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A Controlled Increase in Dietary Phosphate Elevates BP in Healthy Human Subjects

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ABSTRACT

Background Despite epidemiologic evidence for increased cardiovascular morbidity and mortality associated with both high dietary and serum phosphate in humans with normal renal function, no controlled phosphate intervention studies of systemic hemodynamics have been reported. Higher serum 25(OH) vitamin D levels are associated with better cardiovascular outcomes, but vitamin D increases intestinal phosphate absorption.

Methods We conducted a prospective outpatient study with blinded assessment in 20 young adults with normal renal function randomized to high phosphate (regular diet plus 1 mmol/kg body wt per day of Na as neutral sodium phosphate) or low phosphate (regular diet plus lanthanum, 750 mg thrice/day, plus 0.7 mmol/kg body wt per day of Na as NaCl) for 11 weeks. After 6 weeks, all subjects received vitamin D_3 (600,000 U) by intramuscular injection. Outcome parameters were 24-hour ambulatory systolic and diastolic BP (SBP and DBP), pulse rate (PR), biomarkers, and measures of endothelial and arterial function.

Results Compared with the low-phosphate diet group, the high-phosphate diet group had a significant increase in mean±SEM fasting plasma phosphate concentration (0.23±0.11 mmol/L); 24-hour SBP and DBP (+4.1; 95% confidence interval [95% CI], 2.1 to 6.1; and +3.2; 95% CI, 1.2 to 5.2 mm Hg, respectively); mean 24-hour PR (+4.0; 95% CI, 2.0 to 6.0 beats/min); and urinary metanephrine and normetanephrine excretion (54; 95% CI, 50 to 70; and 122; 95% CI, 85 to 159 μg/24 hr, respectively). Vitamin D had no effect on any of these parameters. Neither high- nor low-phosphate diet nor vitamin D affected endothelial function or arterial elasticity.

Conclusions Increased phosphate intake (controlled for sodium) significantly increases SBP, DBP, and PR in humans with normal renal function, in part, by increasing sympathoadrenergic activity.

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Vascular calcification is a cardiovascular risk factor leading to increased mortality in both CKD and general populations.[1] Both traditional (hypertension, diabetes, dyslipidemia) and nontraditional risk factors (inflammation, oxidative stress, advanced glycation end products, hyperphosphatemia, elevated FGF-23 levels)[2] are important in inducing vascular calcification. Elevated extracellular phosphate concentration has been found in observational epidemiologic assessments to play a highly suggestive role in initiating and exacerbating vascular disease and cardiovascular mortality in patients with CKD.[3,4]

The negative effect on cardiovascular morbidity/mortality of high dietary phosphate load/hyperphosphatemia may, however, also affect the general population[5,6]:

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The age-associated decrease in GFR could contribute to a slowly progressive increase in net phosphate load per nephron and associated incremental hyperphosphatemia, but such experiments would require accurate measured GFR and control of diurnal phosphate sampling time effects.

Mostly because of its use in processed "fast foods," phosphate intake has risen in recent decades and is now far above the current recommended daily allowance for inorganic phosphate as established by the National Academy of Medicine (700 mg).

Independent associations of fasting plasma phosphate concentration and dietary phosphate loads with cardiac calcification, left ventricular hypertrophy, cardiovascular events, and death are evident in the general population, even in younger adults, and cardiovascular event risk accelerates with phosphate levels >1.15–1.47 mmol/L.[7–11]

$1,25(OH)_2D$ stimulates intestinal phosphate absorption, thereby increasing the systemic phosphate load.[12] Lower 25 (OH) vitamin D levels or decreased supply of dietary vitamin D are associated with increased cardiovascular morbidity and cardiovascular as well as all-cause mortality.[13–16] Therefore, it is unclear whether the routinely recommended vitamin D supplements might protect against cardiovascular phosphate toxicity or aggravate it *via* increasing phosphate absorption.

We examined in young healthy subjects with normal renal function, by randomized single-blind design, the effects of low-phosphate (LP) and high-phosphate (HP) intake for 6 weeks on a variety of cardiovascular hemodynamic effects (*i.e.*, 24-hour BP and pulse profiles and measures of endothelial and arterial function) and cardiovascular risk and aging markers, as well as phosphate homeostasis. In addition, we evaluated the effect of supplemental vitamin D_3 on these parameters with continued LP and HP intake for another 5 weeks.

Significance Statement

Dietary intake of phosphate has increased significantly in recent years mostly due to increased intake of processed food. Despite strong epidemiologic evidence for increased cardiovascular morbidity and mortality associated with increased dietary phosphate intake in healthy humans, no intervention studies with controlled phosphate intake have been reported. This exploratory, physiologic study shows that a controlled increase in dietary phosphate (within the range observed in a representative United States population) significantly and reversibly increases BP and pulse rate in young, healthy human adults. The effect is associated with a phosphate-specific increase in sympathoadrenergic activity. These results provide an important explanation for the association of dietary phosphate intake with increased cardiovascular morbidity and mortality in the general population. These conclusions, if supported by larger studies in diverse populations, are of public health importance.

METHODS

Study Protocol

We evaluated the chronic effects of decreasing and increasing the dietary phosphate load without and with vitamin D_3 supplementation on cardiovascular outcome parameters, *i.e.*, 24-hour systolic and diastolic BP (SBP and DBP) and mean pulse rate, reactive hyperemia index as a measure of endothelial function, and aortic pulse wave velocity (PWV) and the augmentation index (AI) as a measure of arterial elasticity.

In a prospective, exploratory, single-blind outpatient study design (see Figure 1), 20 young, healthy human subjects of both sexes were studied. Exclusion criteria were: any evidence for kidney disease; electrolyte or acid-base disturbance; office BP≥125/80 mm Hg; ingestion of any drug (prescribed, over the counter, or illicit) during the 3 months before and during the study; smoking; excessive alcohol intake (more than 1 drink/d); any evidence for adrenal (24-hour urinary cortisol and aldosterone excretion), thyroid, or parathyroid dysfunction; or 25(OH)D serum concentrations <50 or >100 nmol/L.

The subjects were randomized by a research administrator, unblocked, and unstratified 1:1 to either the HP group (n=10), ingesting regular diet plus 1 mmol sodium per kg body weight per os as neutral sodium phosphate (yielding 0.55 mmol P per mmol Na) in three divided doses per day with meals, or to the LP group (n=10), ingesting a regular diet and the intestinal phosphate binder lanthanum (Fosrenol, 750 mg; Shire Pharmaceuticals, Lexington, MA, in three per os divided doses with meals). The LP group was supplemented with 0.7 mmol/kg body wt per day NaCl commensurate with the excess sodium intake of the HP group assuming an intestinal absorption of sodium phosphate of 70%.[17] After 6 weeks of LP or HP loading, all subjects received a single intramuscular injection of vitamin D_3 (600,000 U) and were studied for another 5 weeks. There were three visits each on three consecutive days at baseline, at the end of week 6, and at the end of week 11 (5 weeks after vitamin D administration). There were additional visits every 2 weeks to assess tolerance, safety, and compliance and a close-out visit at the end of week 12. After study unblinding, the HP group was re-examined (24-hour ambulatory BP measurement [ABPM] and tests of endothelial and arterial function only) in a recovery visit on regular diet 2 months after the close-out visit.

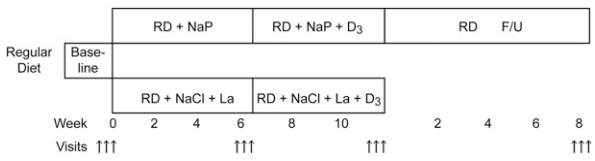

Figure 1. Flowchart of study protocol. Each arrow denotes daily complete assessments (three consecutive days/evaluations per study period). F/U, follow-up; La, lanthanum; NaCl, sodium chloride; NaP, neutral sodium phosphate; RD, regular diet.

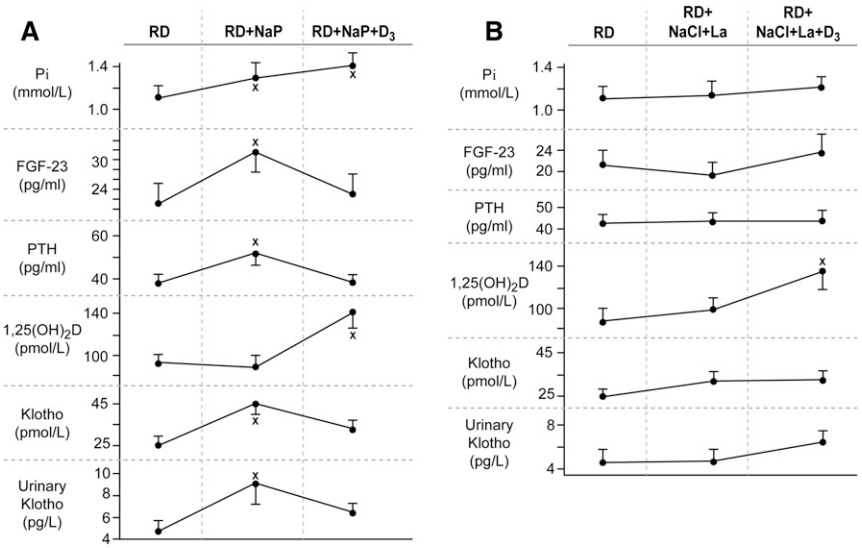

Figure 2. High phosphate diet increases FGF-24, PTH, and both plasma and urinary Klotho, while low phosphate diet has no effect. (A) Effect of HP diet: Baseline (RD), after 6 weeks of HP diet (RD+NaP), and 5 weeks after supplemental vitamin D_3 (600,000 U in one single intramuscular dose) and continued HP diet. (B) Effect of LP diet: Baseline (RD), after 6 weeks of ingestion of the phosphate binding substance lanthanum (750 mg per os three times a day) with supplemental NaCl, and 5 weeks after supplemental vitamin D3 (600,000 U in one single i.m. dose) and continued lanthanum and NaCl administration. *P at least <0.05 compared with baseline (RD). La, lanthanum; NaCl, sodium chloride; NaP, neutral sodium phosphate (0.55 mmol/kg body wt phosphate per day); PTH, parathyroid hormone; RD, regular diet.

Measurements

Twenty-four-hour ABPMs were made at the nondominant arm using the commercial Schiller BR-102 device with vendor software. BP measurements were made every 15 minutes (06:00–22:00 hours) and every 30 minutes during the nighttime (22:00–06:00 hours), consistent with ABPM method guidance.[18]

Arterial pressure waveforms were recorded and analyzed using established techniques using vendor software (Sphygmocor; AtCor Medical, Australia). In brief, the aortic PWV (m/s) is estimated by calculating the time between the foot of the pressure wave and the inflection point. Augmentation represents the difference between the second reflected systolic peak and first systolic peak of the central pressure waveform, and AI is defined as the augmentation increment expressed as a percentage of the pulse pressure and is a measure of systemic arterial stiffness.[19]

Endothelial function was analyzed by determining the reactive hyperemia index, measured as flow-mediated vasodilation after 5 minutes of tourniquet-induced hypoxia using the Endopat (Itamar, Israel) pulse arterial volume change as assessed with a fingertip sensor and vendor software. The tourniquet was applied to the dominant arm and finger probes to both hands, the nondominant side serving as control.

Before these measurements, the fasting subjects were lying comfortably for 30 minutes in a noise-shielded, dimly lit room, at a constant room temperature of 22°C–24°C and constant humidity of 58%–62%. Measurements were always performed between 08:30 AM and 11:00 AM. In our study lab, the interassay coefficient for PWV measurements was 5.9%, and 8.7% for reactive hypoxia index.

Biochemical Analysis

Fasting samples for plasma electrolyte and blood acid-base measurements and for serum endocrine and aging markers were drawn after preheating the forearm to 43°C without use of tourniquets between 07:00 AM and 08:00 AM. Twenty-four-hour urines (three consecutive days of each study period) were collected under paraffin oil with thymol as a preservative. Plasma and urine electrolytes were measured in the hospital routine lab.

$1,25(OH)_2D$ was measured by ELISA (Immundiagnostik AG, Bensheim, Germany), 25(OH) vitamin D by the liquid chromatography–mass spectrometry method (MassChrom), and intact PTH and insulin by the Unicel Dxl 800 immunoassay system (Beckman Coulter).

Human FGF-23 (second generation, dual antibody method to detect both C-terminal and intact FGF-23) was measured using an ELISA kit (#60–6100; Immunotopics, San Clemente, CA). Serum αKlotho protein was precipitated from the serum using the sb-106 Fab, reported to recognize both vector HEK cell–expressed human full-length membrane Klotho (Isoform 1; Uniprot Consortium, Switzerland) and Klotho extracellular domain (ECD) (α cut) as nominal 130-kD bands. After gel electrophoresis, the immune-precipitated αKlotho protein was further detected on immunoblot using the clone KM2076 antibody (Transgenic Inc., Kobe, Japan). This antibody was generated from KL1 domain immunization in rats and was reported to detect (western blot) an approximately 130-kD human renal membrane-associated klotho and thus may detect human full-length membrane klotho (Isoform 1), proteolytic ECD (α cut) near 130 kD, or the secreted alternatively spliced isoform at a lower molecular mass (Isoform 2; Uniprot).[20,21] KM2076 is predicted to not detect a putative C terminus β cut Klotho (KL2 domain). The serum 130 kD band was estimated on the basis of murine rKlotho isoform 1 standard as a density and thus likely represents the α cut protein ECD. The rKlotho protein standard was obtained from a CHO cell line that stably expressed the full-length Klotho (CHOKL, Isoform 1), obtained as a gift from Kyowa Hakko Kogyo Co. Ltd.[22]

Urine Klotho was measured by adding urine to 4× lithium dodecyl sulfate sample buffer containing a final concentration of 100 mM dithiothreitol. The boiled samples were run on a 4%–14% Bis-Tris gel (NuPage; Invitrogen), immunoblotted using clone KM2076 antibody, and reported as density of the 130-kD band.

Plasma renin and aldosterone and urinary aldosterone concentrations were measured by chemiluminescence immunoassay

Table 1. Fasting mean plasma electrolyte, uric acid, urea, creatinine, and blood bicarbonate concentrations in HP and LP groups without and with vitamin D_3 at baseline, W6, and W11

Period/Group	Na^+	K^+	Cl^-	HCO_3^-	Ca_{tot}	Ca^{++}	Pi	Mg	Uric Acid (μmol/L)	Urea	Creatinine (μmol/L)
HP baseline	141±6	4.1±0.6	101±9	24.8±1.5	2.30±0.34	1.19±0.25	1.11±0.32	0.84±0.28	295±34	4.40±0.70	67.5±17.6
LP baseline	141±6	4.1±0.6	101±12	24.5±2.5	2.30±0.62	1.19±0.22	1.12±0.34	0.82±0.31	296±47	4.41±0.95	67.6±18.6
HP W6	142±9	4.3±0.6	100±9	25.8±3.6	2.30±0.65	1.20±0.19	1.30[a]±0.47	0.80±0.35	283±53	4.17±1.15	65.2±18.9
LP W6	141±6	4.2±0.6	101±4	24.5±3.2	2.30±0.50	1.19±0.29	1.15±0.39	0.85±0.38	282±69	4.29±1.25	66.0±18.2
HP plus D3 W11	141±9	4.3±0.6	101±4	25.7±2.3	2.31±0.49	1.18±0.39	1.41[b]±0.45	0.8±0.33	279±60	4.37±1.45	67.5±18.1
LP plus D3 W11	141±6	4.4±0.6	100±3	24.9±2.8	2.30±0.60	1.18±0.25	1.22±0.38	0.8±0.41	284±62	4.21±1.44	66.3±19.0

Data are mean±SD, values are millimoles per liter unless indicated otherwise. HP, HP group; LP, LP group; W, week; Ca_{tot}, total calcium.
[a] P=0.03 compared with own baseline (intragroup) and 0.03 compared with LP (intergroup).
[b] P=0.02 compared with baseline and 0.03 compared with LP.

(Bioscientia, Ingelheim, Germany). Urinary total metanephrine and normetanephrine (comprising free and conjugated compounds) were measured by HPLC after acid hydrolysis, and urinary free cortisol was measured by liquid chromatography–mass spectrometry.

The serum calcification propensity test (T50) was performed using a Nephelostar nephelometer (BMG Labtech, Offenburg, Germany).[23] Serum oxidized LDL and SOD1 were measured by ELISA, antioxidative capacity and serum lipid peroxide concentration were measured photometrically, and urinary 8-oxo-2-deoxy guanosine was measured by ELISA (all: Biovis, Limbur, Offheim, Germany).

After enrollment, subjects received advice to ingest a stable self-selected diet.

All values given in "Results" are the means of three measurements made on these three separate, consecutive days in each period.

Investigators were blinded to treatment assignment of each study subject throughout the study.

The protocol was approved by the ethics committee of both Cantons of Basel (Switzerland).

Methods and safety considerations of the vitamin D dosing are described in the Supplemental Material.

Statistical Analyses

Sample size determination was empiric without consideration of power or effect size. Values given are means with 95% confidence intervals (95% CIs) for the cardiovascular outcome parameters. All other values are mean±SD or SEM, as indicated. Statistical significance was tested with ANOVA for repeated measures for intragroup comparison and with one-way ANOVA for intergroup comparisons.

RESULTS

Study population: 53 human subjects were screened and 20 were included into the study at 1:1 randomization (HP group/LP group). All randomized subjects completed the study. Reasons for exclusion were: Loss of interest in participation due to occupational duties (n=9), study perceived as too long and too demanding (n=21), vitamin D deficiency (n=1), and concomitant medications (n=2). Subject characteristics are listed in Supplemental Table 1. Compliance rate (inspection of unused agents) for the ingestion of oral study drugs (NaPO4, lanthanum, and NaCl) was 96% in the HP group and 95% in the LP group.

HP induced a significant intra- and intergroup rise in fasting plasma phosphate concentration (+0.19±0.09 mmol/L, P=0.03, Figure 2A, Table 1) and 24-hour mean urinary phosphate excretion rate (+21.0±7.2 mmol/24 hr, P<0.01, Table 2). Neither value was further affected significantly by the D_3 supplement. The LP diet's lanthanum supplement decreased urinary phosphate excretion nonsignificantly (−5.9±2.3 mmol/24 hr) without affecting fasting plasma phosphate concentration. In contrast to HP, D_3 in the LP group increased

Table 2. Twenty-four-hour urinary excretion rates of electrolytes, creatinine, and albumin in HP and LP groups without and with vitamin D_3 at baseline, W6, and W11

Period/Group	Na^+	K^+	Cl^-	Ca	Pi	Mg	Creatinine	Albumin (mg/24 h)	Body Weight (kg)
HP baseline	119±8	55±4	106±8	3.4±0.2	20.5±2.2	3.3±0.3	7.8±1.1	5.2±2.2	64.5±5.6
LP baseline	117±7	53±5	107±7	3.3±0.2	21.9±2.4	3.2±0.4	7.6±1.2	5.3±2.4	63.7±4.8
HP W6	145[a]±9	59±6	97±6	2.8±0.1	41.5[b]±4.1	3.2±0.2	7.1±1.0	3.4±2.3	65.1±5.8
LP W6	149[a]±8	52±5	139[c]±8	3.1±0.3	14.6±1.8	3.3±0.2	7.1±1.2	5.0±2.5	64.5±4.4
HP plus D_3 W11	142[a]±10	59±4	102±7	3.0±0.2	42.5[b]±3.4	3.3±0.3	6.5±0.9	4.3±2.6	64.6±5.1
LP plus D_3 W11	149[a]±9	57±5	133[c]±7	3.0±0.2	21.4[d]±2.9	3.3±0.2	6.6±0.9	2.5±2.4	64.4±4.5

Data are mean±SD, values are millimoles (milliequivalents per 24 h unless indicated otherwise). HP, HP group; LP, LP group; W, week.
[a]$P<0.03$ compared with own baseline.
[b]$P<0.01$ for intra- and intergroup comparisons.
[c]$P<0.02$ compared with own baseline.
[d]$P=0.04$ for comparison of LP plus D_3 to LP without D_3.

urinary phosphate excretion significantly (+6.8±3.1 mmol/24 hr, $P=0.04$). Table 2 also shows that 24-hour urinary sodium excretion rates were not different among HP and LP groups, confirming the protocol's intent for a similar daily sodium load throughout the study among both groups.

Figure 2, A and B, and Table 3 show that HP induced significant intra- and intergroup increases in the serum concentrations of PTH, FGF-23, and αKlotho and urinary Klotho, whereas LP without and with D_3 had no effect. In response to vitamin D_3, serum 25(OH)D and $1,25(OH)_2D$ increased significantly in both groups, whereas mean serum PTH and FGF-23 decreased in the HP group. Both PTH and $1,25(OH)_2D$ have been shown to increase FGF-23 serum concentration.[24,25] The baseline values for urinary phosphate excretion of approximately 21 mmol/24 hr are similar to reported United States and European subjects on self-selected diets.[26,27]

Figure 3A and Table 4 illustrate that HP induced significant inter- and intragroup increases in both mean 24-hour SBP and DBP and mean 24-hour pulse rate, whereas LP had no effect. HP increased 24-hour mean SBP by +4.1 mm Hg (95% CI, 2.1 to 6.1), 24-hour mean DBP by +3.2 (95% CI, 1.2 to 5.2) mm Hg, and mean 24-hour pulse rate by +4.0 (95% CI, 2.0 to 6.0) beats/min. Because these increases were resistant to further change by supplemental vitamin D_3, subjects in the HP group were restudied and reversibility of short-term dietary phosphate–induced increases in BP and pulse rate was documented. Figure 3B shows the circadian rhythm of mean arterial pressure. Sympathoadrenergic activity, as assessed by 24-hour urinary excretion of metanephrine and normetanephrine, was stimulated by HP: Urinary metanephrine and normetanephrine excretion increased by 54 (95% CI, 28 to 70) and 122 (95% CI, 85 to 159) μg/24 hr, respectively, whereas 24-hour urinary excretion rates of aldosterone, free cortisol, and plasma renin/aldosterone concentration were not affected by either HP or LP (Figure 3A, Table 4). Figure 3C shows a scatter plot of the individual mean 24-hour arterial BP changes in the HP group.

As illustrated in Table 5, in both HP and LP groups without and with vitamin D, no measurable effects on endothelial function and/or arterial elasticity were found.

With the exception of increases in lipid peroxide serum concentration and urinary excretion rate of 8-oxo-2'-deoxy-guanosine, modulation of dietary Pi loading did not affect markers of cardiovascular risk, oxidative stress, and senescence (Table 6). No adverse events were reported.

DISCUSSION

These are—to our knowledge—the first reported hemodynamic data from a prospective dietary phosphate interventional study in humans with normal renal function. The principal finding is that increasing the systemic phosphate load by an amount sufficient to significantly increase fasting plasma phosphate concentration induces significant increases in both SBP and DBP as well as pulse

Table 3. Mean serum concentrations of phosphate-regulating hormones and urinary excretion of Klotho under HP and LP dietary conditions without and with supplemental D_3

Group/Period	Serum 25(OH)D (nmol/L)	Serum $1,25(OH)_2D$ (pmol/L)	Serum iPTH (pg/ml)	Serum FGF-23 (pg/ml)	Serum αKlotho (pmol/L)	Urinary αKlotho (ng/g Creatinine)
HP baseline	63.0±6.4	92.4±8.1	41.4±4.8	21.2±3.8	24.6±3.7	7.9±1.8
LP Baseline	63.5±7.4	93.7±8.4	41.8±3.9	21.3±2.9	24.6±2.9	7.2±1.5
HP W6	68.3±5.8	89.1±9.9	51.7[a]±6.2	31.4[a]±3.6	45.0[a]±5.2	12.6[a]±3.0
LP W6	67.3±7.6	99.4±10.4	42.4±4.4	19.1±2.4	31.6±4.6	7.1±1.3
HP plus D_3 W11	113.6[b]±12.4	141.6[b]±15.2	41.3[c]±4.5	23.1[c]±3.6	32.0[c]±4.7	7.8±2.0
LP plus D_3 W11	104.9[b]±14.2	132.9[b]±14.8	43.1±5.2	22.8±3.0	31.8±4.4	6.2±1.3

Values are mean±SEM. iPTH, intact parathyroid hormone; HP, high phosphate group; LP, low phosphate group; W, week.
[a]$P<0.02$ compared with own baseline, and <0.025 for comparison to LP group.
[b]$P<0.01$ in comparison with baseline and HP/LP without D_3 supplementation.
[c]$P<0.03$ for the comparison of HP without to HP with D_3 supplemented.

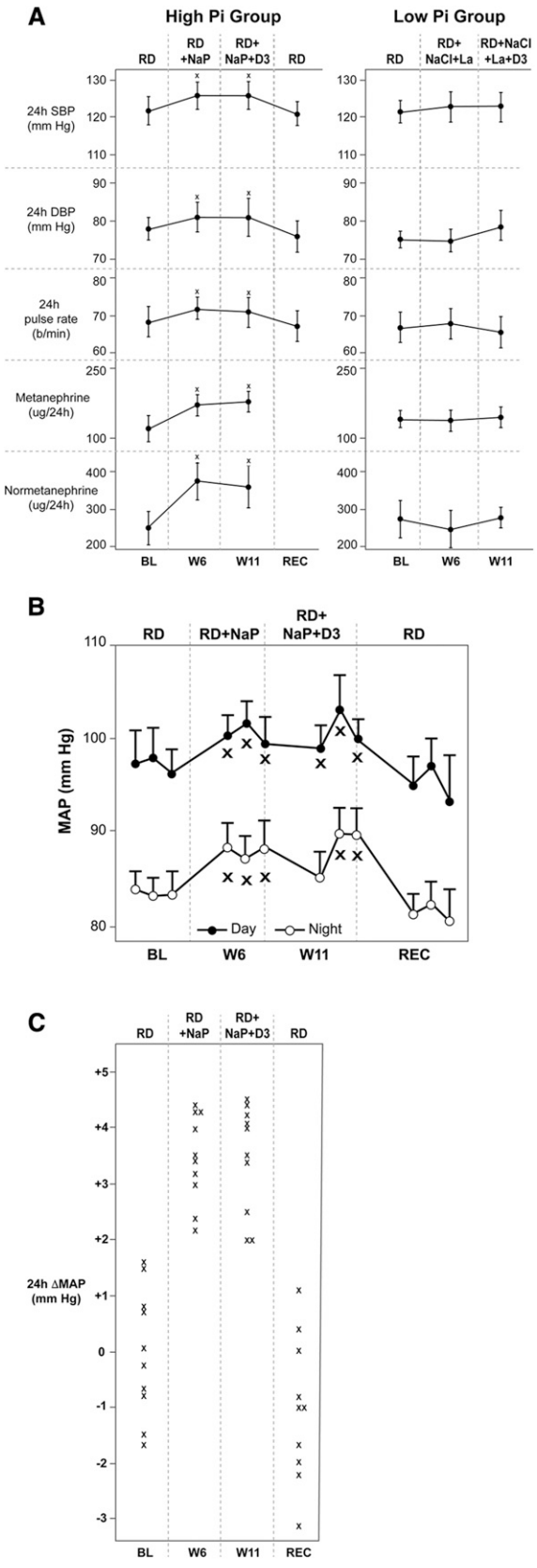

Figure 3. HP diet increases mean 24-hour SBP, DBP, and pulse rate with preserved circadian oscillation, and in parallel with significant increases in urinary metanephrine and normetanephrine excretion rates. Vitamin D does not reverse the hypertensiongenic effect in the short term (5 weeks). (A) See Figure 2 legend for description of periods. All values are means of three consecutive daily measurements during the last 3 days of each period. Recovery values are from measurements 2 months after week 11 to show reversibility and with the subjects on regular diet only. **P* at least <0.05 for both intra- and intergroup comparisons. (B) Effect of HP diet on day and night mean arterial BP and mean pulse rates. **P* at least <0.05 for the comparisons to both baseline and recovery. (C) Per subject changes in MAP in the HP group. BL, baseline; D_3, vitamin D_3; HP, HP diet; La, lanthanum; LP, LP diet; MAP, mean arterial pressure; NaCl, sodium chloride; NaP, neutral sodium phosphate; PTH, parathyroid hormone; RD, regular diet; REC, recovery; W6 and W11, week 6 and 11, respectively.

rate. The effect is experimentally specific to the ingested phosphate because the subjects were well controlled for the increase in sodium load. These results are in accordance with all but one[28] report in normotensive and spontaneously hypertensive rats (both with normal renal function), that an HP diet elevated BP and augmented the exercise pressor reflex function.[29–31] Our studies also offer an explanation—at least in part—of the mechanism of the phosphate-induced hypertensinogenic effect: Although plasma renin/aldosterone concentrations and 24-hour excretion rates for both aldosterone and free cortisol were unchanged, 24-hour urinary excretion rates of metanephrine and normetanephrine increased significantly, explaining the additional observation of significantly increased mean 24-hour pulse rate. Even small increases in sympathetic nervous system activity may be sufficient to cause significant hypertension because an HP diet in mice has been reported to potentiate phenylephrine-induced vasoconstriction in *ex vivo* aortic rings.[32] In addition, increased extracellular phosphate concentration, *per se*, has also been demonstrated to result in vasoconstriction in murine aortic rings *in vitro*.[33] It will be of interest to investigate whether phosphate exerts its sympathetic effect *via* a primary central or peripheral stimulation of the sympathetic nervous system and whether the effect of phosphate is direct or mediated *via* unknown endocrine/paracrine factors that are modulated by phosphate intake. The finding of increases in both PTH and FGF-23 in the HP group, yet persistence of the hypertensinogenic and heart rate effects when PTH and FGF-23 levels had normalized, suggests that at least those effects in tandem may not be pivotal hemodynamic mediators for the present results.

The basis for sympathetic activation by an HP diet remains to be determined. One potential mechanism for phosphate-induced sympathetic activation is the presence of phosphate-responsive NaPi2-like transporters in the rat amygdaloid region of the central nervous system as identified by immunocytochemistry.[34] Infusion of very low (20 nM) phosphate concentrations into the third ventricle resulted in a profound suppression of NaPi-2 protein expression as compared with vehicle perfusion, providing at least an afferent arm for potential systemic phosphate effects on brain/sympathetic function.

Table 4. Mean 24-h SBP and DBP and pulse rate as well as mean 24-h urinary excretion rates of metanephrine, normetanephrine, aldosterone, and cortisol and mean plasma concentrations of renin and aldosterone under LP and HP dietary conditions without and with supplemental D_3

Variable	24-h SBP (mm Hg)	24-h DBP (mm Hg)	24-h Mean Pulse Rate (Beats/Min)	Metanephrine (μg/24 h)	Metanephrine (μg/gr Creatinine)	Normetanephrine (μg/24 h)	Normetanephrine (μg/g creatinine)	Aldosterone (nmol/24 h)	Free Cortisol (nmol/24 h)	Plasma Renin (ng/L)	Plasma Aldosterone (nmol/L)
HP baseline	122 (118 to 126	78 (75 to 81)	68 (64 to 72)	147±86	18.5±11.2	252±107	33.2±14.1	61±53	24±10	9.42±6.80	555±245
LP Baseline	122 (119 to 127)	76 (74 to 78)	67 (63 to 71)	141±107	18.3±14.2	271±119	35.6±14.6	70±44	26±13	9.73±7.92	579±265
HP W6	126[a] (123 to 129)	81[a] (77 to 85)	72 (69 to 75)[a]	201[a]±145	28.0[a]±20.3	374[a]±147	52.2[a]±19.9	55±41	29±15	8.98±8.31	525±279
LP W6	123 (119 to 127)	75 (72 to 78)	68 (64 to 72)	132±91	18.3±13.0	241±130	33.8±18.3	64±51	23±15	9.23±8.82	536±226
HP plus D3 W11	126[a] (122 to 130)	81[a] (76 to 86)	71 (67 to 75)[a]	198[a]±139	29.8[a]±21.2	358[a]±139	54.0[a]±21.3	68±48	31±19	8.10±8.62	507±207
LP plus D3 W11	123 (119 to 127)	79 (75 to 83)	66 (62 to 70)	144±63	19.5±8.9	280±132	42.4±20.0	75±51	27±13	7.78±7.51	505±221

Values are means plus 95% CIs (BP and pulse rate) and mean±SD (endocrine parameters). HP, HP group; LP, LP group; W, week.
[a]$P<0.03$ for intergroup and intragroup comparisons.

Although not investigated in this study, an additional explanation for phosphate-induced autonomic increase in BP is phosphate-induced arterial or visceral inflammation[35] resulting in local sympathetic afferent activation.[36] Human vascular smooth muscle cells when incubated for 9 days in an HP medium produced increased levels of reactive oxygen and nitrogen species as well as inflammatory cytokines (*e.g.*, TNFα, IL6, and ICAM-1), a finding not modulated by the presence of physiologic $1,25(OH)_2D$ concentrations.[35] Peripheral vascular or visceral inflammation provides an afferent autonomic signal that can activate efferent/systemic sympathetic outflow.[36] Notably, however, we did not find an effect of HP on high-sensitivity C-reactive protein serum concentration (Table 6).

The phosphate-induced sympathetic activity in this study did not induce detectable changes in arterial elasticity as analyzed by PWV and AI, confirming and extending the previous observation that acute and short-term increases of dietary phosphate did not affect PWV in non-CKD subjects.[35] In contrast to the findings in this chronic study, acute phosphate loading in humans has been reported to impair endothelial function by disruption of the nitric oxide pathway.[37,38] It is possible that a more chronic phosphate exposure induces adaptive or regulatory mechanisms able to restore endothelial function.

The phosphate-induced increase in BP appears to depend on chronicity of phosphate loading. In a previous acute study of neutral phosphate loading (intravenously and enterally) for 36 hours, also with appropriate Na load control, we did not detect significant differences in 24-hour BP measurements and pulse rates during phosphate loading and in the first 24 hours thereafter (unpublished data, R. Scanni et al.).[17] Thus, delineation of the exact time course of the hypertensinogenic effect of dietary phosphate and of increased sympathetic tone may yield additional insight into the mechanisms involved.

Cardiovascular risk markers and markers of oxidative stress ("aging") were not affected by modulating dietary phosphate or by the supplementation of vitamin D_3. The exception was the increase of lipid peroxide serum concentration and urinary 8-oxo-2-deoxyguanosine in both LP and HP groups that could, however, be caused by the increase in sodium intake in both LP and HP.[39]

Significance of Observed Increase in BP and Pulse Rate

The morbidity and mortality implications of phosphate-induced BP elevations with a mean increment in SBP of approximately 4 mm Hg and DBP of approximately 3 mm Hg are substantial. Each 20 mm Hg increment in SBP and 10 mm Hg increment in DBP is associated with a doubling in the risk of death caused by stroke, heart disease, or other vascular disease.[40,41] The general population risk ratio (RR) data for elevated pulse rate are also highly significant and independent of age.[36] On the basis of a recent meta-analysis of 1.2 million persons aged 25–90, the RR for resting pulse rate for both cardiovascular (CV) and all-cause mortality exhibited a linear rise for values above 45 beats per minute (bpm) (RR, 1.08,

Table 5. Effects of low (upper panel) and high Pi dietary intake (lower panel) without and with vitamin D_3 on parameters of endothelial function and arterial elasticity

Endothelial and Arterial Parameters	Baseline	W6	W11 (Plus D3)	Recovery
Low Pi dietary intake				
Reactive hyperemia index	1.67 (1.30 to 2.04)	1.53 (1.30 to 1.86)	1.76 (1.40 to 2.12)	ND
AI, %	−10.6 (−2.0 to −19.2)	−7.4 (−2 to −12.8)	−10.7 (−5.7 to 16.4)	ND
PWV, m/s	5.1 (4.4 to 5.8)	5.3 (4.5 to 6.1)	5.1 (4.4 to 5.8)	ND
High Pi dietary intake				
Reactive hyperemia index	1.93 (1.48 to 2.38)	1.84 (1.60 to 2.08)	1.86 (1.50 to 2.22)	1.92 (1.70 to 2.14)
AI, %	−10.1 (−2.0 to 18.2)	−8.1 (−3.2 to −13.0)	−4.9 (+1.0 to 10.8)	−7.8 (0 to 15.6)
PWV, m/s	5.7 (4.7 to 6.7)	5.8 (4.9 to 6.7)	5.7 (4.8 to 6.6)	5.8 (4.9 to 6.7)

Values are means with 95% CIs in brackets. Recovery measurements (2 mo after the high-Pi plus D_3 period+1 week) were only performed in the high-Pi group. D_3, vitamin D_3; W, week; Pi, phosphorus.

1.09 per 10-bpm increment, respectively, $P<0.01$, each), attributed to sympathetic nervous system activity.[42] Thus, although this study lacks middle-aged and elderly subjects that exhibit the highest CV event rates, strong observational data support both BP and pulse rate CV morbidity and mortality effects even for small increments and, where data are available, across all adult age groups for both sexes.

It is also important to point out that the controlled increase in phosphate load induced in this study (renal excretion increased from a mean of 615 to 1245 mg per day, or from 20.5 to 41.5 mmol/d, see Table 3) is not excessive, but is well within the range that a majority of a representative United States general population consumes each day (National Health And Nutrition Examination Survey III).[6]

Modulators of Phosphate Homeostasis

FGF-23/PTH/1,25(OH)$_2$D

In response to HP, serum phosphate, PTH, FGF-23, and α-Klotho and urinary Klotho increased significantly, whereas 1,25(OH)$_2$D serum concentrations did not change. After supplementation with 600,000 U vitamin D_3, 25(OH)D and 1,25(OH)$_2$D increased significantly suggesting that, at least in part, D_3 period elevations in 1,25(OH)$_2$D during both arms were driven by rising 25(OH)D levels. PTH and FGF-23 (and serum and urine Klotho) decreased significantly in the D_3 period of HP. PTH stimulates and FGF-23 inhibits 1α hydroxylase and both 1,25(OH)$_2$D and PTH have been shown to increase FGF-23.[24,25] Thus, under the HP conditions of these experiments, our D_3 period results are best explained by a dominant effect of PTH on FGF-23/Klotho because FGF-23 and serum and urine Klotho fell in parallel with falling PTH concentrations, despite continued HP, a further significant increase in plasma phosphate concentration, and persistently high 1,25(OH)$_2$D levels. These changes in aggregate lend further support to the notion that complete adaptation to phosphate loading requires PTH.[17,43]

It is possible that the sympathetic activation observed herein is responsible in part for the FGF-23 increase, because acute dosing with the β adrenergic agonist, isoproterenol, produced large increases in femur FGF-23 expression and administration of the β blocker, propranolol, prevented the circadian increase in femur FGF-23 expression.[44] Propranolol pretreatment, however, had no effect on HP-diet–induced femur FGF-23 expression. The mechanism of β adrenergic control of FGF-23 production was attributed to a cAMP response element in the FGF-23 promotor as assessed in osteoblastic UMR-106 cells.[44]

Klotho

The hyperphosphatemic increase in serum and urinary Klotho as well as FGF-23 represent the first demonstration in humans of a phosphate-driven increase in Klotho and suggest that elevated Klotho levels reflect a chronic adaptation to phosphate inasmuch as acute phosphate loading in humans documented an increase in serum FGF-23 but found no change in serum Klotho.[38] Recombinant Klotho administration to FGF-23 knockout mice is reported to exhibit a phosphaturic effect,[45] albeit with n=4, raising the possibility that proximal tubule effects of klotho are not confined to its role as coreceptor with membrane FGFR1c for circulating FGF-23 agonism.[46] The present results with elevated Klotho and FGF-23 levels leave open the question as to whether both of the reported proximal tubule effects of the Klotho response to hyperphosphatemia [*i.e.*, phosphaturia and decreased 1,25(OH)$_2$D] are dependent on or independent of increased FGF-23 in humans. The elevations in serum and urinary Klotho levels in response to hyperphosphatemia in humans reinforce the mounting evidence for Klotho's key role as a homeostatic phosphatonin and, potentially, for homeostatic beneficial effects in multiple organs.

The elevation of serum 1,25(OH)$_2$D after D_3 administration did not detectably stimulate intestinal phosphate absorption in HP and induced only a modest stimulatory effect under LP conditions. Thus, the effect of vitamin D on intestinal phosphate absorption in humans is small, compatible with the small effect found in rats.[12]

In conclusion, this study demonstrates that chronic, *i.e.*, weeks of high dietary phosphate intake as sodium phosphate increases sympathoadrenergic activity and consequently increases SBP, DBP, and pulse rate in young humans with normal renal function. Thereby, it provides at least one important explanation for the association of dietary phosphate intake and/or plasma phosphate concentrations with increased cardiovascular morbidity and mortality, irrespective of renal

Table 6. Effect of HP and LP dietary conditions without and with vitamin D_3 on fasting markers of cardiovascular risk, oxidative stress, and aging as well as the calcifying propensity of blood (T50)

Variable	Insulin (IU/L)	HOMA-IR	Glucose (mmol/L)	HbA_{1C} (%)	hsCRP (mg/dl)	Fibrinogen (mmol/L)	D-Dimers (μg/ml)	Homocysteine (μmol/L)	Albumin (g/L)	Triglycerides (mmol/L)	Total Cholesterol (mmol/L)	oxLDL (ng/ml)	Antiox. Capacity (μmol/L)	Lipid Peroxides (μmol/L)	Superoxide Dismutase (ng/ml)	8-oxo-2deoxy guanosine (urine) (μg/L)	T50[a] (Min)
HP baseline	9.2±1.4	1.71±0.17	3.99±0.33	5.2±0.2	1.38±0.11	2.9±0.2	0.22±0.06	11.0±1.4	46.0±1.1	0.99±0.09	4.44±0.20	385±27	253±22	385±38	4.26±0.31	3.39±0.21	340±32
LP baseline	9.4±1.2	1.74±0.15	4.02±0.31	5.2±0.2	1.42±0.12	2.9±0.2	0.24±0.07	10.8±1.5	45.9±1.2	1.00±0.07	4.45±0.23	419±30	256±25	398±33	4.27±0.27	3.39±0.22	300±34
HP W6	8.2±0.9	1.44±0.14	3.87±0.38	5.1±0.3	2.10±0.16	2.9±0.1	0.20±0.05	12.2±1.3	46.9±1.0	0.95±0.06	4.51±0.20	362±28	236±22	521[b]±42	4.84±0.30	5.55[b]±0.41	305±40
LP W6	9.1±1.1	1.64±0.14	3.92±0.35	5.2±0.3	1.58±0.11	2.9±0.2	0.26±0.06	10.9±1.2	46.8±0.9	0.93±0.07	4.50±0.22	397±32	234±24	478[b]±44	4.72±0.26	5.98[b]±0.42	274±29
HP plus D3 W11	9.4±1.2	1.92±0.24	4.16±0.25	5.1±0.2	1.39±0.12	2.8±0.1	0.28±0.05	12.0±1.3	46.0±0.9	0.97±0.06	4.50±0.24	435±33	240±21	449[b]±47	3.83±0.36	5.42[b]±0.44	308±39
LP plus D3 W11	9.3±1.0	1.82±0.18	4.04±0.29	5.1±0.2	1.79±0.14	2.8±0.1	0.30±0.06	12.3±1.2	46.3±1.0	0.95±0.05	4.6±0.23	439±37	241±19	448[b]±45	4.43±0.41	6.00[b]±0.47	259±27

Values are mean±SEM. HOMA-IR, homeostatis model assessment insulin resistance; HbA_{1C}, glycosylated hemoglobin; hsCRP, high-sensitivity C-reactive protein; oxLDL, oxidized low density lipoprotein; Antiox., antioxidant; W, week.

[a] $n=7$ in both groups.

[b] $P<0.05$ for the intragroup comparison with baseline.

function. These conclusions, although of high public health importance, require support of larger studies in more diverse populations.

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DISCLOSURES

None.

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