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High-phosphorus diet controlled for sodium elevates blood pressure in healthy adults via volume expansion

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Abstract

Whether increasing exposure to dietary phosphorus can lead to adverse clinical outcomes in healthy people is not clear. In this open-label prospective cross-over study, we are to explore the impact of various dietary phosphorus intake on mineral, sodium metabolisms and blood pressure in young healthy adults. There were 3 separate study periods of 5 days, each with a 5 days washout period between different diets interventions. Six young healthy male volunteers with normal nutrition status were recruited in Phase I Clinical Research Center and sequentially exposed to the following diets: (a) normal-phosphorus diet (NPD): 1500 mg/d, (b) low-phosphorus diet (LPD): 500 mg/d, (c) high-phosphorus diet (HPD): 2300 mg/d. HPD induced a significant rise in daily average serum phosphate (1.47 ± 0.02 mmol/L [4.56 ± 0.06 mg/dl]) compared to NPD (1.34 ± 0.02 mmol/L [4.15 ± 0.06 mg/dL]) and LPD (1.17 ± 0.02 mmol/L [3.63 ± 0.06 mg/dL]) ($p < .05$). Daily average levels of serum parathyroid hormone and fibroblast growth factor 23 in HPD were significantly higher, and serum $1,25(OH)_2D_3$ was remarkably lower than those in LPD. HPD induced a significant decrease in daily average serum aldosterone and an increase in daily average atrial natriuretic peptide level compared to LPD. The 24-hour urine volume in HPD subjects was less than that in LPD subjects. HPD significantly increased daily average systolic blood pressure by 6.02 ± 1.24 mm Hg compared to NPD and by 8.58 ± 1.24mm Hg compared to LPD ($p < .05$). Our study provides the first evidence that 5-day high-phosphorus diet can induce elevation in SBP in young healthy adults, which may due to volume expansion.

1 | INTRODUCTION

Hyperphosphatemia is one of the most common complications among patients with chronic kidney disease (CKD), which is closely related to cardiovascular disease (CVD) and mortality.[1-3] Moreover, recent large epidemiologic studies suggest that mild elevations of serum phosphate (Pi), even within the normal range, are associated with an increased cardiovascular disease (CVD) risk even in general population.[4-6] Thus, how to maintain the steady phosphate metabolism and avoid the increase of phosphate loading in general population has become an increasingly concerned public health problem.[7]

Maintenance of phosphate homeostasis depends on the regulation of phosphate handling by intestine, bone and kidney.[7,8] For general populations with healthy bone and normal renal function,

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dietary intervention might be the main way to control the normal and stable levels of serum Pi.[7] However, dietary phosphorus intake continues to increase in recent decades, resulting from the growing application of inorganic phosphate additives in processed foods, which are highly absorbable from the gastrointestinal tract. The phosphorus intake is now far more above the current daily recommendation and has largely exceeded the nutrient needs of healthy population.[9,10] Increasing studies have confirmed that excessive dietary phosphorus intake could lead to the change of serum Pi and have adverse consequences on cardiovascular systems in general populations.[9,11-13] The basic mechanisms linked to cardiovascular damage caused by dietary phosphorus have been demonstrated to include phosphorus-regulating hormones, especially fibroblast growth factor 23 (FGF23) and parathyroid hormone (PTH).[14,15]

Although numerous studies have demonstrated the association of dietary phosphorus intake and serum Pi with left ventricular structure and function, cardiovascular events, and death in the general population, little attention has been paid to the potential role of dietary phosphorus excess in the pathogenesis of blood pressure (BP). Some studies have found that people with more dietary phosphorus intake had reduced BP or lower risk of hypertension.[16-18] However, these studies either depended on food frequency questionnaires [16] or were cross-sectional.[17,18] A recent systematic review of randomized trials and observational studies did not find an association of dietary phosphorus intake with BP.[19] On the contrary, a small amount of experimental studies had implicated that chronic dietary phosphorus loading elevated BP in rats.[20-22] A secondary analysis of PREMIER Study demonstrated the longitudinal (6 months) association between dietary intake of phosphorus (total, plant, animal, and added) with BP.[23] A recent prospective study has demonstrated that in healthy young adults, increased phosphorus intake for 11 weeks induced a significant increase in 24-hour ambulatory BP and pulse rate.[24] So the exact effects of dietary phosphorus intake on BP remain to be explored.

In the current prospective crossover-controlled study performed in young healthy subjects, we investigated the effects of normal-phosphorus diet (NPD), low-phosphorus diet (LPD), and high-phosphorus diet (HPD) for 5 days on BP, as well as phosphate and sodium homeostasis to probe into its possible mechanisms.

2 | MATERIALS AND METHODS

2.1 | Study subjects

This study is an exploratory study, referring to the principle of phase I clinical trial, and the sample size is about 6-10. So, six young healthy male volunteers with normal nutrition status and without any medication use were recruited. The study was approved by the Ethics Committee on Human Research of Huashan Hospital, Fudan University. During dietary intervention, and all subjects were hospitalized in Phase I Clinical Research Center (Huashan Hosptial, Fudan University, Shanghai, China), which obtained the ISO 17 025 quality certification. Written informed consent was obtained from each patient.

2.2 | Study design

This study was conducted as an open-label crossover-controlled study, which has been registered in ClinicalTrials.gov (#NCT03208075). Before coming into our study, all subjects should be on their usual diets for at least 5 days. Then, there were 3 separate study periods of 5 days, each with a 5 days washout period between different diets interventions (Figure 1A). All subjects were sequentially exposed to the following diets which were prescribed and prepared by Nutrition Department at Huashan Hospital: (a) NPD: 1500 mg/d, (b) LPD: 500 mg/d, and (c) HPD: 2300 mg/d. The HPD was achieved by supplementing the NPD with a solution of neutral sodium phosphate (4.85:1 mixture of Na_2HPO_4 and NaH_2PO_4, 40 ml/d) in divided doses with lunch and dinner.[25] The LPD was achieved by reducing protein content to lower phosphorus content without using any phosphate binder. As protein content was low in LPD, carbohydrate intake was increased to ensure that calorie intake was consistent with NPD and HPD. The intakes of sodium (Na) were constantly maintained by administering additional sodium chloride 2.73 g (which contained Na 1073 mg) during NPD and LPD equal to the amount administered during HPD. Dietary analysis was performed using China Food Composition Database.

The subjects were kept blind to diet interventions through the study. During the 5-day hospitalization with diet intervention, the subjects were stipulated to get up at 7:00, sleep at 22:30, have breakfast at 7:30, lunch at 11:30 and dinner at 17:30, respectively (Figure 1B). The meals should be completed in half an hour. The amount of drinking distilled water for each patient was 2500 ml/d. The subjects were free to perform light physical activities in the ward, but were forbidden to go out and eat other foods. During the discharge washout periods, the subjects were asked to take their usual diets, avoiding significant changes.

On the first day of each diet intervention, the measurements of fasting blood and urine samples collected at 04:00 were used as baseline values. Then at the end of each intervention, subjects underwent blood and urine assessments, and body weight and BP measurement at 10 time points (08:00, 10:00, 12:00, 14:00, 16:00, 18:00, 20:00, 24:00, 04:00, 08:00) from 08:00 on the 5th day to 08:00 on the 6th day (Figure 1B).

2.3 | Blood pressure measurement

BP was measured using the Omron U16 oscillometer monitor that has been validated with a cuff size appropriate for the arm. After the subjects rested quietly for 5 minutes in a seated position with the arm at the level of the heart, BP was measured three times in a row, with an interval of 30 seconds. The average of these three recordings was taken. All observers had received an additional

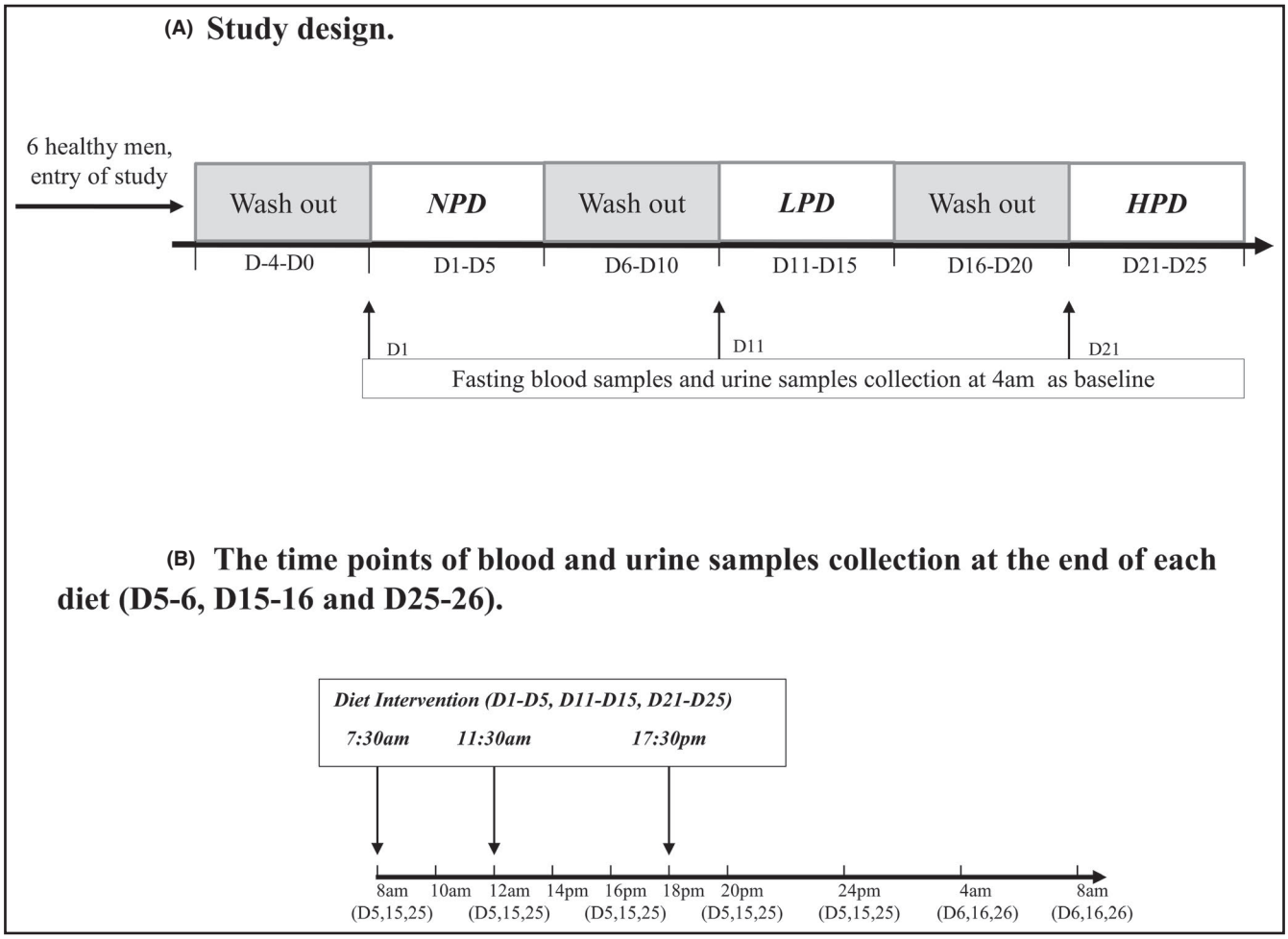

FIGURE 1 Study design (A) and the time points of blood and urine samples collection at the end of each diet (D5-6, D15-16, and D25-26) (B). Abbreviations: NPD, Normal-phosphorus diet; LPD, Low-phosphorus diet; HPD, High-phosphorus diet

training of BP measurement skills and were blind to interventional assignment.

2.4 | Biochemical assessments

Serum biochemical indicators such as electrolytes, blood urea nitrogen (BUN), serum creatinine (Scr) etc, as well as urinary electrolytes and creatinine were analyzed using a modular system from Roche Diagnostics (Mannheim, Germany). Tubular reabsorption of Pi (TRPi), calcium (TRCa), and Na (TRNa) were calculated using the following formula: *TRPi/Ca/Na(%)=100× [1-(urinary Pi/Ca/Na (mmol/L) ×Scr (umol/L))/(Ucr (umol/L) ×serum Pi/Ca/Na (mmol/L))]*. Pi and Na balance gap was calculated as follows: *Pi (or Na) balance gap (mg) = Pi (or Na) balance gap at the previous time point (mg) - the urinary Pi (or Na) excretion at the current time point (mg)*. As for Pi *(or Na)* balance gap at 08:00, 12:00, and 18:00, the corresponding dietary Pi *(or Na)* intake per meal multiply by intestinal absorption rate were added to the above formula. Intestinal absorption rate was calculated by 24-hour urinary Pi *(or Na)* excretion divided by dietary Pi *(or Na)* intake.

The serum levels of intact PTH (Immutopics), C-terminal FGF23 (Immutopics), a-klotho (IBL), 1,25 dihydroxy vitamin D (1,25D) (Immunodiagnostic Systems), a-atrial natriuretic peptide (ANP) (Phoenix Pharmaceuticals), brain natriuretic peptide-32 (BNP-32) (Peninsula Lab), Copeptin (the carboxyl terminal of arginine vasopressin (AVP) and was a sensitive surrogate marker for AVP release) (Phoenix Pharmaceuticals), active renin (LDN), Angiotensin II (AngII) (Phoenix Pharmaceuticals), and aldosterone (LDN) were measured by ELISA according to the manufacturers' protocols. The samples for measuring AngII were pre-treated by passing through a Centricon-10 column with a cutoff of >10 000 Da (Amicon).

2.5 | Statistical analysis

Data are presented as means ± SE. The values at 04:00 after interventions were compared with baseline values (at 04:00 before interventions) by paired t test for self-comparison to examine intra-group changes before and after dietary intervention. Differences were analyzed by one-way ANOVA followed by a Tukey-Kramer test among groups to examine intergroup changes. To evaluate the presence

of circadian rhythm, we used a double repeated-measure ANOVA. Repeated factors were diet (NPD, LPD, and HPD) and hour of a day (08:00, 10:00, 12:00, 14:00, 16:00, 18:00, 20:00, 24:00, 04:00, 08:00). Subjects were treated as random effects, and the covariance structure was set as unstructured for diet by autoregressive for hour of day. We tested diet × time interactions to determine whether the diurnal pattern of indicators differed across diets. For all tests, two-tailed p values of < .05 were considered statistically significant. All analyses were performed with SPSS statistic 20.0.

3 | RESULTS

3.1 | Study population and dietary intervention

Six young male volunteers with normal nutrition status and biochemical indexes were enrolled (Supplemental Table S1). Briefly, mean age, systolic blood pressure (SBP), and estimated glomerular filtration rate were 29 ± 2 years, 111.83 ± 5.19 mmHg, and 114.69 ± 2.89 ml/min^2/1.73 m^{2,} respectively. All subjects successfully completed the three interventions without any adverse events. No significant differences were observed regarding to nutrient components including total calories, calcium, and sodium (Table 1). The intakes of electrolytes were verified by 24-hour urine excretions. HPD and NPD induced a significant intra-group increase in serum BUN, while LPD induced a significant intergroup decrease, which might be due to the high protein content in HPD and NPD. No significant differences were seen in other nutritional characters (Supplemental Table S2).

3.2 | Changes in serum Pi and urinary Pi excretion

HPD induced a significant intergroup rise in fasting serum Pi, daily average serum Pi, and urinary phosphate/urinary creatinine ($U_{Pi/Cr}$) compared to NPD and LPD intervention (Table 2, Supplemental Table S3). Daily average TRPi decreased significantly in HPD compared to LPD (Supplemental Table S3).

Serum Pi concentrations differed significantly by diet (Figure 2A, $p_{\text{diet effect}}$ < 0.001). Although the circadian pattern of serum Pi also differed by diet (Figure 2A, $p_{\text{diet×time interaction}}$ = 0.013), there were still two peaks at 14:00-16:00 and 04:00 and a nadir at 10:00. Serum Pi levels at 08:00-12:00 were observed to be significantly different only between HPD and LPD, and serum Pi concentrations were higher in the afternoon, indicating daily average values of serum Pi might be more precise to reflect the dietary phosphorus loading than the fasting morning values.

There was a significantly increased $U_{Pi/Cr}$ level in HPD compared with NPD and LPD ($p_{\text{diet effect}}$ < 0.001), and the circadian pattern of $U_{Pi/Cr}$ also differed by diet ($p_{\text{diet×time interaction}}$ = 0.004) (Figure 2B). The 24-hour urinary Pi excretion in HPD (35.87 ± 2.88 mmol/24 h [1111.97 ± 89.28 mg/24 h]) was significantly higher than those in NPD (30.48 ± 1.03 mmol/24 h [944.88 ± 31.93 mg/24 h]) and LPD (15.54 ± 0.79 mmol/24 h [481.74 ± 24.49 mg/24 h]) (p < .05) (Figure 2C). Pi balance gaps of the 3 diets were positive from 08:00 to 04:00 the next day, peaked at 12:00 after lunch and 18:00 after dinner. These positive Pi balances suggested that dietary phosphorus loading cannot be excrete timely and sufficiently. After 18:00, with no further dietary phosphorus loading, Pi balance gaps were gradually decreased and reached zero balance at 08:00 the next day. Moreover, there was a significantly increased Pi balance gap in HPD compared with NPD and LPD ($p_{\text{diet effect}}$ < 0.001), suggesting HPD may lead to more postprandial Pi accumulation (Figure 2D).

3.3 | Changes in serum calcium (Ca), urinary Ca excretion, and circulating hormones related to mineral metabolism

HPD induced a significant decrease of daily average serum Ca compared with LPD (Supplemental Table S4). Moreover, HPD led to a significant decrease in urinary calcium/urinary creatinine ($U_{Ca/Cr}$) and 24-hour urinary Ca excretion, as well as an increase in the daily average level of TRCa compared with NPD and LPD (Supplemental Table S3, Supplemental Figure S1).

Daily average levels of serum PTH and FGF23 in HPD were significantly higher (Table 2), and serum 1,25D was remarkably lower than those in LPD, whereas there was no difference in serum α-klotho level among 3 diets (Supplemental Table S4).

3.4 | Changes in serum Na and urinary Na excretion

Although HPD induced a slight intergroup increase in daily average serum Na compared to LPD and LPD led an intra-group decrease in fasting serum Na compared to baseline (Table 2), the circadian

TABLE 1 Diet composition of three intervention diet

Diet		Calories (kcal)	Protein (g)	Phosphorus (mg)	Calcium (mg)	Sodium (mg)
NPD	2177.04 ± 22.61		112.36 ± 1.34	1479.30 ± 19.94	858.54 ± 18.04	3006.78 ± 86.07
LPD	2154.14 ± 6.51		50.64 ± 1.17[a]	496.04 ± 6.49[a]	794.72 ± 35.46	2949.18 ± 24.56
HPD	2172.18 ± 23.61		110.40 ± 1.77[b]	2221.14 ± 10.67[ab]	779.04 ± 47.56	3029.70 ± 85.66

Note:: Values expressed as Mean ± SE [a]p < .05 vs NPD; [b]p < .05 vs LPD.

Abbreviations: HPD, High-phosphorus diet; LPD, Low-phosphorus diet; NPD, Normal-phosphorus diet.

TABLE 2 Effect of dietary phosphorus on serum concentrations and blood pressure in healthy men

	Pi mmol/L (mg/dl)	Na mmol/L	iPTH pg/ml	FGF23 pg/ml	a-ANP ng/ml	Aldosterone pg/ml	SBP mm Hg	DBP mm Hg
NPD								
Baseline[a]	1.44 ± 0.02 [4.46 ± 0.06]	138.22 ± 0.21	33.98 ± 2.10	49.85 ± 4.02	0.55 ± 0.01	825.32 ± 25.67	110.02 ± 3.21	71.48 ± 2.98
End of diet								
Fasting[b]	1.47 ± 0.03 [4.56 ± 0.09]	138.17 ± 0.75	34.97 ± 3.06	50.21 ± 5.48	0.54 ± 0.02	827.33 ± 39.36	107.00 ± 4.28	74.17 ± 3.75
Daily average[c]	1.34 ± 0.02 [4.15 ± 0.06]	138.54 ± 0.29	33.57 ± 1.33	50.53 ± 1.72	0.54 ± 0.01	913.77 ± 10.32	108.33 ± 1.15	69.75 ± 0.92
LPD								
Baseline[a]	1.48 ± 0.03 [4.59 ± 0.09]	138.52 ± 0.15	34.42 ± 1.98	50.22 ± 5.08	0.54 ± 0.02	829.42 ± 26.33	109.64 ± 2.98	71.09 ± 2.94
End of diet								
Fasting[b]	1.51 ± 0.02 [4.68 ± 0.06]	136.63 ± 0.19*,***	33.37 ± 2.08	45.44 ± 6.07	0.56 ± 0.02	874.30 ± 37.77	95.33 ± 2.11***	70.83 ± 2.71
Daily average[c]	1.17 ± 0.02 [3.63 ± 0.06]*	136.96 ± 0.27*	28.74 ± 1.86	48.89 ± 1.98	0.53 ± 0.01	894.85 ± 14.46	105.03 ± 1.09	69.40 ± 0.82
HPD								
Baseline[a]	1.47 ± 0.02 [4.56 ± 0.06]	138.50 ± 0.14	34.21 ± 2.41	49.53 ± 4.93	0.55 ± 0.02	829.29 ± 26.26	107.17 ± 2.39	71.25 ± 2.91
End of diet								
Fasting[b]	1.66 ± 0.01 [5.15 ± 0.03]*,**,***	138.13 ± 0.33**	40.42 ± 8.19	59.30 ± 7.66	0.62 ± 0.02	741.67 ± 28.90**	112.17 ± 4.29**	71.67 ± 2.11
Daily average[c]	1.47 ± 0.02 [4.56 ± 0.06]*,**	138.97 ± 0.22**	36.12 ± 2.26**	57.00 ± 2.69**	0.66 ± 0.01*,**	721.41 ± 15.66*,**	114.00 ± 1.36*,**	70.52 ± 0.83

Note: Values expressed as Mean ± SE.

Abbreviations: a-ANP, a-Atrial natriuretic peptide; DBP, diastolic blood pressure; FGF23, fibroblast growth factor 23; HPD, High-phosphorus diet; iPTH, intact parathyroid hormone; LPD, Low-phosphorus diet; Na, serum sodium; NPD, Normal-phosphorus diet; Pi, serum phosphate; SBP, systolic blood pressure.

[a]Baseline value was the value at 04:00 on the D1, D11, and D21 before diet intervention.

[b]Fasting value was the value at 04:00 on the D6, D16, and D26 at the end of diet intervention.

[c]Daily average value was calculated by the ten time points values from 08:00 (D5, D15, D25) to 08:00 (D6, D16, D26).

$^{*}p < .05$ vs NPD.

$^{**}p < .05$ vs LPD.

$^{***}p < .05$ vs the baseline in the same diet intervention.

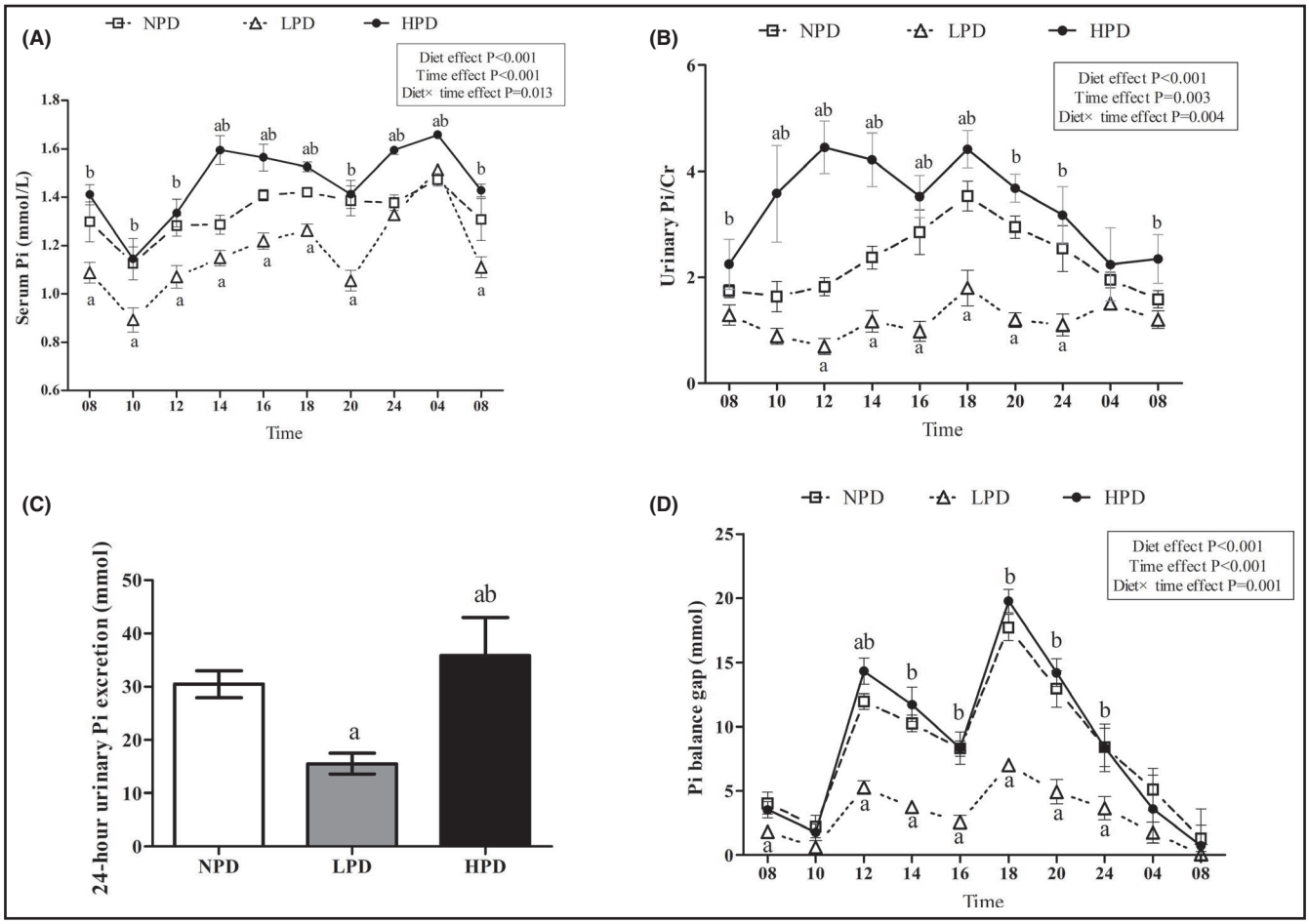

FIGURE 2 Changes of serum phosphate (Pi) (A), urinary phosphate/urinary creatinine (urinary Pi/Cr) (B), and Pi balance gap (D) in healthy male subjects (n = 6) at different time points across normal-phosphorus diet (NPD), low-phosphorus diet (LPD), and high-phosphorus diet (HPD). 24-hour urinary Pi excretion between NPD, LPD, and HPD (C). Depicted are mean values ± SE $^{a}p < .05$ vs NPD; $^{b}p < .05$ vs LPD

rhythm curve showed that there was no marked difference at 16:00-24:00 between the three groups (Figure 3A).

There was no significantly difference in urinary sodium/urinary creatinine ($U_{Na/Cr}$) level between the 3 diets ($P_{diet\ effect}$ = 0.265) (Figure 3B, Supplemental Table S3), and there was no significant difference in 24-hour urinary Na excretion among NPD (117.02 ± 3.41 mmol/24 h), LPD (118.23 ± 1.39 mmol/24 h), and HPD (116.20 ± 1.27 mmol/24 h) groups ($p > .05$) (Figure 3C). The circadian rhythm curves of Na balance in the 3 diets were similar to those of Pi balance, which showed two peaks after lunch and dinner, and Na balance gaps were gradually decreased after dinner and reached zero balance at 08:00 the next day. Although dietary Na intakes of the 3 diets were the same, Na balance gaps of HPD group were significantly higher than those of LPD and NPD group ($p_{diet\ effect}$ = 0.006), suggesting HPD might bring to more Na loading (Figure 3D).

HPD induced less 24-hour urine volume (1558.49 ± 85.38 ml/24 h) than NPD (2367.70 ± 49.35 mL/24 h) and LPD (2612.43 ± 133.36 ml/24 h) ($p < .05$) (Figure 3E). Accordingly, although the difference was not statistically significant, the body weight of HPD group (63.48 ± 0.89 kg) tended to be higher compared with NPD (63.15 ± 0.93 kg) and LPD (63.05 ± 0.92 kg).

3.5 | Changes in circulating hormones related to sodium metabolism

HPD induced a significant elevation in the daily average ANP level compared to NPD and LPD (Table 2). The serum ANP from 14:00 to 18:00 was significantly higher in HPD compared to NPD and LPD (Figure 4A). HPD induced a significant intergroup decrease in daily average serum aldosterone concentration (Table 2). The circadian pattern of serum aldosterone differed significantly by diet ($p_{diet\times time\ interaction} < 0.001$) (Figure 4B). The active renin and Ang II level tended to decrease in HPD group compared with NPD and LPD group, although not reaching statistically significant. No significant differences of daily average levels of serum BNP and AVP were observed among 3 diets (Supplemental Table S4).

3.6 | Changes in blood pressure

HPD significantly increased daily average SBP by 6.02 ± 1.24 mm Hg compared to NPD and by 8.58 ± 1.24 mm Hg compared to LPD,

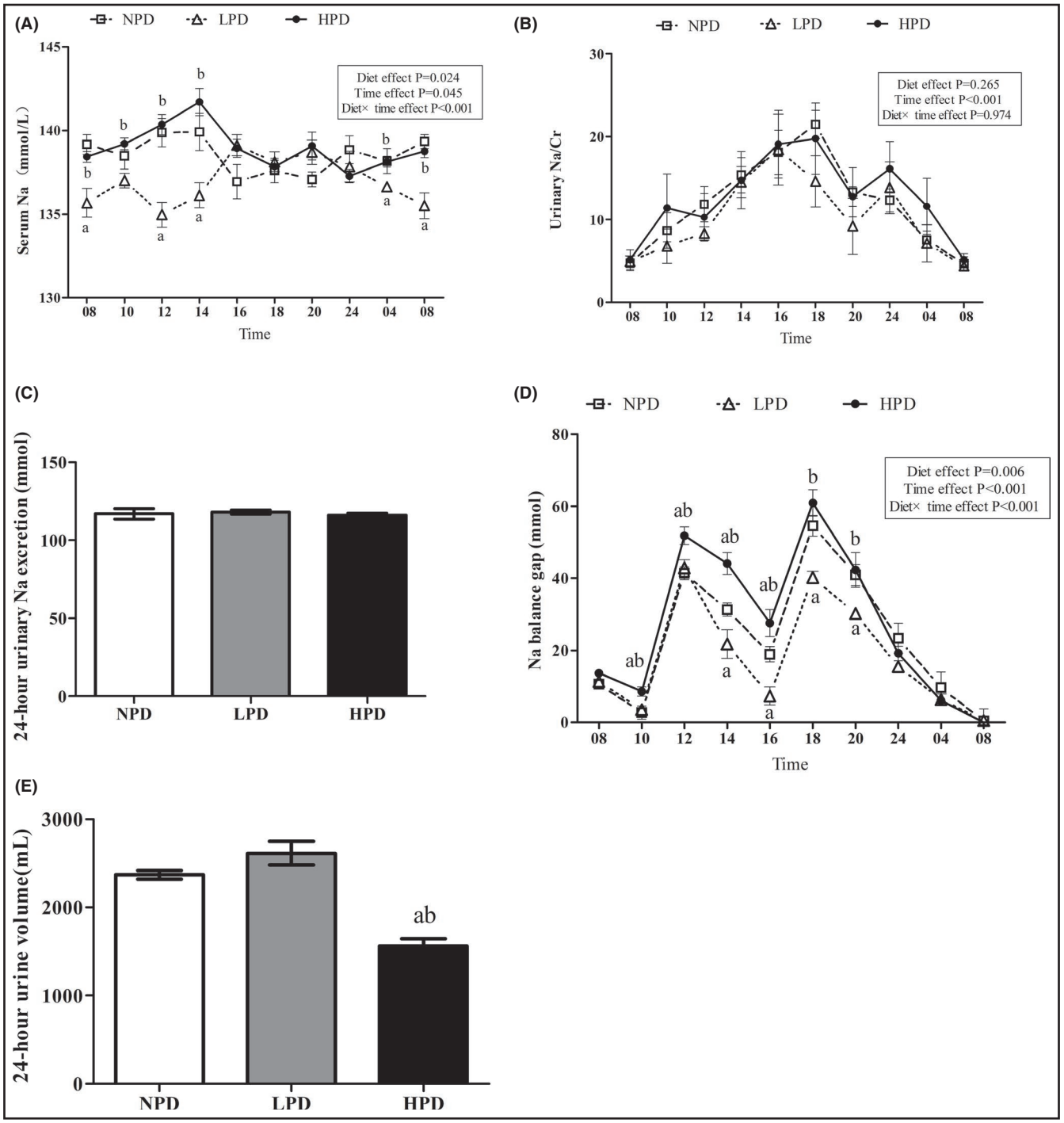

FIGURE 3 Changes of serum sodium (Na) (A), urinary sodium/urinary creatinine (urinary Na/Cr) (B), Na balance gap (D) in healthy male subjects (n = 6) at different time points across normal-phosphorus diet (NPD), low-phosphorus diet (LPD) and high-phosphorus diet (HPD). 24-hour urinary Na excretion (C) and 24-hour urine volume (E) between NPD, LPD, and HPD. Depicted are mean values ± SE $^{a}p < .05$ vs NPD; $^{b}p < .05$ vs LPD

respectively. Meanwhile, LPD decreased SBP in the fasting state compared with baseline level (Table 2), but there was no orthostatic hypotension. The circadian pattern of SBP differed significantly by diet ($p_{\text{diet×time interaction}} = 0.027$) (Figure 4C). There was no significant difference in diastolic blood pressure (DBP) among the 3 diets (Table 2, Figure 4D).

4 | DISCUSSION

To our knowledge, this study is the first reported prospective crossover controlled trial in young healthy adults, to evaluate the effects of absolute dietary phosphorus interventional on mineral and sodium metabolisms, and the effects on BP. The primary finding is that

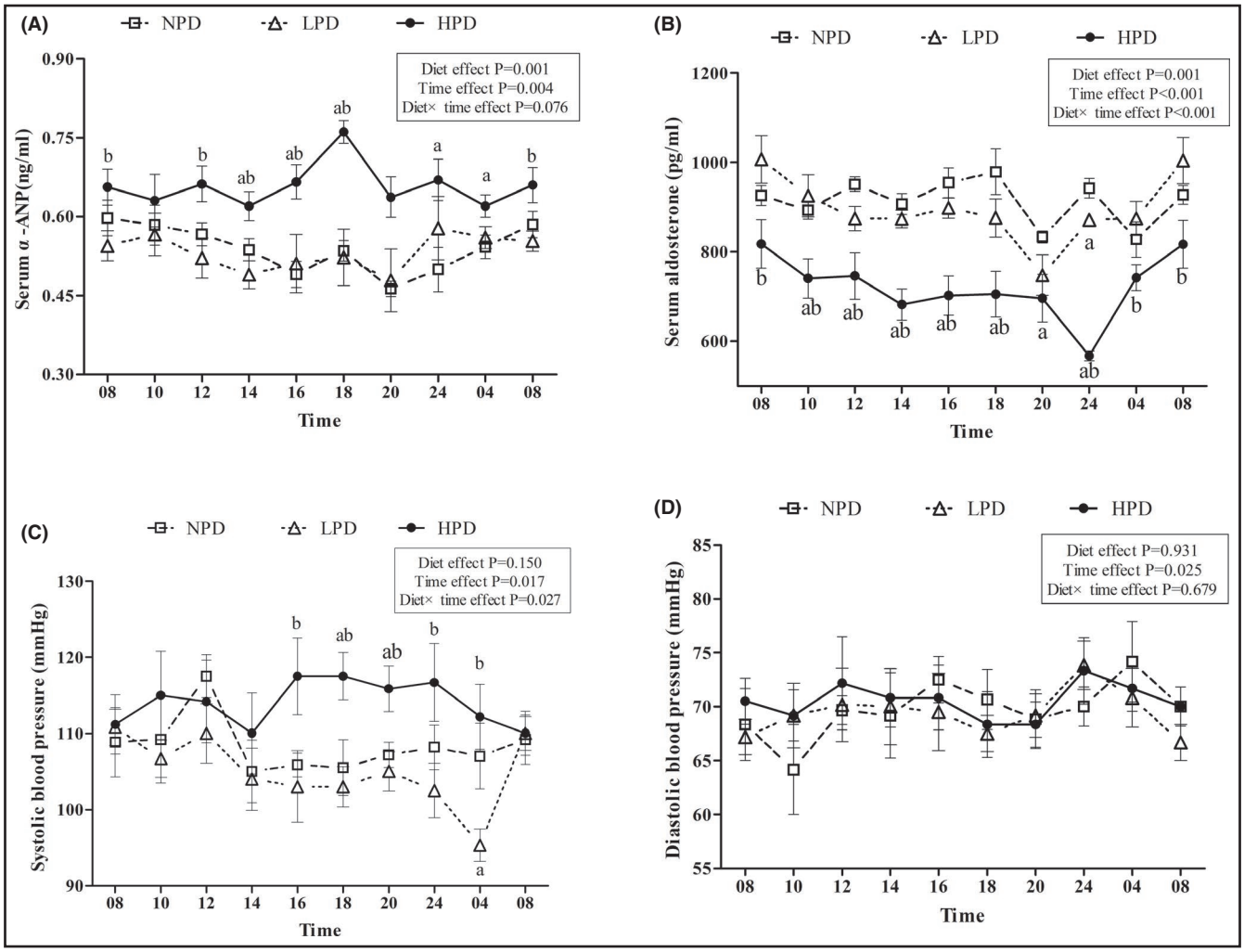

FIGURE 4 Changes of serum a-atrial natriuretic peptide (a-ANP) (A), aldosterone (B), systolic blood pressure (C), and diastolic blood pressure (D) in healthy male subjects (n = 6) at different time points across normal-phosphorus diet (NPD), low-phosphorus diet (LPD), and high-phosphorus diet (HPD). Depicted are mean values ± SE $^{a}p < .05$ vs NPD; $^{b}p < .05$ vs LPD

5-day high-phosphorus intake not only increases Pi loading, but also increases SBP, which may be through promoting volume expansion.

One of the strengths of our study was the availability of measurements of serum Pi at multiple time points across the day, concurrent availability of urine Pi and known phosphaturic hormones, to evaluate the Pi loading comprehensively. Firstly, fasting morning serum Pi was routinely used to evaluate Pi loading, but more evidence revealed that it could not reflect the actual Pi loading. As shown in our and other studies,[12,26-28] there was a biphasic circadian rhythm of serum Pi, showing a decline in the morning and a rise in the afternoon. Supplementation of dietary phosphorus induced no significant change in morning fasting serum Pi, but the daily average serum Pi increased significantly.[11,26,29-32] So, the daily average serum Pi measurement might be more precise and more likely to detect differences than fasting morning specimen.

Secondly, elevations in Pi loading are often associated with changes in phosphorus-regulating hormones such as PTH, FGF23, and vitamin D.[11,26,29-33] PTH is considered to be the fastest hormone mediating the kidney's response to dietary phosphorus. High-phosphorus diet significantly increased the daily average serum PTH and FGF23 levels, which were consistent with previous reports,[29-33] while high-phosphorus diet decreased the levels of 1,25D in our and other studies.[11,26,29] Although high-phosphorus diet induced significant changes in the hormones such as FGF23 and 1,25D, the morning fasting serum Pi remained unchanged in some studies,[29,31,32] indicating phosphorus-regulating hormones might be important in reflecting Pi loading than fasting morning serum Pi.

The third parameter to evaluate Pi loading is urinary Pi excretion. Although bone release and gut absorption are important in establishing the filtered load of Pi, the renal threshold for Pi reabsorption in the proximal tubule is the most important in determining the steady serum Pi concentration. PTH and FGF23, the two dominant phosphaturic hormones, may increase urinary Pi excretion.[34] In our study, it was novel to find that although PTH, FGF23, and urinary Pi excretion increased in response to 5-day HPD, both fasting serum Pi and daily average serum Pi were still significantly increased, suggesting the compensatory increase in urinary Pi excretion was not able

to excrete excess Pi from diet timely and sufficiently, thus resulting in Pi accumulation.

As we mentioned, inconsistent findings exist for the association between dietary phosphorus intake and BP. One reason might be that most of these studies were cross-sectional or were not interventional, and the dietary phosphorus intake data came from food questionnaires. Recently, a prospective study found increased phosphorus intake for 11 weeks significantly increases SBP, DBP, and pulse rate in healthy adults, in part, by increasing sympathoadrenergic activity.[24] Notably, the principal finding of our study was that even 5 days of high-phosphorus diet (controlled for appropriate dietary sodium) could induce significant increase in SBP in healthy young adults.

It is interesting to explore the possible mechanism of 5-day phosphorus loading induced hypertensinogenic effect, which may be different from the previous mentioned chronic animal and human studies.[20-22,24] Dietary protein intake could influence glomerular filtration rate, which might affect BP. However, although serum creatinine was lower and estimated glomerular filtration rate (eGFR) seemed higher in HPD than in LPD and NPD, there was no significant difference in eGFR among the three groups. Therefore, renal hemodynamic changes might have little effect on BP in the study. The activation of renin-angiotensin-aldosterone system (RAS) is central to many common pathologic conditions including hypertension, heart failure, and renal disease. It was found that high-phosphorus diet for 4 weeks increased arterial blood pressure through an increase in renin and Ang II in rats,[20] while plasma renin/aldosterone and 24-hour urinary aldosterone excretion were unchanged despite a significant increase in 24-hour BP induced by increased dietary phosphorus for at least 6 weeks in healthy human adults.[24]

In contrast in our study, it was found that high-phosphorus intake induced a decreasing trend of serum renin and Ang II, a significant decrease in serum aldosterone, and a remarkable increase in ANP. As we know, aldosterone and natriuretic peptide are two major systems in response to the changes in effective arterial blood volume by regulating both systemic vascular resistance and Na excretion. As the sodium and volume conserving hormone, aldosterone increases renal tubular Na^+ reabsorption through augmented membrane abundance of the epithelial Na^+ channel (ENaC) and activating NaCl-cotransporter (NCC) in the distal parts of the nephron. The natriuretic peptides are vasodilators and increase sodium excretion by inhibiting the NCC and ENaC of the distal tubules. In response to volume expansion, natriuretic peptide secretion is increased while aldosterone is decreased, changes that promote urinary excretion of the larger sodium loading. So, the reduced levels of aldosterone and increased levels of ANP in our subjects indicated a state of volume expansion. Accordingly, although the water intake was similar among three groups, the 24-hour urine volume in HPD subjects was less than that in LPD subjects, suggesting more water balance gap in HPD subjects. Although there was no statistical difference in the body weight among three groups, the body weight in HPD subjects tended to be higher than that in LPD subjects. These evidences suggested the increased SBP might be due to volume expansion.

As we know, the common basis for volume expansion is Na retention. Since the sodium intakes and 24-hour urinary sodium excretions were identical among the three groups, suggesting the total sodium balance was similar. But when we calculated circadian sodium balance changes among three groups and found that the sodium balance gaps in HPD group were higher than those in LPD group during 10:00-20:00. Although the above-mentioned sodium-regulating hormones changed in order to increase urinary Na excretion, there is a slight lag in equilibration, which might result in a period of more sodium loading in HPD group than that in LPD.

Phosphorus-regulating hormones can also play role in phosphate-induced Na retention. Andrukhova et al found that increased circulating FGF23 could augment distal renal tubular NaCl-cotransporter expression and activity, which led to renal Na retention, volume expansion, and hypertension in mice.[35] This study suggested that FGF23 was not only a phosphaturic, but also a Na^+-conserving hormone involved in volume and BP homeostasis. In addition, animal and human studies supported a clinically relevant interaction between PTH level and aldosterone and suggested an impact of the interaction on cardiovascular health.[36-39] We speculated that in our study, the increased FGF23 and PTH in HPD group might play a role in Na conservation.

Another possible explanation for phosphate-induced Na retention may be the synergistic absorption of sodium and phosphorus in the intestine. Evidence showed that high sodium diet in normal rats could increase the absorption of phosphorus in the intestine.[40] The use of sodium/proton exchanger type 3 (NHE3) inhibitor not only inhibited the absorption of sodium, but also inhibited the absorption of phosphorus in the intestines of rats.[41] Since the synergistic effects of sodium and phosphorus intestinal absorption existed, whether the increased phosphorus absorption might promote more Na absorption through some unknown mechanisms remained to be further explored.

High-phosphorus intake induced BP elevations with a mean increment in SBP of about 8.58mmHg in healthy adults is of great significance. The new US guideline for treatment and prevention of hypertension reduced the threshold to define hypertension and target BP goal to 130/80 mm Hg,[42] instead of less than 140/90 mm Hg which was first proposed more than two decades ago. The rationale for the new guidelines was based on strong and consistent evidence that lower BP was associated with lower cardiovascular risk, and even small increments in BP effect cardiovascular events.[43,44] Thus, although this study subjects are healthy men, the morbidity and mortality implications of phosphate-induced BP elevations are substantial.

Some study limitations should be considered in the interpretation of the results. Firstly, the diet interventions were administered sequentially in a fixed order in all subjects. Although it was a non-randomized cross-over study, there was a 5-day wash out period between each intervention, which would minimize the carry-over effect. Secondly, the study was only single blinded. Although it was not blind to the investigators, the subjects and the people who measured the key outcomes such as blood pressure were blinded to

the interventional assignment, which would avoid the measurement bias. Thirdly, although the sample size of six in our study was relatively small, we had anyways significant results. Fourthly, the participants in our study were males exclusively. So, large sample-size randomized clinical trials in both sexes will be needed in further studies.

In conclusion, our study provides the first evidence that 5-day high-phosphorus diet can induce elevation in SBP in young healthy adults, which may due to—at least in part—volume expansion. Our findings may provide a mechanistic explanation for the association of dietary phosphorus intake with cardiovascular risk and mortality in subjects with and without CKD. If our findings are confirmed in larger and more diverse population, it may lead to revision of food labeling to include phosphorus content and also lead to a new paradigm in preventing hypertension.

CONFLICT OF INTEREST

There are no conflicts of interest.

AUTHOR CONTRIBUTIONS

JC and JZ designed the research; JZ and XD conducted the research; JZ and HY wrote the manuscript; JZ, MW, QZ, and JL analyzed the data; and JC had primary responsibility for the final content. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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