol 1992;44:131–136. [PubMed: 1587378]
38. Uemura H, Irahara M, Yoneda N, et al. Close correlation between estrogen treatment and renal phosphate reabsorption capacity. J Clin Endocrinol Metab 2000;85:1215–1219. [PubMed: 10720065]
39. Beers KW, Thompson MA, Chini EN, et al. Beta-estradiol inhibits Na^+-P(i) cotransport across renal brush border membranes from ovariectomized rats. Biochem Biophys Res Commun 1996;221:442–445. [PubMed: 8619874]
40. Hagenfeldt Y, Eriksson HA. The estrogen receptor in the rat kidney. Ontogeny, properties and effects of gonadectomy on its concentration. J Steroid Biochem 1988;31:49–56. [PubMed: 3398528]
41. Davidoff M, Caffier H, Schiebler TH. Steroid hormone binding receptors in the rat kidney. Histochemistry 1980;69:39–48. [PubMed: 7440259]
42. Edwards DP. Regulation of signal transduction pathways by estrogen and progesterone. Annu Rev Physiol 2005;67:335–376. [PubMed: 15709962]
43. Nawaz Z, Lonard DM, Dennis AP, et al. Proteasome-dependent degradation of the human estrogen receptor. Proc Natl Acad Sci USA 1999;96:1858–1862. [PubMed: 10051559]
44. Riggs BL, Jowsey J, Kelly PJ, et al. Effect of sex hormones on bone in primary osteoporosis. J Clin Invest 1969;48:1065–1072. [PubMed: 5771187]
45. Stock JL, Coderre JA, Posillico JT. Effects of estrogen on mineral metabolism in postmenopausal women as evaluated by multiple assays measuring parathyrin bioactivity. Clin Chem 1989;35:18–22. [PubMed: 2535973]
46. Palmer K, Gray JM. Central vs peripheral effects of estrogen on food intake and lipoprotein lipase activity in ovariectomized rats. Physiol Behav 1986;37:187–189. [PubMed: 3737718]
47. Nance DM. The developmental and neural determinants of the effects of estrogen on feeding behavior in the rat: a theoretical perspective. Neurosci Biobehav Rev 1983;7:189–211. [PubMed: 6348605]
48. Wade GN, Gray JM. Gonadal effects on food intake and adiposity: a metabolic hypothesis. Physiol Behav 1979;22:583–593. [PubMed: 379889]
49. Lee SH, Mouradian MM. Up-regulation of D1A dopamine receptor gene transcription by estrogen. Mol Cell Endocrinol 1999;156:151–157. [PubMed: 10612433]
50. Agrati P, Ma ZQ, Patrone C, et al. Dopaminergic phenotype induced by oestrogens in a human neuroblastoma cell line. Eur J Neurosci 1997;9:1008–1016. [PubMed: 9182953]
51. Shimada T, Urakawa I, Yamazaki Y, et al. Impairment in NaPi-IIa transport activity should correlate with the decrease in its expression to account for increased Pi excretion in EST-injected rats. Biochem Biophys Res Commun 2004;314:409–414. [PubMed: 14733920]
52. Murono EP, Kirdani RY, Sandberg AA. Specific estradiol-17 beta binding component in adult rat kidney. J Steroid Biochem 1979;11:1347–1351. [PubMed: 513757]
53. Wijayaratne AL, McDonnell DP. The human estrogen receptor-alpha is a ubiquitinated protein whose stability is affected differentially by agonists, antagonists, and selective estrogen receptor modulators. J Biol Chem 2001;276:35684–35692. [PubMed: 11473106]
54. Calligé M, Richard-Foy H. Ligand-induced estrogen receptor alpha degradation by the proteasome: new actors? Nucl Recept Signal 2006;4:e004. [PubMed: 16604167]
55. Amlal H, Faroqui S, Balasubramaniam A, et al. Estrogen up-regulates neuropeptide Y Y1 receptor expression in a human breast cancer cell line. Cancer Res 2006;66:3706–3714. [PubMed: 16585197]
56. Björnström L, Sjöberg M. Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes. Mol Endocrinol 2005;19:833–842. [PubMed: 15695368]
57. Funakoshi T, Yanai A, Shinoda K, et al. G protein-coupled receptor 30 is an estrogen receptor in the plasma membrane. Biochem Biophys Res Commun 2006;346:904–910. [PubMed: 16780796]

58. Filardo EJ, Quinn JA, Frackelton AR Jr, et al. Estrogen action via the G protein-coupled receptor, GPR30: stimulation of adenylyl cyclase and cAMP-mediated attenuation of the epidermal growth factor receptor-to-MAPK signaling axis. Mol Endocrinol 2002;16:70–84. [PubMed: 11773440]

59. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. Anal Biochem 1987;162:156–159. [PubMed: 2440339]

60. Church GM, Gilbert W. Genomic sequencing. Proc Natl Acad Sci USA 1984;81:1991–1995. [PubMed: 6326095]

61. Wilke C, Sheriff S, Soleimani M, et al. Vasopressin-independent regulation of collecting duct aquaporin-2 in food deprivation. Kidney Int 2005;67:201–216. [PubMed: 15610244]

62. Amlal H, Wilke C. Resistance of mTAL Na^+-dependent transporters and collecting duct aquaporins to dehydration in 7-month-old rats. Kidney Int 2003;64:544–554. [PubMed: 12846749]

63. Zajicek HK, Wang H, Puttaparthi K, et al. Glycosphingolipids modulate renal phosphate transport in potassium deficiency. Kidney Int 2001;60:694–704. [PubMed: 11473652]

64. Sorribas V, Halaihel N, Puttaparthi K, et al. Gentamicin causes endocytosis of Na/Pi cotransporter protein (NaPi-2). Kidney Int 2001;59:1024–1036. [PubMed: 11231357]

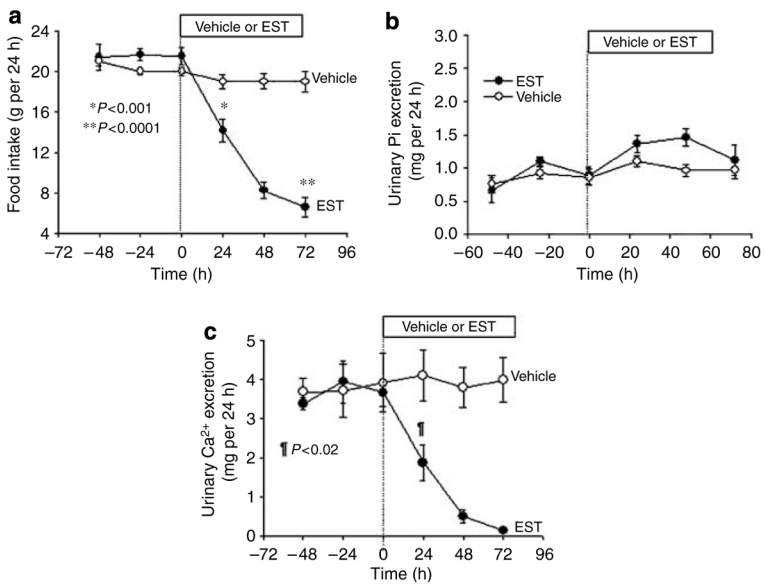

Figure 1. Effects of EST on food intake and urinary Pi and calcium excretion

OVX rats were placed in metabolic cages and had free access to regular rat chow and distilled water. After 3 days, rats were injected with EST or its vehicle, and (**a**) food intake, (**b**) urinary inorganic phosphate (Pi) excretion, and (**c**) urinary calcium excretion were monitored daily for an additional 3 days. $n = 4$ rats in each group. Significance between baseline and EST treatment at different time points is indicated.

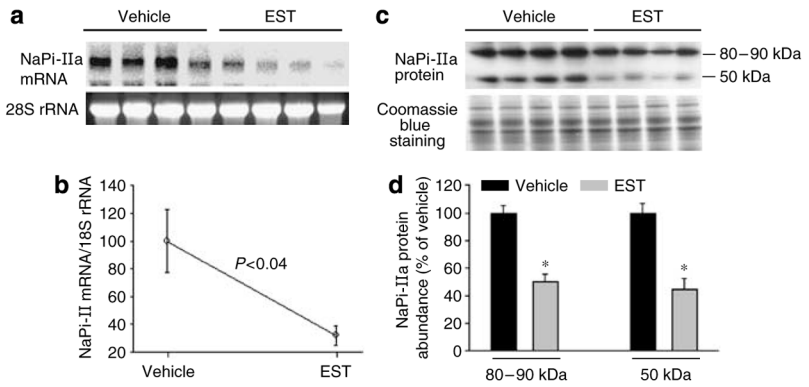

Figure 2. mRNA expression and protein abundance of NaPi-IIa

(**a**) Northern hybridization of NaPi-IIa mRNA in the renal cortex of rats injected with EST vs vehicle. 28S rRNA was used as a constitutive gene for the control of the equity of RNA loading into northern gels. (**b**) Corresponding densitometric analysis showing the mean of NaPi-IIa mRNA-to-28S rRNA ratio. (**c**) Immunoblot showing the abundance of NaPi-IIa protein in membrane fractions isolated from renal cortex of vehicle- and EST-treated rats (upper panel), and a Coomassie blue-stained gel picture showing the equity of protein loading (lower panel). (**d**) The average of the densitometric analysis of NaPi-IIa bands (50 and 80–90 kDa). Each lane was loaded with 30 μg of total RNA or 25 μg membrane proteins from a different rat. $n = 4$ rats in each group, $^{*}P<0.01$ vs vehicle.

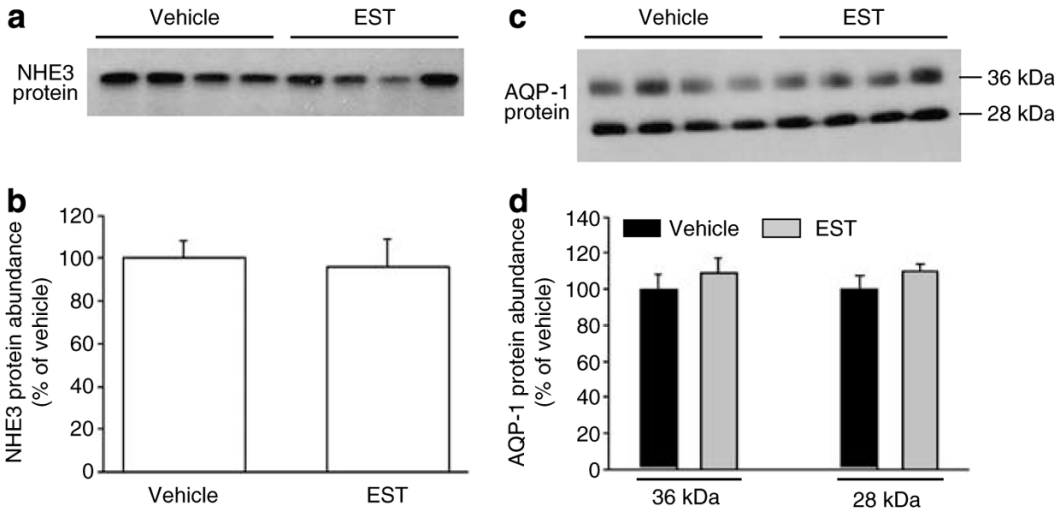

Figure 3. Protein abundance of NHE3 and AQP-1

(**a** and **c**) Immunoblots showing the abundance of NHE3 and AQP-1 water channel proteins in membrane fractions isolated from renal cortex of vehicle- and EST-treated rats. (**b** and **d**) The average of the densitometric analysis of NHE3 and AQP-1 (28 kDa) bands. *n* = 4 rats in each group. Each lane was loaded with 40 μg (NHE3) or 5 μg (AQP-1) of membrane proteins from a different rat.

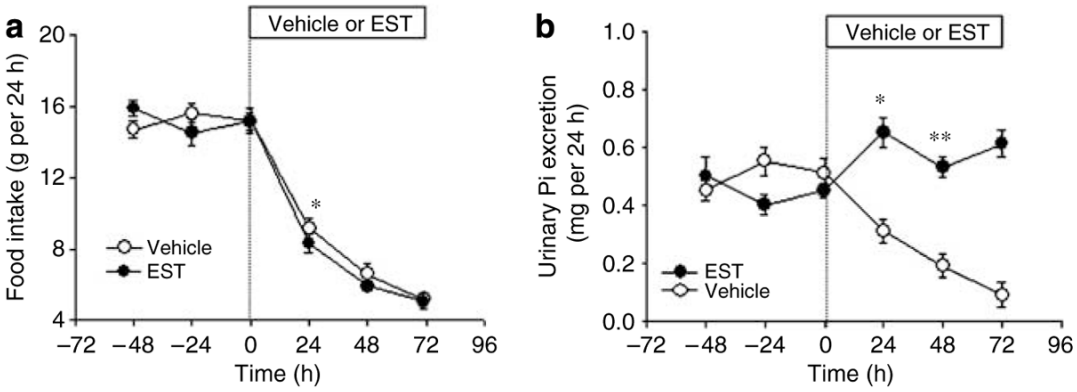

Figure 4. Effects of EST on food intake and urinary Pi excretion in a PF protocol

OVX rats were placed in metabolic cages and had free access to regular rat chow and distilled water. After 3 days, rats were injected with EST or its vehicle. At time zero, vehicle-treated rats received the same amount of food (PF) that is consumed by EST-injected rats. (**a**) Food intake and (**b**) urinary inorganic phosphate (Pi) excretion were monitored daily for an additional 3 days. $n = 4$ rats in each group. Significance between baselines and EST or vehicle treatments at different time points is indicated. *$P<0.01$ and **$P<0.001$ vs vehicle.

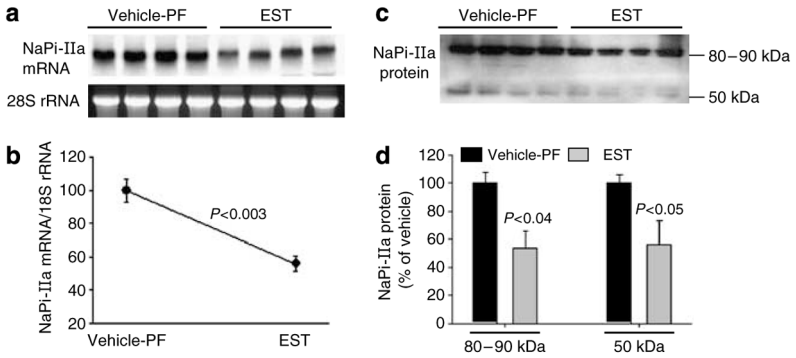

Figure 5. mRNA expression and protein abundance of NaPi-IIa in a PF protocol

(**a**) Northern hybridization of NaPi-IIa mRNA in the renal cortex of rats injected with EST vs vehicle. (**b**) Corresponding densitometric analysis showing the mean of NaPi-IIa mRNA-to-28S rRNA ratio. (**c**) Immunoblot showing the abundance of NaPi-IIa protein in the renal cortex of vehicle- and EST-treated rats. (**d**) The average of the densitometric analysis of NaPi-IIa bands (50 and 80–90 kDa). Each lane was loaded with 30 μg of total RNA or 25 μg membrane proteins from a different rat. $n = 4$ rats in each group. Significance between groups is indicated.

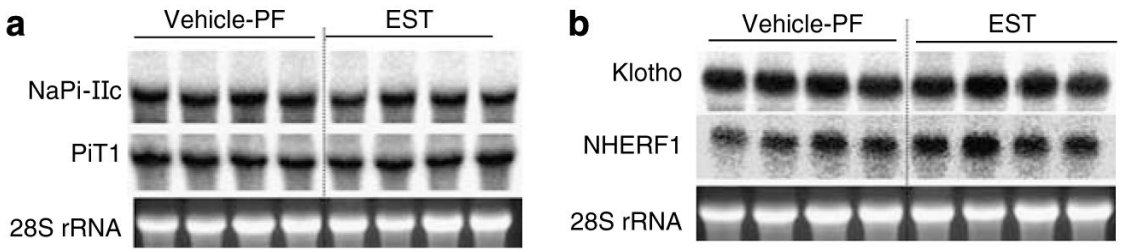

Figure 6. mRNA expression of NaPi-IIc, PiT1 Klotho, and NHERF1 in the renal cortex of OVX rats

(**a**) Northern hybridization of NaPi-IIc and PiT1 and 28S rRNA in the renal cortex of rats injected with EST vs vehicle. (**b**) Northern hybridization of klotho and NHERF1 and 28S rRNA in the renal cortex of rats injected with EST vs vehicle. Each lane was loaded with 30 μg of total RNA harvested from a different rat. $n = 4$ rats in each group.

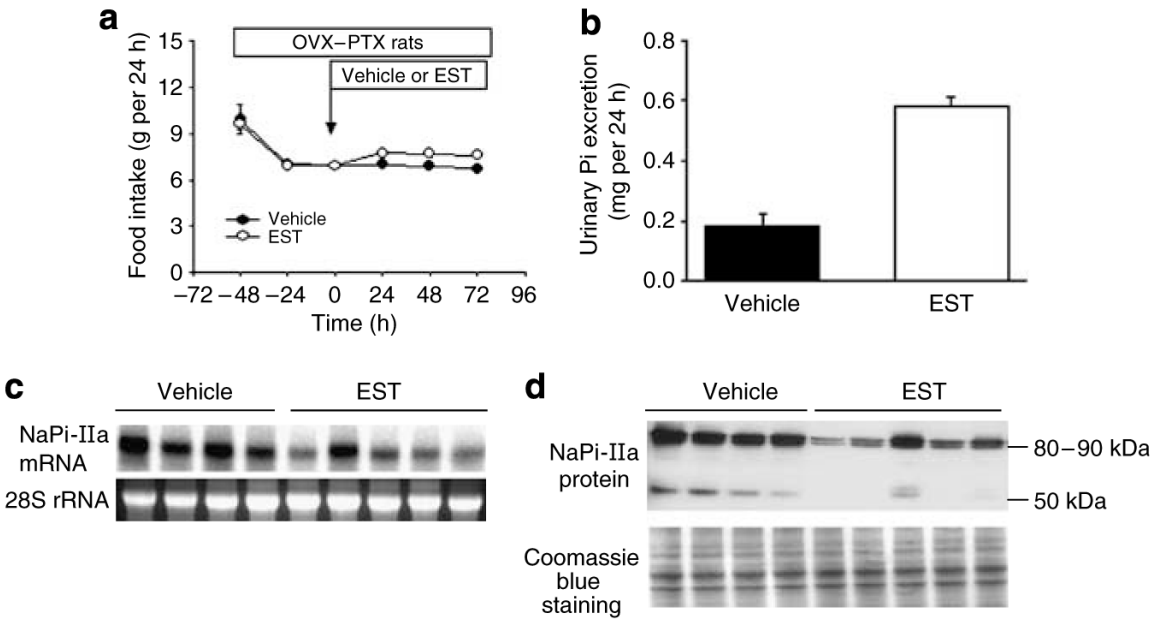

Figure 7. Food intake, Pi excretion, and NaPi-IIa expression in OVX–PTX rats

OVX–PTX rats were placed in metabolic cages and had free access to food and water. After 3 days, rats were injected with EST or its vehicle and monitored for another 3 days. (**a**) Food intake measured daily. (**b**) Urinary Pi excretion measured on the last day of the treatment. (**c**) Northern hybridization of NaPi-IIa mRNA in the renal cortex of rats injected with EST vs vehicle. (**d**) Immunoblot showing the abundance of NaPi-IIa protein in the renal cortex of vehicle- and EST-treated rats. Each lane was loaded with 30 μg of total RNA or 25 μg membrane proteins from a different rat. n = 4–5 rats in each group.

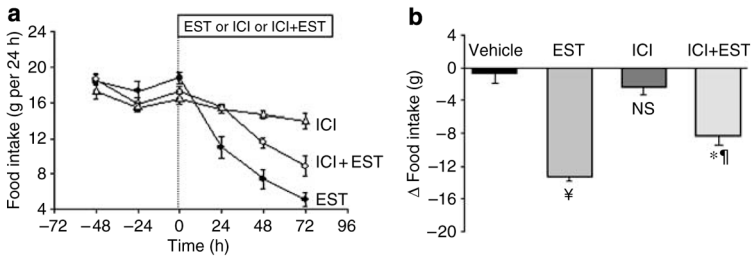

Figure 8. Effect of EST on food intake in the presence or absence of ER*α* inhibitor

(**a**) OVX rats were placed in metabolic cages and had free access to food and distilled water. After 3 days, rats were divided into four groups and treated as follows: vehicle, EST alone, ICI182,780 alone, and ICI + EST as described in Materials and Methods. Food intake was then monitored daily for another 3 days. (**b**) Δ Food intake is calculated as the difference between baseline food intake (time zero) and food intake on the last day of the treatment (time 72 h). $^{¥}P<0.001$ vs vehicle, $*P<0.02$ vs ICI, $P<0.04$ vs EST. NS indicates not significant with $P>0.05$ vs vehicle.

Table 1

Blood composition of OVX rats treated with EST or its vehicle for 3 days

	Glucose (mg/dl)	BUN (mg/dl)	Creatinine (mg/dl)	Na^+ (mEq/l)	K^+ (mEq/l)	Cl^- (mEq/l)	CO_2 (mEq/l)	Pi (mEq/l)
Vehicle	154±8.34	20±1.5	0.35±0.03	142±0.81	5.65±0.10	100±0.65	20±2.7	7.78±0.29
EST	139±3.6	17±1.6	0.36±0.02	140±0.20	5.62±0.20	100±0.50	20±0.40	7.02±0.17
P	NS	NS	NS	NS	NS	NS	NS	0.049

BUN, blood urea nitrogen; EST, estrogen; NS, nonsignificant; OVX, ovariectomized.

Data are mean±s.e., *n*=4 rats in each group.

Table 2

Effects of EST on NaPi-IIa mRNA expression and protein abundance in the presence of ERα blocker ICI182,780

	NaPi-IIa	
	mRNA	**Protein**
Vehicle	100±5.5	100±6
ICI182,780	85±3.4	95±4
ICI+EST	46±1.36[a,b]	51±7[a,c]

ERα, estrogen receptor-α; EST, estrogen.

Data are mean±s.e. expressed as % of vehicle. n=3–4 rats in each group.

[a] $P<0.001$ vs ICI alone.

[b] $P<0.0001$ vs vehicle.

[c] $P<0.03$ vs vehicle.