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The Human Response to Acute Enteral and Parenteral Phosphate Loads

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ABSTRACT

The human response to acute phosphate (PO4) loading is poorly characterized, and it is unknown whether an intestinal phosphate sensor mechanism exists. Here, we characterized the human mineral and endocrine response to parenteral and duodenal acute phosphate loads. Healthy human participants underwent 36 hours of intravenous (IV; 1.15 [low dose] and 2.30 [high dose] mmol of PO4/kg per 24 hours) or duodenal (1.53 mmol of PO4/kg per 24 hours) neutral sodium PO4 loading. Control experiments used equimolar NaCl loads. Maximum PO4 urinary excretory responses occurred between 12 and 24 hours and were similar for low-dose IV and duodenal infusion. Hyperphosphatemic responses were also temporally and quantitatively similar for low-dose IV and duodenal PO4 infusion. Fractional renal PO4 clearance increased approximately 6-fold (high-dose IV group) and 4-fold (low-dose IV and duodenal groups), and significant reductions in plasma PO4 concentrations relative to peak values occurred by 36 hours, despite persistent PO4 loading. After cessation of loading, frank hypophosphatemia occurred. The earliest phosphaturic response occurred after plasma PO4 and parathyroid hormone concentrations increased. Plasma fibroblast growth factor-23 concentration increased after the onset of phosphaturia, followed by a decrease in plasma 1,25(OH)2D levels; α-Klotho levels did not change. Contrary to results in rodents, we found no evidence for intestinal-specific phosphaturic control mechanisms in humans. Complete urinary phosphate recovery in the IV loading groups provides evidence against any important extrarenal response to acute PO4 loads.

J Am Soc Nephrol 25: 2730–2739, 2014. doi: 10.1681/ASN.2013101076

Plasma phosphate (PO4) concentration is regulated within narrow limits and is the result of intestinal absorption, influx into and efflux from bone, renal excretion, and modest intestinal secretion.[1] Rapid changes in transcellular distribution are effected primarily by systemic acid-base equilibrium and hormones such as insulin and catecholamines.[2] $1,25(OH)_2D$ stimulates intestinal PO4 absorption,[4] while parathyroid hormone (PTH) and osteocyte-derived fibroblast growth factor-23 (FGF-23) are the best-characterized phosphaturic factors. Both inhibit proximal tubular sodium-dependent PO4 reabsorption (*via* Na/PO4 cotransport, NaPi2a and NaPi2c).[5,6] In addition, α-Klotho is a renal transmembrane and secreted protein that functions as an FGF-23 coreceptor and is phosphaturic independently of FGF-23.[7] However, the relative roles of other phosphaturic agents, such as secreted frizzled related protein (sFRP-4), matrix extracellular phosphoglycoprotein, and FGF-8 are not yet defined in humans.

$1,25(OH)_2D$, PTH, and FGF-23 regulate PO4 metabolism within a complex system of positive and negative feedback mechanisms (for review, see Bergwitz and Jüppner[8]). Increases in plasma PO4 concentration stimulate PTH secretion, while proximal tubule 1α-hydroxylase and, therefore,

Received October 14, 2013. Accepted March 13, 2014.

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Published online ahead of print. Publication date available at www.jasn.org.

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production of 1,25$(OH)_2$D are inhibited, resulting in both increased renal excretion of PO4 and inhibition of intestinal PO4 resorption. Dietary PO4 loading increases plasma FGF-23 in mice,[9] but the FGF-23 response in humans is unclear because both increases and no changes were reported in short- and long-term phosphate-loading protocols.[10–15] A hyperphosphatemia-mediated increase in FGF-23, if confirmed in humans, might also directly inhibit sodium-dependent intestinal PO4 resorption by reducing intestinal NaPi2b transporter protein. However, when examined in mice, the effect of FGF-23 to suppress intestinal NaPi2b protein was vitamin D receptor dependent.[16] The net effect of PO4 loading on 1,25$(OH)_2$D in healthy humans is difficult to predict because hyperphosphatemia and increased FGF-23 were demonstrated to inhibit while increased PTH was shown to stimulate 1α-hydroxylase.[17–19] In addition, FGF-23 is positively regulated by 1,25$(OH)_2$D, PTH, and Klotho.[20–22] This complexity of the PO4–FGF-23 cybernetic system, coupled with the indeterminate evidence for an FGF-23 response to phosphate loads in humans, makes it difficult to predict the relative importance of these four hormones and the temporal relation of their response in the elimination of a PO4 load.

The sensing mechanisms by which changes in extracellular PO4 concentration signal to elicit changes in PO4 transporter activities and hormone secretion are largely unknown. In mice responding to increases and decreases in phosphate intake, a PO4 response element has been reported in the *NPT2a* gene, and an associated transcription factor, μE3, has been identified.[23] Nevertheless, it remains unclear whether the most proximal phosphate sensing signal is extracellular or intracellular.[24] Uncertainties remain regarding PO4 sensing mechanisms in the parathyroid gland, osteocytes/osteoblasts, and renal cortical 1α-hydroxylase.[25]

Recent experiments in rats have demonstrated an intestinal PO4 sensing mechanism that results in acute phosphaturia. A duodenal luminal PO4 infusion in rats produced a PO4 anion–specific phosphaturic response before measureable increases in plasma PO4, FGF-23, PTH, and secreted frizzled related protein concentrations.[26] Taken together with the acute phosphaturic response to systemic application of PO4-exposed homogenates of duodenal mucosa, potent intestinal phosphaturic factors were postulated. The authors suggested that the intestinal response to dietary PO4 loads might prevent the deleterious systemic effects of PO4 loads in humans and that it might also be important for chronic renal regulation of human PO4 homeostasis. The nature of this apparent intestinal sensing mechanism in rodents and whether it exists in humans are unknown.

The aims of the present studies were, therefore, to characterize in healthy humans the renal, systemic, and endocrine responses to an acute PO4 load and to investigate its dependency on the dose and the route of administration (*i.e.,* enteral versus parenteral). To address these issues, we evaluated the PO4 excretory response and its endocrine correlates using two quantitatively different intravenous (IV) PO4 loads in healthy humans. We compared these effects to the response evoked by a similar systemic PO4 load administered directly into the duodenum.

RESULTS

As shown in Figure 1, A–C, plasma PO4 was significantly increased at 2 hours after the start of both IV and duodenal infusions. Quantitatively, the duodenal and the low-dose intravenous load of PO4 induced an almost identical rise and time course in plasma PO4. An acute steady state was reached between 4 and 24 hours. After that time point, plasma PO4 concentrations decreased (a significant change in the intravenous protocols) despite constant PO4 infusion rates.

As expected, the plasma ionized calcium concentration significantly decreased by 0.18±0.03 mmol/L after 24 hours in the IV high-dose group and to a smaller but also significant extent in both the duodenal and IV low-dose groups (Figure 1, A–C). Consistent with the reduced filtered calcium load and PTH response, urinary calcium excretion decreased significantly in all PO4-treated groups compared with NaCl controls.

In response to both hyperphosphatemia and hypocalcemia, PTH increased significantly by 2 hours after the beginning of the high-dose IV PO4 load in the IV high-dose group and reached a maximum after 24 hours. In the IV low-dose and duodenal groups, PTH increased significantly between 8 and 12 hours (Figure 1). α-Klotho levels were not affected significantly by any of the three PO4 loading protocols. FGF-23 increased after the rise in PTH in all three groups. In particular, in the duodenal perfusate group, both plasma PO4 and serum PTH, but not FGF-23, concentrations were significantly increased when urinary phosphate excretion started to rise significantly after 4 hours (see Figure 2). 1,25$(OH)_2$D decreased significantly even later (*i.e.,* after 36 hours in both IV-loaded groups and after 48 hours in the intestinally loaded group) (Figure 1). In all groups, plasma 1,25$(OH)_2$D returned to baseline values within 36 hours after the end of all PO4 loading periods. The PO4 load induced no significant changes in insulin and IGF-1 serum concentrations at any time point of the PO4 loads in all three PO4 loading protocols and the NaCl control experiments (data not shown).

Figure 2 shows no difference in the urinary excretory pattern among the low-dose IV and duodenal PO4 loads. Comparing urinary PO4 excretion with baseline values, urinary excretion increased significantly in the 2- and 4-hour collection periods in participants loaded with the lower IV PO4 dose. In contradistinction, the rise in urinary PO4 became significant only after 4 hours in the duodenal perfusate group. The maximum increase in excretion rate was reached between 12 and 24 hours from the beginning of the infusion in the IV-low dose (7.5±1.7 mmol/2 hours) and the duodenal (6.9±0.5 mmol/2 hours) groups.

Figure 3, A–D, delineates the timed urinary PO4 elimination as a function of the change in plasma [PO4] 2, 4, 8, and 12 hours after the start of the three different PO4 loading

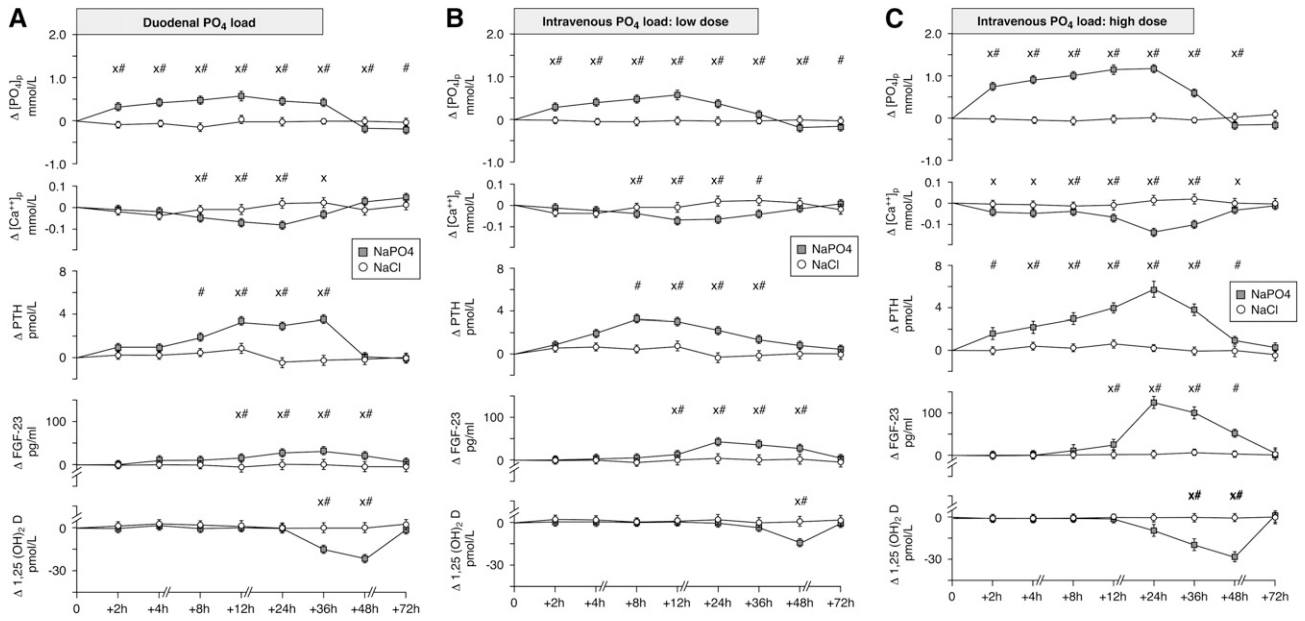

Figure 1. Intavenous and duodenal PO4 loading induce qualitatively similar effects on plasma PO4 and phosphrtropic hormones. (A–C) Effect of 36 hours of duodenal PO4 (1.53 mmol/kg body wt per 24 hours), of low-dose IV (1.15 mmol/kg body wt per 24 hours), and high-dose IV (2.30 mmol/kg body wt per 24 hours) loading on changes in plasma [PO4] and ionized [Ca], as well as changes in intact PTH) FGF-23, and 1,25$(OH)_2$D. Filled symbols are the protocols using neutral sodium PO4, open symbols are the protocols using equimolar amounts of NaCl. $\times P<0.05$ for the comparison to own baseline; $\#P<0.05$ for the comparison to the NaCl control.

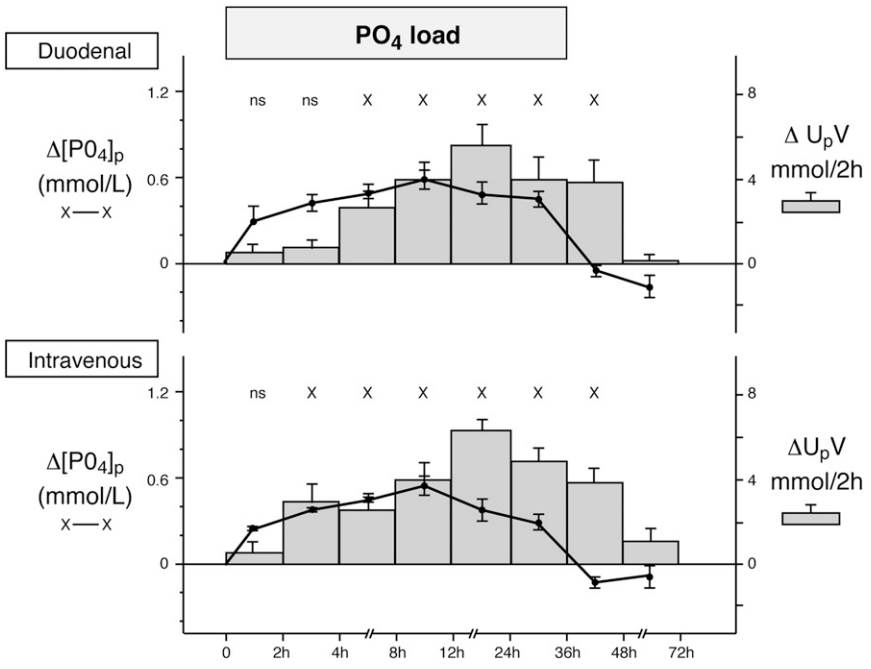

Figure 2. No difference in the urinary excretory pattern among the low-dose IV and duodenal PO4 loads. Time course of the increase in plasma [PO4] and the mean increase of urinary PO4 excretion per 2 hours during (0–36 hours) and after (36–72 hours) PO4 loading in the duodenal (upper panel) and low-dose IV group (lower panel). $\times P<0.05$ for the comparison to own baseline. UpV, denotes the amount in mmols of PO4 excreted during the colllection period.

protocols. In the duodenal perfusate group, urinary PO4 excretion lagged behind the low-dose IV group at 4 hours despite an identical increase in plasma [PO4]. At all other time points, there was no significant difference among these two groups. The figure also highlights a clear dose-response relationship among phosphate load and increases in plasma [PO4] as well as urinary PO4 excretion (increases in the low-dose group were approximately 50% of that observed in the high-dose IV protocol).

Figure 4 shows that the fractional renal PO4 clearance increased progressively in all three groups, with clearance rates during recovery (*i.e.*, 48 and 72 hours) still significantly above baseline values. The sharp increase in renal clearance induced a later decrease from peak plasma [PO4] despite constant PO4 infusion rates (Figure 1, A–C, Table 1) and caused frank hypophosphatemia in the recovery periods.

Figure 5 shows the cumulative increase in urinary phosphate excretion and the magnitude of the phosphate load for all three protocols by comparing the duodenal infusion group with the combined IV loading groups. Observations for these

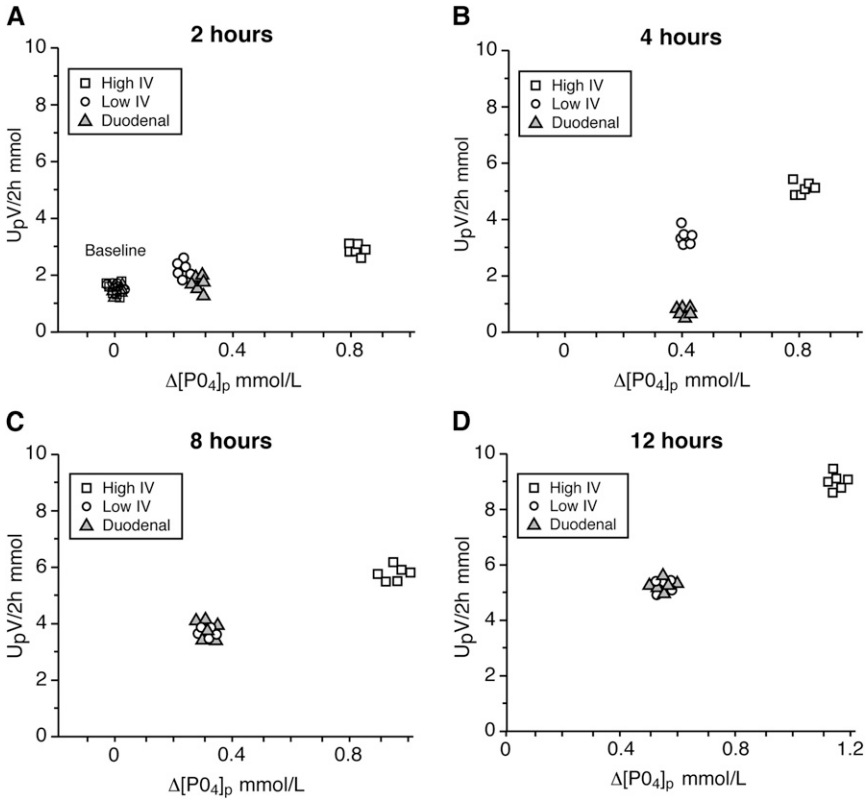

Figure 3. Timed renal PO4 excretion is similarly dependent on plasma [PO4] in duodenal, low and high dose intravenous groups. (A–D) Relationships of the individual changes in urinary PO4 excretion per 2 hours on the corresponding changes in plasma [PO4] in all experimental groups. Time points are 2, 4, 8, and 12 hours after the beginning of PO4 loading. UpV, denotes the amount in mmols of PO4 excreted in urine for the given plasma [PO4].

calculations were carried out for a total of 120 hours after the beginning of the PO4 loading. For the intravenous low- and high-dose groups, urinary recovery of PO4 accounted for 100% of the PO4 administered, thus excluding an extrarenal elimination mechanism (*e.g.,* gastrointestinal secretion). In the duodenal perfusion group, 73% of the PO4 administered was recovered in the urine. Thus, we can estimate fractional gastrointestinal absorption rate of PO4 at approximately 70%, a value in close agreement to the earlier observations by Fine *et al.*[27]

As illustrated by Table 1, neutral PO4 loading induced metabolic alkalosis in all groups, while the high-dose NaCl control induced mild metabolic acidosis. As described previously[27,28] and shown in Table 2, the metabolic alkalosis in response to neutral phosphate loading was of renal origin as renal net acid excretion (NAE) increased significantly, largely because of a significant rise in urinary titratable acid excretion. Metabolic alkalosis (increase in plasma HCO_3^- in the first 24 hours) was nominally more pronounced (ΔHCO3, 1.7±0.4 in duodenal group versus 0.5±0.3 in the low-dose IV group; P=0.041) in the duodenal perfusate group with a nominally but not statistically significantly higher renal NAE response (ΔNAE, 45±8 mEq/24 hours in the duodenal group versus 37±9 mEq/24 hours in the low-dose IV group; $P<0.06$ for the first 24 hours) compared with the low-dose intravenous group.

DISCUSSION

The present studies analyzed the renal and systemic mineral, acid-base, electrolyte, and endocrine responses to two different intravenous PO4 loads and compared the effects with those of an intestinal PO4 load. The PO4 loading studies were conducted with further detailed control by a protocol administering equimolar amounts of sodium as NaCl using a blinded cross-over design. The main results were as follows: (*1*) In healthy humans, similar systemic PO4 loads elicited a nearly identical phosphaturic response, both quantitatively and temporally, irrespective of the mode of administration (IV versus intestinal). (*2*) Systemic PO4 loads (both IV and intestinal) elicited dose-dependent changes in plasma PO4 concentration, PTH, FGF-23, and 1,25 $(OH)_2D$, characterized by a reproducible time course: The early increase in plasma PTH is followed by an increase in plasma FGF-23, with a subsequent reversible suppression of plasma 1,25$(OH)_2D$. Of note, α-Klotho levels did not change significantly. (*3*) On the basis of the progressive rise in fractional PO4 clearance rates, the adaptive renal phosphaturic response was of sufficient potency as to return elevated plasma [PO4] toward normal levels despite constant PO4 loading. In addition, renal PO4 clearance remained elevated sufficiently in the recovery period that it resulted in frank hypophosphatemia through at least 36 hours of observation. (*4*) The studies confirm intestinal absorption of PO4 to be approximately 73% of the administered intestinal dose[27] and demonstrate complete and exclusive renal excretion of IV PO4 loads, with no evidence for extrarenal elimination (*e.g.,* gut secretion).

The two different IV PO4 loads resulted in rapid and dose-dependent increases in plasma [PO4] (Figure 1), with PTH being the first of the phosphaturic hormones to rise. The PTH response was due in part to hyperphosphatemia (early rise in the absence of significant ionized hypocalcemia in the duodenal PO4 loading protocol; Figure 1)[28,29] and to the expected hypocalcemia in response to hyperphosphatemia/PO4 load. The rise in FGF-23 occurred with a latency of about 10 hours after the first documented significant rise in plasma PO4 and also distinctly later than the first documented significant rise in PTH and in phosphaturia in all protocols. In addition, the

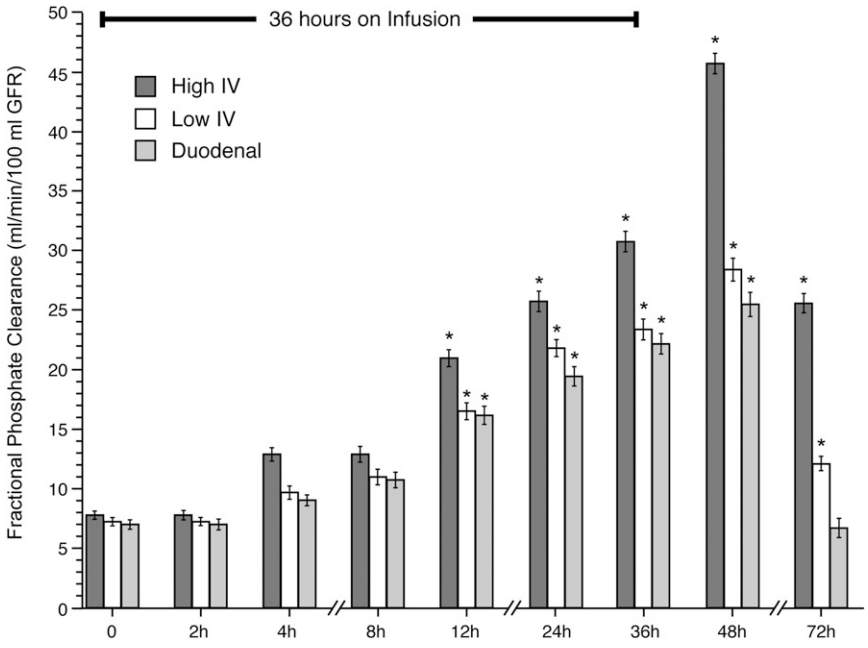

Figure 4. Fractional renal phosphate clearance progressively increases during constant PO4 loading in all three groups. Renal fractional PO4 clearance (per 100 ml GFR) during (0–36 hours) and after (36–72 hours) PO4 loading in all three experimental groups. *$P<0.05$ for comparison to own baseline.

rise in FGF-23 occurred independently of α-Klotho, which we found to be unaffected by the PO4 loads (Table 3). Thus, if PO4 *per se* is an important direct secretagogue for FGF-23,[14] the temporal response of FGF-23 to a phosphate load appears to be much slower than for effects on PTH. In addition or alternatively, PO4 might not be an important signal for FGF-23 secretion and the FGF-23 response might critically depend on the antecedent rise in PTH, at least in human participants with normal renal and parathyroid function. Such an interpretation is in accord with previous cell culture experiments which found that osteocytes/osteoblasts cannot be stimulated directly by extracellular PO4 to produce FGF-23.[25] Similarly, through use of a murine adenine/high PO4 diet model of CKD, it was reported that prior parathyroidectomy abrogated the CKD and hyperphosphatemia-induced increase in plasma FGF-23 concentration.[30]

PTH, *via* PTH receptor1 (PTHR1) activation, is reported to increase murine osteocyte FGF-23 expression *in vitro* and to increase murine plasma FGF-23 concentration *via* a PTHR1 ligand (*e.g.,* PTH) or constitutive activation of PTHR1.[31] Similarly, in humans potent PTHR1-mediated increases in plasma FGF-23 concentration have been demonstrated in Jansen metaphyseal chondroplasia, a disorder characterized by constitutive activating mutations in *PTHR1*. It would, therefore, be of considerable mechanistic interest to differentiate the relative potencies of a primary extracellular PO4 and primary PTH signals by investigating the FGF-23 response to PO4 loads in hypoparathyroid patients or patients with total parathyroidectomy.

A later event in the response to all investigated PO4 loads, was the significant and reversible decrease in $1,25(OH)_2D$ (Figure 1). This decrease is best explained both by the increase in FGF-23 and by hyperphosphatemia, which quantitatively overrides a concomitant stimulatory effect of PTH on proximal tubule 1α-hydroxylase. FGF-23 acts on 1α-hydroxylase to reduce the serum levels of $1,25(OH)_2D$.[32,33] In addition, PO4 is an independent inhibitor of 1,25(OH)2D production.[19]

The almost perfect correspondence between the increase in plasma PO4 and the phosphaturic response in the IV low-dose and duodenal groups suggests that, in humans, there are no significant differences in PO4 handling that depend on the route of administration. In particular, the phosphaturic response to the duodenal PO4 load occurred after the significant rise in plasma PO4 concentration, which resulted in an increase in the filtered load, in parallel with the phosphaturic effect of PTH. If anything, the phosphaturic response to the intestinal PO4 load occurred later than the response to the similar IV load, suggesting that—in contrast to the findings in rats[26]—there is no evidence in healthy humans that a quantitatively important intestinal signal might exhibit an independent phosphaturic effect. Within the ethical constraints of a study examining healthy humans, we could not analyze the phosphaturic response continuously (*i.e.,* by placing an indwelling urinary catheter). Therefore, we could have missed a very early phosphaturic response and thus a difference among the renal response to the IV or intestinal PO4 loads. However, as illustrated by Figure 2, such a mechanism, even if present in humans, is likely to be of minor quantitative importance and, therefore, of questionable clinical or physiologic importance. We have no obvious explanation for the observation that the significant change in phosphaturia occurred slightly later in the duodenal than in the low-dose IV group despite similar increases in plasma [PO4].

The late fall in plasma PO4 concentrations despite persistent and constant PO4 loading conditions is evident in all protocols (Figure 1). As illustrated by Figure 4, renal adaptation resulted in a progressive increase in renal fractional PO4 clearance. This observation indicates that in persons with normal renal function the phosphaturic response is so potent that normophosphatemia is restored within 1–2 days (altered renal set-point for PO4 excretion) despite continued high phosphate loads. To definitively characterize this response, longer periods (beyond 36 hours) of constant PO4 loading should be evaluated. In addition, the high fractional PO4 clearance was responsible for renal hypophosphatemia in the recovery period.

Table 1. Effect of IV and duodenal PO4 loading and of NaCl on plasma electrolytes, creatinine clearance, body weight, and blood acid-base parameters.

Variable	Baseline					24 hr					48 hr					72 hr				
	IV High Dose	NaCl High Dose	IV Low Dose	Duodenal	NaCl Low Dose	IV High Dose	NaCl High Dose	IV Low Dose	Duodenal	NaCl Low Dose	IV High Dose	NaCl High Dose	IV Low Dose	Duodenal	NaCl Low Dose	IV High Dose	NaCl High Dose	IV Low Dose	Duodenal	NaCl Low Dose
Na^+ (mmol/L)	138.1± 0.5	138.4± 0.4	137.7± 0.5	138.9± 0.5	138.3± 0.4	138.7± 0.9	139.3± 0.3	139.3± 0.3	139.6± 0.6	137.7± 0.9	138.2± 0.6	138.8± 0.7	138.1± 0.5	138.4± 0.6	18.7± 0.6	139.7± 0.4	139.2± 0.5	139.2± 0.4	139.3± 0.9	138.7± 0.3
K^+ (mmol/L)	3.68± 0.1	3.73± 0.1	3.59± 0.1	3.51± 0.1	3.66± 0.1	3.07± 0.1	3.87± 0.1	3.53± 0.03	3.28± 0.1[a]	3.77± 0.1	3.66± 0.05	3.71± 0.06	3.51± 0.04	3.47± 0.04	3.65± 0.05	3.71± 0.1	3.81± 0.1	3.80± 0.1	3.63± 0.1	3.7± 0.1
Ca^{++} (mmol/L)	1.17± 0.02	1.17± 0.03	1.18± 0.03	1.19± 0.03	1.18± 0.04	1.06± 0.05[a,b]	1.15± 0.03	1.12± 0.04[a]	1.11± 0.03	1.17± 0.04	1.12± 0.04[a]	1.17± 0.02	1.18± 0.04	1.21± 0.04	1.19± 0.03	1.19± 0.03	1.17± 0.03	1.18± 0.02	1.20± 0.04	1.19± 0.03
PO_4^{2-} (mmol/L)	1.07± 0.14	1.08± 0.13	1.09± 0.13	1.08± 0.15	1.07± 0.12	2.17± 0.19[a,b]	1.13± 0.15	1.69± 0.17[a,b]	1.53± 0.14[a,b]	1.10± 0.14	0.97± 0.18[a,b]	1.12± 0.17	0.99± 0.15	0.95± 0.14[a,b]	1.10± 0.12	1.00± 0.16	1.09± 0.17	1.04± 0.14[a]	0.98± 0.15[a]	1.08± 0.14
Creatinine clearance (ml/min)	165±3	171±8	167± 11	170± 11	150± 14	189± 13	176±8	162± 11	180± 14	175± 15	185± 12	179± 13	165± 14	178± 12	162± 14	164± 10	153±7	159± 11	171± 16	146± 16
Body weight (kg)	72.5± 2.4	73.4± 2.6	73.0± 2.2	72.7± 2.4	73.4± 2.6	73.4± 2.5	74.4± 2.8	73.7± 2.1	73.0± 2.5	73.1± 2.3	73.2± 2.3	74.4± 2.9	73.2± 2.4	73.2± 2.4	73.0± 2.2	72.3± 2.5	73.6± 2.4	72.7± 2.3	72.6± 2.5	72.8± 2.3
H^+ (nmol/L)	39.66± 0.52	40.18± 0.40	39.39± 0.47	40.37± 0.73	39.63± 0.45	36.66± 0.85[a,b]	42.99± 0.26	39.19± 0.37	37.01± 0.92[a,b]	40.98± 1.18	37.25± 0.69[a,b]	41.75± 0.37	40.00± 0.32	38.52± 0.55[a,b]	39.77± 0.22	40.53± 1.03	40.40± 0.30	40.17± 0.40	39.23± 0.50	39.80± 0.10
HCO_3^- (mmol/L)	25.7± 0.4	25.6± 0.5	24.6± 0.4	24.4± 0.7	24.5± 0.4	26.5± 0.7[a]	24.1± 0.5	25.1± 0.3	26.1± 0.9[a,b,c]	23.3± 0.9	26.1± 0.5	24.8± 0.6	25.4± 0.4	25.1± 0.6	24.5± 0.6	24.67± 0.2	24.93± 0.5	24.43± 0.3	23.67± 0.9	25.03± 0.4

Values are expressed as the mean±SEM.

[a] $P<0.05$ versus placebo.

[b] $P<0.05$ versus own baseline.

[c] $P<0.05$ for the comparison to the IV low-dose group.

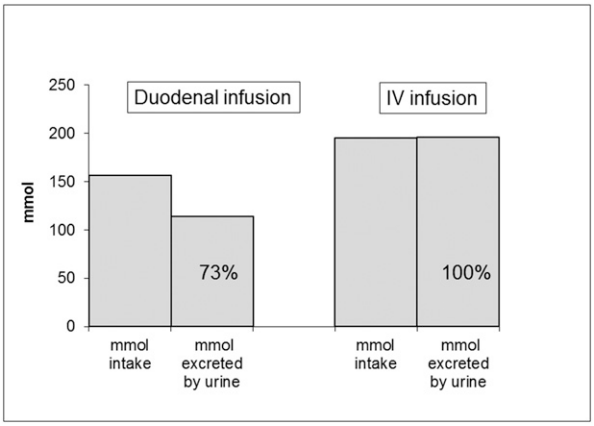

Figure 5. Intestinal PO4 absorption is 73%, while the administered PO4 load are exclusively eliminated by the kidney. Comparison of the cumulative urinary PO4 excretion with infused amounts of PO4 in the duodenal (left two bars) and the mean of both IV (right two bars) infusion groups. Observations were carried for 120 hours after beginning of PO4 loading.

On the basis of the ratio between PO4 intake (duodenal infusion+dietary intake) and the sum of daily urinary PO4 excretion rates (Figure 3), we found that the intestinal absorption rate of PO4 was 73%, a value identical to the value previously reported by Fine *et al.*[27] In the IV protocols, the sums of urinary PO4 excretion was identical to the excess sum of PO4 infused. Thus, the kidney excreted 100% of the IV-infused PO4 (Figure 5), and, therefore, there was no evidence for extrarenal (*i.e.,* gastrointestinal) disposal of phosphate under the conditions of these experiments.

All participants receiving neutral PO4 *via* IV route or into the duodenum developed acute metabolic alkalosis (Table 1), confirming previous, more chronic experiments performed in humans[28] and in rats.[29] The metabolic alkalosis is generated by the provision of increased PO4 buffer to the distal nephron with increased buffering of secreted protons.[29,34]

In summary and conclusion, in healthy humans similar systemic PO4 loads elicit a nearly identical phosphaturic response, both quantitatively and temporally, irrespective of the mode of administration (IV versus intestinal). Systemic PO4 loads elicit dose-dependent changes in plasma PO4 concentration, the renal PO4 excretory response as well as PTH, FGF-23, and 1,25$(OH)_2$D, but not of α-Klotho. Finally, we found no evidence for a quantitatively important intestinal sensing and effector mechanism that would elicit renal phosphaturia independent of changes in plasma PO4 concentration.

CONCISE METHODS

We evaluated, in healthy humans, the endocrine and electrolyte response to acute IV and intestinal PO4 loads using a prospective, double-blind, NaCl-controlled, single-crossover design. Participants exhibiting any abnormality in calcium/PO4 metabolism, primary or secondary hyperparathyroidism, 25$(OH)_2$D serum concentration $<$50 nmol/L, or urinary creatinine clearance $<$65 ml/min were excluded.

Twelve healthy volunteers (eight men and four women; mean age, 24.1$\pm$0.3 years; mean weight, 73$\pm$3.7 kg) were divided into two groups and randomly assigned to the IV low-dose PO4 or IV high-dose PO4 group. Six additional healthy volunteers (three men and three women; mean age, 23.4$\pm$0.3 years; mean weight, 72$\pm$4.1 kg) were randomly assigned to the intestinal PO4 load group. All participants were admitted to a metabolic unit for the entire study (3 weeks). They were allowed only minimal ambulation, had a studio for themselves, and could study/perform mental activities.

The volunteers consumed a constant diet during control, experimental, washout, and recovery periods providing per kilogram of body weight the following: 0.36 mmol of PO4, 0.16 mmol of calcium, and 30.3 kcal per day. During the experimental period, they received continuous IV infusion of neutral sodium PO4 (or equivalent amounts of sodium as NaCl) in two different doses (1.15 and 2.30 mmol PO4/kg body wt per 24 hours, respectively) for 36 hours. After the end of the infusion, observations were carried out for a further 84 hours (total, 120 hours or 5 days). PO4 loading was started when participants were considered to be in a metabolic steady state on the basis of a renal PO4 excretion of 18$\pm$3 mmol/24 hours and daily variation of plasma [HCO_3^-] by $<$1 mmol/L. Gastrointestinal absorption of PO4 was anticipated to be approximately 73%.

In an attempt to achieve a similar systemic PO4 load, we therefore chose to administer a duodenal dose of 1.53 mmol (47 mg/kg body wt per 24 hours over 36 hours (*i.e.,* about 33% more than in the low-dose IV infusion protocol). Higher doses of oral PO4 have the potential to provoke diarrhea. For this reason, we did not administer a duodenal load high enough to induce a similar systemic PO4 load as anticipated in the high-dose IV protocol. For continuous, controlled intestinal PO4 loading, a nasoduodenal tube was placed under fluoroscopy during propofol-induced sedation into pars 2 of the duodenum. The tube was taped to one nostril and removed after the 36-hour infusion period.

Analysis

Daily fasting arterialized venous blood samples were obtained from a heated hand or forearm vein.[34] Twenty-four-hour urine was collected daily and stored in plastic bottles containing mineral oil and thymol-chloroform preservative. On the infusion days (neutral NaPO4 or 0.9% NaCl), additional timed urine and blood samples were collected (see Figures 1 and 2). Electrolyte values in plasma and urine were determined as described previously.[35–37] Blood and urine pH and PCO_2 were measured with a Radiometer (Copenhagen model ABL 500 or 700) and HCO_3 calculated. Urinary ammonium was analyzed by ion chromatography.[38] Titratable acidity in urine was calculated from urinary PO4 excretion, urine pH, and blood pH, with the pK' of PO4 corrected for ionic strength by the method of Schwartz *et al.*[39] Urinary NAE was calculated as ammonium plus titratable acid minus bicarbonate excretion. We measured 1,25$(OH)_2$D by ELISA (Immundiagnostik AG, Bensheim, Germany), 25(OH) vitamin D by the liquid chromatograph-mass spectometry/mass spectometry method (MassChrom), c-terminal FGF-23 by ELISA kit (Kainos Laboratories, Inc.), IGF-1 by the Immulite method, and PTH and insulin by the Unicel Dxl 800 immunoassay system (Beckman Coulter). α-Klotho was measured by ELISA (Immunobiology

Table 2. Effect of IV and duodenal PO4 loading and of NaCl loading on 24-hour urinary electrolyte and NAE

Variable	Baseline					24 hr					48 hr					72 hr				
	IV High Dose	NaCl High Dose	IV Low Dose	Duodenal	NaCl Low Dose	IV High Dose	NaCl High Dose	IV Low Dose	Duodenal	NaCl Low Dose	IV High Dose	NaCl High Dose	IV Low Dose	Duodenal	NaCl Low Dose	IV High Dose	NaCl High Dose	IV Low Dose	Duodenal	NaCl Low Dose
Na^+ (mmol/24 hr)	81± 11	105± 7	96± 12	116±8	86± 11	243± 34 [a]	178± 15 [a]	148± 9 [a]	104± 10[b]	183± 30[a]	309± 22[a]	369±[18a]	246±[29a]	187± 22 [a,b]	331± 36[a]	224± 16[b]	244± 13[b]	244± 15	180± 23[b]	196± 32[b]
K^+ (mmol/24 hr)	70±5	57±4	68±	42±3	47±3	65±5	58±4	51±7	38±5	53±5	70±5	70±3	546	32±5	60±5	61±6	60±4	43±3	41±7	57±6
Ca^{2+} (mmol/24 hr)	3±1	3±1	4±1	5±1	4±1	2±1[b]	4±1	3±1[b]	4±1[b]	6±1	1±0.1[b]	5±1	3±1[b]	3±1[b]	6±1	3±1	4±0.4	4±1	5±1	4±1
PO_4^- (mmol/24 hr)	20±2	20±1	16±2	16±2	17±3	106± 27[a,b]	24±1	60± 10[a,b]	56±4 [a,b]	23±2	107± 12[a,b]	21±1	63± 5 [a,b]	64± 4 [a,b]	17±3	35± 2 [a,b]	17±1	25± 3 [a,b]	24±2 [a,b]	15± 18
FeCa (%)	1.2± 0.2	1.2± 0.2	1.4± 0.3	1.5± 0.2	1.6± 0.3	0.8± 0.2[a]	1.4± 0.3	1.2± 0.3	1.5± 0.3	2.0± 0.4	0.5±0.[2a]	1.0.8± 0.3	1.1± 0.[3a]	1.1± 0.3[a]	1.1± 0.2	1.3± 0.4	1.7± 0.4	1.6± 0.3	1.7± 0.3	1.7± 0.3
Fractional PO4 clearance (ml/min per 100 ml GFR)	7.8± 0.7	7.20± 0.6	7.1 ± 0.7	7.0± 0.8	7.3± 0.6	25.8+ 1.7[a,b]	7.4± 0.9	21.9± 1.4[a,b]	19.4 ± 1.6[a,b]	8.1± 0.8	45.7+ 1.7 [a,b]	8.2± 0.9	28.2± 1.9 [a,b]	25.4± 2.0 [a,b]	7.8± 0.7	25.5+ 1.6 [a,b]	7.5± 0.7	12.0 ± 1.2 [a,b]	6.7± 1.6	7.8± 0.8
NH_4^+ (mmol/24 hr)	39±2	38±3	39±4	48±5	35±4	40±3[b]	52±4[a]	44±5	63±6	56±6[a]	24±2	41±3	26±2	33±5[a,b]	37±5	28± 3[a,b]	42±4	30±2	32± 5[a,b]	36±4
Titratable acidity (mmol/24 hr)	13±1	12±1	10±1	12±2	11±1	58±17[a]	10±1	43±6[a]	46±3 [a,b]	15±1	34±5 [a,b]	10±1[a]	26±2[a]	35±13[b]	10±2	14±1[b]	9±1[a]	12±2	8±2	9±1
Urinary pH	6.028± 0.224	6.036± 0.199	5.932± 0.132	5.833± 0.244	6.028± 0255	6.377± 0.264[a]	6.071± 0.237	6.040± 0.217	5.664± 0.268	5.716± 0.281	6.777± 0.239[a,b]	6.156± 0.298	6.572± 0.235[a,b]	6.113± 0.213	6.206± 0.252	6.326± 0.0256	6.470± 0.201	6.470± 0.245	5.877± 0.218	6.026± 0.276
HCO_3^- (mmol/24 hr)	4±2	4±1	3±1	0.2± 0.03	3±1	10±2	5±1	4±1[b]	3±2	8±3[a]	36±4[a,b]	11±2[a]	16±2[a,b]	6±2	5±2	17±3[a]	8±2[a]	13±3[a,b]	6±3	4±1
NAE (mmol/24 hr)	48±5	46±3	46±4	60±5	43±5	88±19	57±8	83±10	105± 8[a,b]	63±1	22± 11[a,b]	40±3	36±5	62±5	42±6	25±6[a,b]	43±3[a]	29±5	34±6[a]	41±3

Values are expressed as the mean±SEM.

[a] $P<0.05$ versus own baseline.

[b] $P<0.05$ versus placebo.

Table 3. Effect of neutral sodium PO4 loading on serum α-Klotho levels

Study period	Serum α-Klotho Levels (pg/ml)						
	Baseline	2 hr	4 hr	8 hr	12 hr	24 hr	48 hr
High-dose PO4	1392±416	1287±442	1274±416	1473±468	1498±455	1443±429	1157±377
High-dose NaCl	1651 ±533	1729±546	1547±520	1573±481	1547±507	1495±494	1573±533
Low-dose PO4	976 ±403	884 ±364	1053±416	1092±429	1014±403	1016±442	1053±416
Low-dose NaCl	1079±442	1001±418	1020±457	1105±410	1053±444	1001±398	1209±427
Duodenal PO4	962 ±442	910±455	871±481	1053±403	1048±442	962±455	1079±494

Values are means±SD. Phosphate loading occurred after baseline until 36 hours. None of the values were significant both for the inter- and intragroup comparisons.

Laboratories, Inc., Minneapolis, MN).[40] All determinations were performed in duplicate.

The study protocols were approved by the ethics committee of the University Basel, Switzerland. All participants volunteered for the studies, gave informed consent, and were paid for their participation.

Statistical Analyses

Results are mean±SEM unless indicated otherwise. Statistical comparisons were made by *t* test or ANOVA for repeated measurements where appropriate. Statistical analysis was performed using SPSS for Windows NT Version 20 (SPSS Inc., Chicago, IL).

ACKNOWLEDGMENTS

The help of gastroenterologists Horst Haack and Niklaus Schaub for placing the duodenal tubes is gratefully acknowledged. The authors thank the team of the clinical research unit (Annie Kofmel, Helga Schneider, Andrea Kloetzer, Marisa Zimmermann) for their expert technical help and empathic work with the human volunteers. We thank Orson Moe and Johanne Pastor for analyzing serum α-Klotho levels.

This work was supported by the Swiss National Science Foundation, NCCR Kidney Homeostasis (R.K.).

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement 246539.

DISCLOSURES

None.

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