Excessive dietary phosphorus intake impairs endothelial function in young healthy men: a time- and dose-dependent study

Tamae Nishi[1,4] *, Emi Shuto[2]*, Mariko Ogawa[2], Miho Ohya[2], Misaki Nakanishi[2], Masashi Masuda[1], Misaki Katsumoto[1], Hisami Yamanaka-Okumura[1], Tohru Sakai[2], Eiji Takeda[1], Hiroshi Sakaue[3], and Yutaka Taketani[1]

[1]Department of Clinical Nutrition and Food Management, [2]Department of Applied Nutrition, and [3]Department of Nutrition and Metabolism, Institute of Health Biosciences, University of Tokushima Graduate School, Tokushima 770-8503, Japan, [4]Department of Gastroenterology, Hepatology and Nutrition, Kurashiki Medical Center, Kurashiki, Japan

Corresponding Author: Yutaka Taketani, Ph.D

Professor, Department of Clinical Nutrition and Food Management

17 Institute of Health Biosciences, University of Tokushima Graduate School

18 3-18-15, Kuramoto-cho, Tokushima 770-8503, Japan

19 Phone: +81-88-633-9597

20 FAX: +81-88-633-7094

21 e-mail: taketani@tokushima-u.ac.jp

22

23

24 Disclosure: No conflicts of interest are declared.

25

26 *These authors contributed equally to this work.

27

28 Abstract

29 Excessive dietary phosphorus (P) has been speculated to be a risk
30 factor for cardiovascular disease (CVD). Here, we performed a
31 double-blinded crossover study to investigate the time- and
32 dose-dependent effects of dietary P intake on endothelial function
33 in healthy subjects. Sixteen healthy male volunteers were given
34 meals containing 400, 800, and 1200 mg P (P400, P800, and P1200
35 meals, respectively) with at least 7 days between doses. There
36 were no differences in nutritional composition among the
37 experimental diets except for P content. Blood biochemistry data
38 and flow-mediated dilation (%FMD) of the brachial artery were
39 measured while fasted, at 0h, 1h, 2h, and 4h after meal ingestion,
40 and the next morning while fasted. The P800 and P1200 meals
41 significantly increased serum P levels at 1–4h after ingestion. A
42 significant decrease in %FMD was observed between 1–4h, while
43 the P400 meal did not affect %FMD. We observed no differences

among meals in serum P levels or %FMD the next morning. A significant negative correlation was observed between %FMD and serum P. These results indicate that excessive dietary P intake can acutely impair endothelial function in healthy people.

Keywords: *phosphorus, endothelial dysfunction, flow-mediated vasodilation, hyperphosphatemia, chronic kidney disease*

INTRODUCTION

Cardiovascular disease (CVD) is the most important complication contributing to reduced life expectancy in patients with chronic kidney disease (CKD) (1-3). Traditional and non-traditional risk factors relating to the pathogenesis of CVD in CKD patients have been identified (4, 5). Hyperphosphatemia has recently been recognized as a mediator between CKD and CVD (6, 7). Hyperphosphatemia is also an emerging problem, not only in CKD patients, but also in the healthy population. Recent studies have demonstrated that higher serum P levels, even those within the normal range, were associated with the development of atherosclerosis and mortality in the population with normal kidney function (8) and in the Framingham Offspring Study participants (9). Onufrak et al. also demonstrated that a high serum P level was associated with thickening of the carotid artery intima media in the general population (10). Hyperphosphatemia can induce the differentiation of vascular smooth

68 muscle cells to osteoblast-like cells that are involved in the medial
69 calcification of the artery, so-called Mönkeberg's arteriosclerosis (11-13). In
70 addition, we and others have demonstrated that hyperphosphatemia can
71 also mediate endothelial dysfunction (14-16), which is a principal cause of
72 atherosclerosis resulting in CVD.
73 Our previous study demonstrated that the ingestion of a high P diet (1200
74 mg P per meal) impaired flow-mediated dilation at 2h after meal ingestion in
75 young healthy men, compared with those given a control diet (400 mg P per
76 meal) (14). In addition, increasing the extracellular P level induced increased
77 oxidative stress and decreased nitric oxide production in bovine thoracic
78 aorta endothelial cells (14). Peng et al. reported that hyperphosphatemia
79 decreased endothelial nitric oxide synthase (eNOS) expression in human
80 umbilical vein endothelial cells (15). DiMarco et al. also demonstrated that
81 an elevation of extracellular P can induce apoptosis via increased oxidative
82 stress in human endothelial cell lines (16). These results suggest that over a
83 high dietary intake of P may contribute to the pathogenesis of CVD. In this

84 study, we performed a double-blinded crossover study to investigate the
85 dose- and time-dependent effects of high dietary P intake on endothelial
86 function in healthy human subjects.
87

METHODS

Subjects

Sixteen male volunteers aged 23.4±2.8 years and without apparent health problems were recruited for this study. The participants showed no evidence of diabetes, abnormal glucose intolerance, obesity, hypertension, kidney diseases, CVD, dyslipidemia, or other bone and mineral disorders. Demographic data for the participants are provided in Table 1. All participants were nonsmokers, had normal blood pressure, consumed <30 g/d alcohol, and took no medications or antioxidant supplements. The eligibility of participants for this study was determined similarly to our previous reports (14, 17).

Study design

The study used a double-blinded crossover design, with the administration of meals containing specific amounts of P to each volunteer on 3 different days, each separated from the other test days by more than 1 week. Figure 1

illustrates the design of the study. On the day before each test day, the subjects were asked to abstain from foods and beverages other than water not containing P after 13:00. They were served a standard dinner at 20:00 on the evening before each test day, and a standard breakfast at 8:30 on each test day. On the test days, subjects were served either a P400 meal (standard lunch, which contained 400 mg of P + placebo supplement (NaCl)), a P800 meal (standard lunch + 400 mg neutralized phosphate supplement), or a P1200 meal (standard lunch + 800 mg neutralized phosphate supplement) for lunch at 12:30. The composition of the test meals and standard dinner and breakfast is provided in our previous study. In brief, the standard dinner consisted of 700 kcal in energy with a protein:fat:carbohydrate ratio in %energy of 15:19:66, and contained 200 mg of calcium (Ca) and 400 mg of P. The standard breakfast consisted of 700 kcal with a protein:fat:carbohydrate ratio in %energy of 14:21:65, and contained 200 mg of Ca and 400 mg of P. Standard lunch consisted of 700 kcal with a protein:fat:carbohydrate ratio in %energy of 14:21:65, and contained 200 mg

120 of Ca and 400 mg of P.

121 We collected blood samples immediately before (0h), and at 1h, 2h, 4h,
122 and 20h after the test meal ingestion. Venous blood was taken from a
123 median cubital vein for the measurement of serum glucose, insulin, P, Ca,
124 Na, K, Cl, intact-PTH (iPTH) and high sensitivity-C reactive protein
125 (hs-CRP) concentrations. All biochemical measurements and analyses were
126 performed by LSI Medience (Tokyo, Japan). Serum
127 monocyte-chemoattractant protein (MCP-1) and fibroblast growth factor 23
128 (FGF23) were measured by CCL2/MCP-1 immunoassay kit (R&D Systems
129 Inc., Minneapolis, MN) and FGF23 ELISA kit (Kinos, Tokyo, Japan),
130 respectively. We also measured blood pressure and flow-mediated dilation
131 (FMD) by using UNEXEF 18G (UNEX Corporation, Aichi, Japan) according
132 to previously published guidelines (18) immediately before (0h), and at 1h,
133 2h, 4h, and 20h after the test meal ingestion.

134 The study protocols were approved by the Ethics Committee of the
135 Tokushima University Hospital. This study has been registered and opened

136 on the UMIN-CTR database in Japan according to the ICMJE guidelines
137 (UMIN000000803, Dietary phosphorus loading trial in human).
138
139 *Statistical analysis*
140 We tested all data for normal distribution of variables of interests by
141 Kolmogorov-Smirnov test before further parametric or non-parametric
142 statistical analysis. If the test judged the data to be normally distributed, we
143 performed subsequent statistical analysis by parametric analysis. If not, we
144 used nonparametric analysis.
145 Serum biochemical measurements and %FMD within groups and the
146 effects of meals on pre-prandial and post-prandial values of these
147 measurements were analyzed by repeated measurements analysis of
148 variance (ANOVA) and post hoc analysis by Bonferroni's method.
149 For the association analysis, we performed a simple regression analysis
150 and estimated Spearman's non-parametric correlation coefficients. We
151 selected the nonparametric procedure, which does not require normally

distributed data or linear associations of the variables of interest.

We performed all statistical analyses using SPSS Statistics 17.0.

RESULTS

1. Dose and time-dependent effects of high dietary P intake on the serum P level and other P metabolism-regulating factors.

In this study, the subjects alternately received P400, P800, or P1200 meals as lunch and the serum levels of P and P metabolism-regulating factors were measured in the morning (fasting), and before and after intake of the test meal (Table 2 and 3). In spite of the differences in P content among the test meals, the serum P level was significantly increased at 1h, 2h, and 4h after the ingestion of the test meals, compared with the pre-prandial serum P level. However, the serum P levels at 1h, 2h, and 4h after ingestion of the P1200 meal were significantly higher than that measured following ingestion of the P400 meal (Figure 2). Area under the curve (AUC) analysis revealed post-prandial changes in the serum P level during the 4h after test meal ingestion; the serum P level was increased accordingly with the increases in phosphorus intake (Figure 3A). In addition, the serum P levels

172 were above the normal range at 1h, 2h, and 4h after ingestion of the P800 or
173 P1200 meals, but not after ingestion of the P400 meal. Serum P levels had
174 reverted to a normal level when measured the next morning after ingestion
175 of the test meals.

176 Serum intact-PTH levels did not show significant differences among the
177 groups; however, they showed a biphasic peak at 1h and 4h after ingestion of
178 the test meals (Table 2), as reported previously (17). The intact-PTH level at
179 4h after ingestion of the P400 and P1200 meals was significantly increased
180 compared with the pre-prandial serum intact-PTH level. The AUC for
181 post-prandial serum intact-PTH changes over 4h increased accordingly with
182 the increases in the intake of P (Figure 3B). The AUC after the ingestion of
183 the P1200 meal was significantly greater than that after the P400 meal
184 ($P<0.05$). FGF23 is also an important P metabolism-regulating hormone. The
185 serum FGF23 level was not increased following ingestion of the test meals
186 (Table 3). Serum Na, K, Cl, Ca, hs-CRP, and MCP-1 levels also were not
187 affected by the experimental increases in P intake (Tables 2 and 3).

188

189 *2. Dose- and time-dependent effects of high dietary P intake on FMD.*

190 We demonstrated that intake of the P1200 meal led to a significant
191 decrease in %FMD compared with that measured following intake of the
192 P400 meal at 2h after meal ingestion (14). Here, we investigated the dose-
193 and time-dependent effects of high dietary P intake on FMD in young
194 healthy men. As shown in the Figure 4, %FMD at 1h, 2h, and 4h after the
195 ingestion of P800 and P1200 meals was significantly decreased compared
196 with that measured following ingestion of the P400 meal. The peak
197 inhibition of FMD by P800 was observed at 2h after meal ingestion, while
198 that by P1200 was at 1h after meal ingestion. In addition, the decrease
199 in %FMD observed after high P intake was recovered by the next morning.
200 The rate of increase in the post-prandial serum P level between 0–4h after
201 meal intake was significantly correlated with the rate of decrease in %FMD
202 (Figure 5).

203

DISCUSSION

In this study, we investigated the time- and dose-dependent effects of high dietary P intake on endothelial function by evaluating %FMD. We found that FMD was rapidly inhibited by high P intake, but began to be recovered at 4h and was normalized by the next morning. We did not find any clear differences between the P800 and P1200 meals in the high dietary P intake-induced inhibition of FMD. However, the P1200 meal inhibited FMD slightly faster than did the P800 diet. In addition, the inhibitory effect of high dietary P intake could be observed at the minimum level of intake of 800 mg of phosphorus in a single meal.

The post-prandial increase in the serum P level was significantly correlated with the degree of impairment of FMD. Our previous work demonstrated that the experimental elevation of the extracellular P level can inhibit nitric oxide production in endothelial cells via increasing oxidative stress and the inhibitory phosphorylation of eNOS (14). Therefore, a transient increase in the serum P level may be enough to lead to a

220 deterioration of endothelial function. Another possible mechanism for the
221 impairment of endothelial function by a high serum P level is via PTH and
222 FGF23. The post-prandial serum PTH level was increased by high dietary P
223 intake in a dose-dependent manner. Primary hyperparathyroidism patients
224 have an impaired FMD (19-22), but the impairment of FMD was ameliorated
225 after parathyroidectomy (21, 20). Parathyroidectomy or Ca channel blockade
226 was reported to restore inhibited eNOS activity in a rat model of CKD (23).
227 On the other hand, FGF23 also can directly impair endothelium-dependent
228 vasodilation by increasing oxidative stress and reducing NO availability (24).
229 However, the serum FGF23 level was not increased after a single ingestion
230 of a high P meal in our study. Thus, the serum FGF23 level did not appear to
231 be related to the decreases in %FMD observed in this study.
232 A transient increase in the serum P level may be an important atherogenic
233 factor. Watari et al. demonstrated that inducing fluctuations in the serum P
234 level by the alternating administration of high or low P diets led to a
235 deterioration of endothelium-dependent vasodilation and an increased

236 expression of VCAM-1 and MCP-1 in the tunica intima (25). The impairment
237 of endothelial function by the alternating administration of high or low P
238 diets was almost same as that produced by the chronic administration of a
239 high P diet (25). Therefore, repeated transient increases in the serum P level
240 may have some of the same adverse effects on endothelial cells as continuous
241 high dietary P intake.

242 A chronic increase in the serum P level is a well-known risk factor for
243 CVD, not only in CKD patients, but also in the general population (6, 10). In
244 addition, Yamamoto et al. reported that a high dietary P intake was
245 associated with left ventricular hypertrophy (26). They concluded that the
246 highest quintile of dietary phosphorus intake (male 1554-5032 mg/day,
247 female 1346-4069 mg/day) was associated with an greater left ventricular
248 hypertrophy compared with the lowest quintile (male 270-687 mg/day,
249 female 251-585 mg/day). A recent study demonstrated that high dietary P
250 intake was associated with all-cause mortality in the NHANES III cohort
251 (27). All-cause mortality was significantly increased in the people with high

252 phosphorus intake (more than 1400 mg/day) compared with low phosphorus
253 intake (less than 1400 mg/day). In our study, standard P400 meal
254 corresponded to 1200 mg of daily phosphorus consumption if the subject
255 consumed the same meal three times per day. On the other hand, the
256 ingestion of P800 or P1200 meal three times per day would be estimated over
257 1,400 mg/day. In this study, the single-time ingestion of P800 or P1200 meal
258 significantly deteriorated endothelial function. Therefore, habitual
259 consumption of high phosphorus diet likes P800 and P1200 meals may
260 increase the risk of cardiovascular disease.

261 High phosphorus diet also causes large fluctuation of serum phosphorus
262 levels. Portale et al. demonstrated that there is a circadian rhythm of the
263 serum P level (28), with the serum P level being at its lowest during the
264 morning fasting state and highest during the night. A high dietary intake of
265 P increased the serum P level during both day and night, except during the
266 morning fasting state. Thus, a chronic high phosphorus diet can widen the
267 amount of difference between the lowest and highest serum P levels present

268 during each circadian cycle. Such large daily fluctuations arising from
269 continuous high dietary P intake may cause endothelial dysfunction in
270 humans, as was previously observed in rodents (25).

271 This study has some limitations. Firstly, this study was carried out with a
272 limited number, gender, and age range of subjects, although the impact of
273 these limitations was reduced by the use of a double-blinded crossover
274 protocol. A further intervention study with a large number of subjects of
275 different ages and genders should be performed to confirm our results in the
276 future. Secondly, we could not fully clarify the effects of FGF23, PTH, or
277 other factors on endothelial dysfunction caused by high dietary P intake. An
278 elevation or fluctuations in the serum P level must directly inhibit
279 endothelial function. However, PTH and FGF23 may be important as
280 mediators of the deterioration of endothelial function produced by chronic
281 high dietary P intake. Thus, a study investigating the effects of the chronic
282 administration of a high P diet is needed to clarify the effects of PTH or
283 FGF23 on the impairment of endothelial function.

284 In conclusion, excessive dietary P intake can acutely impair endothelial
285 function in healthy people. Habitual excessive P intake and the resulting
286 endothelial dysfunction may contribute to the progression of CVD or
287 increased mortality, as is suggested by epidemiological data.
288
289 **ACKNOWLEDGEMENTS**

290 This work was supported by Grants-in-aid for Scientific Research (B)
291 (22300237) from the Japan Society for the promotion of Science (JSPS) and
292 the kidney foundation (JKFB08-22).
293

294 **CONFLICT OF INTEREST**

295 We have no conflicts of interest to declare for this study.
296
297

REFERENCES

1. Levin A: Clinical epidemiology of cardiovascular disease in chronic kidney disease prior to dialysis. Semin Dial 16: 101-105, 2003

2. Kendric J, Chonchol M: Cardiovascular disease in CKD in 2013: Reducing cardiovascular risk--light at the end of the tunnel. Nat Rev Nephrol 10: 71-72, 2014

3. Afsar B, Turkmen K, Covic A, Kanbay M: An update on coronary artery disease and chronic kidney disease. Int J Nephrol 2014: 767424. doi: 10.1155/2014/767424, 2014

4. Zoccali C, Mallamaci F, Tripepi G: Novel cardiovascular risk factors in end-stage renal disease. J Am Soc Nephrol 15: S77-S80, 2004

5. Stenvinkel P, Carrero JJ, Axelsson J, Lindholm B, Heimbürger O, Massy Z: Emerging biomarkers for evaluating cardiovascular risk in the chronic kidney disease patient: how do new pieces fit into the uremic puzzle? Clin J Am Soc Nephrol 3: 505-521, 2008

6. Kanbay M, Goldsmith D, Akcay A, Covic A: Phosphate - the silent

stealthy cardiorenal culprit in all stages of chronic kidney disease: a systematic review. Blood Purif 27: 220-230, 2009

7. Gupta D, Brietzke S, Hayden MR, Kurukulasuriya LR, Sowers JR: Phosphate metabolism in cardiorenal metabolic disease. Cardiorenal Med 1: 261-270, 2011

8. Tonelli M, Sacks F, Pfeffer M, Gao Z, Curhan G: Relation between serum phosphate level and cardiovascular event rate in people with coronary disease. Circulation 112: 2627-2633, 2005

9. Dhingra R, Sullivan LM, Fox CS, Wang TJ, D'Agostino RB Sr, Gaziano JM, Vasan RS: Relations of serum phosphorus and calcium levels to the incidence of cardiovascular disease in the community. Arch Intern Med 167: 879-885, 2007

10. Onufrak SJ, Bellasi A, Shaw LJ, Herzog CA, Cardarelli F, Wilson PW, Vaccarino V, Raggi P: Phosphorus levels are associated with subclinical atherosclerosis in the general population. Atherosclerosis 199: 424-431, 2008

11. Jono S, McKee MD, Murry CE, Shioi A, Nishizawa Y, Mori K, Morii H, Giachelli CM: Phosphate regulation of vascular smooth muscle cell calcification. Cir Res 87: E10-E17, 2000

12. Giachelli CM: Vascular calcification: in vitro evidence for the role of inorganic phosphate. J Am Soc Nephrol 14: S300-S304, 2003

13. Moe SM, Chen NX : Pathophysiology of vascular calcification in chronic kidney disease. Circ Res 95: 560-567, 2004

14. Shuto E, Taketani Y, Tanaka R, Harada N, Isshiki M, Sato M, Nashiki K, Amo K, Yamamoto H, Higashi Y, Nakaya Y, Takeda E: Dietary phosphorus acutely impairs endothelial function. J Am Soc Nephrol 20: 1504-1512, 2009

15. Peng A, Wu T, Zeng C, Rakheja D, Zhu J, Ye T, Hutcheson J, Vaziri ND, Liu Z, Mohan C, Zhou XJ: Adverse effects of simulated hyper- and hypo-phosphatemia on endothelial cell function and viability. PLoS One 6: e23268. doi: 10.1371/journal.pone.0023268, 2011

16. Di Marco GS, Hausberg M, Hillebrand U, Rustemeyer P, Wittkowski W,

346 Lang D, Pavenstädt H: Increased inorganic phosphate induces human
347 endothelial cell apoptosis in vitro. Am J Physiol Renal Physiol 294:
348 F1381-1387, 2008
349 17. Nishida Y, Taketani Y, Yamanaka-Okumura H, Imamura F, Taniguchi A,
350 Sato T, Shuto E, Nashiki K, Arai H, Yamamoto H, Takeda E: Acute effect
351 of oral phosphate loading on serum fibroblast growth factor 23 levels in
352 healthy men. Kidney Int 70: 2141-2147, 2006
353 18. Corretti MC1, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F,
354 Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D,
355 Vallance P, Vita J, Vogel R: Guidelines for the ultrasound assessment of
356 endothelial-dependent flow-mediated vasodilation of the brachial artery:
357 a report of the International Brachial Artery Reactivity Task Force. J Am
358 Coll Cardiol 39: 257-265, 2002
359 19. Kosch M, Hausberg M, Vormbrock K, Kisters K, Rahn KH, Barenbrock
360 M: Studies on flow-mediated vasodilation and intima-media thickness of
361 the brachial artery in patients with primary hyperparathyroidism. Am J

362 Hypertens 13: 759-764, 2000

363 20. Baykan M, Erem C, Erdoğan T, Hacıhasanoğlu A, Gedikli Ö, Kırış A,
364 Küçükosmanoğlu M, Ersöz H, Çelik S: Impairment of flow mediated
365 vasodilatation of brachial artery in patients with primary
366 hyperparathyroidism. Int J Cardiovasc Imaging 23: 323-328, 2007

367 21. Kosch M, Hausberg M, Vombrock K, Kisters K, Gabriels G, Rahn KH,
368 Barenbrock M: Impaired flow-mediated vasodilation of the brachial
369 artery in patients with primary hyperparathyroidism improves after
370 parathyroidectomy. Cardiovasc Res 47: 813-818, 2000

371 22. Carrelli A, Walker MD, Di Tullio MR, Homma S, Zhang C, McMahon DJ,
372 Silverberg SJ: Endothelial function in mild primary hyperparathyroidism.
373 Clin Endocrinol 78: 204-209, 2013

374 23. Vaziri ND, Ni Z, Wang XQ, Oveisi F, Zhou XJ: Downregulation of nitric
375 oxide synthase in chronic renal insufficiency: role of excess PTH. Am J
376 Physiol Renal Physiol 274: F642-F649, 1998

377 24. Silswal N, Touchberry CD, Daniel DR, McCarthy DL, Zhang S, Andresen

J, Stubbs JR, Wacker MJ: FGF23 directly impairs endothelium-dependent vasorelaxation by increasing superoxide levels and reducing nitric oxide bioavailability. Am J Physiol Endocrinol Metab 307: E426-E436, 2014

25. Watari E, Taketani Y, Kitamura T, Tanaka T, Ohminami H, Abuduli M, Harada N, Yamanaka-Okumura H, Yamamoto H, Takeda E: Fluctuating plasma phosphorus level by changes in dietary phosphorus intake induces endothelial dysfunction. J Clin Biochem Nutr 56: 35-42, 2015

26. Yamamoto KT, Robinson-Cohen C, de Oliveira MC, Kostina A, Nettleton JA, Ix JH, Nguyen H, Eng J, Lima JA, Siscovick DS, Weiss NS, Kestenbaum B: Dietary phosphorus is associated with greater left ventricular mass. Kidney Int 83: 707-714, 2013

27. Chang AR, Lazo M, Appel LJ, Gutiérrez OM, Grams ME: High dietary phosphorus intake is associated with all-cause mortality: results from NHANES III. Am J Clin Nutr 99: 320-327, 2014

28. Portale AA, Halloran BP, Morris RC Jr: Dietary intake of phosphorus

modulates the circadian rhythm in serum concentration of phosphorus. Implications for the renal production of 1,25-dihydroxyvitamin D. J Clin Invest 80: 1147, 1154, 1987

Table 1. Baseline characteristics of subjects.

Age	23.4 ± 2.8
Height (cm)	171.5 ± 2.8
Weight (kg)	60.4 ± 5.1
Percentage body fat (%)	14.2 ± 7.0
Body fat (kg)	8.8 ± 3.5
Fat free mass (kg)	51.6 ± 4.5
Muscle mass (kg)	48.9 ± 4.3
Total body water (kg)	35.0 ± 3.4
BMI (kg/m^2)	20.5 ± 2.1

Values are mean±S.E.M., n=16.

420

Table 2. Measurements of blood and urine biochemical markers

		%FMD	SBP (mmHg)	DBP (mmHg)	Glucose (mg/dL)	Insulin (μU/mL)	Na (mEq/L)	K (mEq/L)	Cl (mEq/L)	Ca (mEq/L)	P (mEq/L)	Intact-PTH (pg/dL)
	Normal Range		<130	<85	70 - 109	1.7 - 10.4	137 - 147	3.5 - 5.0	98 - 108	8.4 - 10.4	2.5 - 4.5	10 - 65
P400 meal	Morning	10.6±0.4	117±2.1	70.3±2.1	91.9±2.2	3.93±0.5	141±0.3	4.10±0.1	103±0.5	9.89±0.1	4.02±0.1	41.0±2.9
	Pre-prandial	11.1±0.3	114±2.5	65.9±2.6	83.4±2.4	4.90±0.7	140±0.4	4.31±0.1	103±0.4	9.90±0.1	3.82±0.1	31.9±2.6
	1H	10.2±0.2	116±2.6	63.1±2.1	96.9±3.9	16.5±1.7*	142±0.5	4.04±0.1*	104±0.5	9.65±0.1	4.13±0.1*	33.7±2.1
	2H	9.25±0.3	115±2.4	64.1±1.8	102±2.3	15.4±1.1*	141±0.5	4.08±0.1*	104±0.4	9.72±0.1	4.26±0.1*	32.4±2.2
	4H	10.2±0.4	113±2.2	66.2±1.9	95.2±2.0	5.80±1.0	141±0.4	4.22±0.1	103±0.4	9.78±0.1	4.48±0.1*	40.7±2.5*
	Next morning	10.6±0.3	113±2.1	68.4±1.8	92.3±1.4	3.66±0.3	140±0.3	4.16±0.1	103±0.4	9.86±0.1	3.91±0.1	33.5±1.6
P800 meal	Morning	10.1±0.4	115±2.5	69.9±1.6	89.9±2.0	3.65±0.4	140±0.3	4.08±0.1	102±0.5	9.94±0.1	4.10±0.1	39.0±3.6
	Pre-prandial	10.8±0.2	113±2.8	65.0±2.0	82.6±2.5	6.94±2.0	140±0.4	4.38±0.1	103±0.3	9.84±0.1	3.81±0.1	33.1±2.5
	1H	6.65±0.4*	115±2.5	63.8±1.5	95.9±4.1	16.7±1.6*	141±0.5*	4.08±0.1*	103±0.3	9.59±0.1	4.81±0.1*	39.6±2.1
	2H	5.89±0.5*	113±2.4	62.5±1.8	101±3.9	15.6±1.7*	141±0.4*	4.08±0.1*	102±0.4	9.63±0.1	4.89±0.1*	38.1±2.0
	4H	7.21±0.4*	115±2.3	67.4±1.5	92.1±2.2	4.46±0.4	140±0.3	4.14±0.1	102±0.4	9.71±0.1	4.86±0.1*	39.6±3.3
	Next morning	10.5±0.4	113±2.6	67.3±2.0	91.5±1.5	3.58±0.3	140±0.3	4.22±0.1	102±0.4	9.93±0.1	3.89±0.1	34.7±3.4
P1200 meal	Morning	9.99±0.3	117±2.2	70.3±1.8	90.9±2.0	3.79±0.4	140±0.5	4.14±0.1	102±0.5	9.85±0.1	4.00±0.1	40.8±3.3
	Pre-prandial	10.6±0.3	116±2.6	66.9±2.1	81.1±2.9	5.10±0.9	140±0.5	4.31±0.1	103±0.3	9.89±0.1	3.75±0.1	33.6±2.7
	1H	5.28±0.4*	115±2.4	63.1±1.8	102±4.6	21.2±2.6*	141±0.5*	3.99±0.1*	103±0.4	9.59±0.1*	5.02±0.2*	41.7±2.5
	2H	5.62±0.4*	116±2.4	64.9±1.7	97.1±3.3	14.1±1.0*	141±0.5*	4.01±0.1*	103±0.4	9.54±0.1*	5.26±0.2*	41.2±2.3
	4H	7.06±0.4*	115±2.2	67.4±1.9	93.3±2.4	4.59±0.5	141±0.4	3.98±0.1*	102±0.3	9.66±0.1	5.23±0.1*	45.9±2.7*
	Next morning	10.6±0.3	116±2.4	67.3±1.4	91.6±1.7	3.83±0.4	141±0.4	4.11±0.1	103±0.3	9.81±0.1	3.93±0.1	32.5±2.1

P400, P400 meal; P800, P800 meal; P1200, P1200 meal; %FMD, %flow-mediated dilation; SBP, systolic blood pressure; DBP, diastolic blood pressure; Cre, creatinine. Values are mean±S.E.M. for 16 subjects. $P<0.05$ vs pre-prandial in the same meal.

421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436

437 **Table 3**. Effects of high dietary phosphorus intake on serum hs-CRP, MCP-1,
438 and FGF23 levels.

		Pre-prandial	4h	Next morning
P400 meal	hs-CRP (mg/dL)	0.035±0.0	0.034±0.0	0.029±0.0
	MCP-1 (pg/dL)	165.8±7.2	164.3±6.5	166.2±6.1
	FGF23 (pg/mL)	41.6±16.5	35.7±16.7	45.0±15.5
P800 meal	hs-CRP (mg/dL)	0.048±0.0	0.043±0.0	0.035±0.0
	MCP-1 (pg/dL)	165.7±6.7	157.2±6.8	160.9±5.9
	FGF23 (pg/mL)	50.8±13.5	39.3±15.6	40.7±14.7
P1200 meal	hs-CRP (mg/dL)	0.062±0.0	0.061±0.0	0.052±0.0
	MCP-1 (pg/dL)	165.8±8.3	154.7±7.8	160.9±7.7
	FGF23 (pg/mL)	60.6±16.7	40.1±16.2	49.2±18.4

439 Abbreviations are hs-CRP, high sensitive-C reactive protein; MCP-1,
440 monocyte/macrophage chemoattractant protein-1; FGF23, fibroblast growth
441 factor 23.
442
443
444

445 **Figure legends**

446 **Figure 1.** Study schema. The three test meals containing different amounts
447 of P were served at 12:30 on the test day. The subjects were allowed only the
448 standardized water and meals that we supplied after 14:00 on the day before
449 the test day. Asterisks indicate the times at which blood collection and FMD
450 measurements were performed.

451

452 **Figure 2.** Effects of high dietary P intake (open diamond, P400 meal; open
453 square, P800 meal; open triangle, P1200 meal) on the serum P level before
454 and after ingestion of test meals. Data are mean±S.E.M. for 16 subjects.

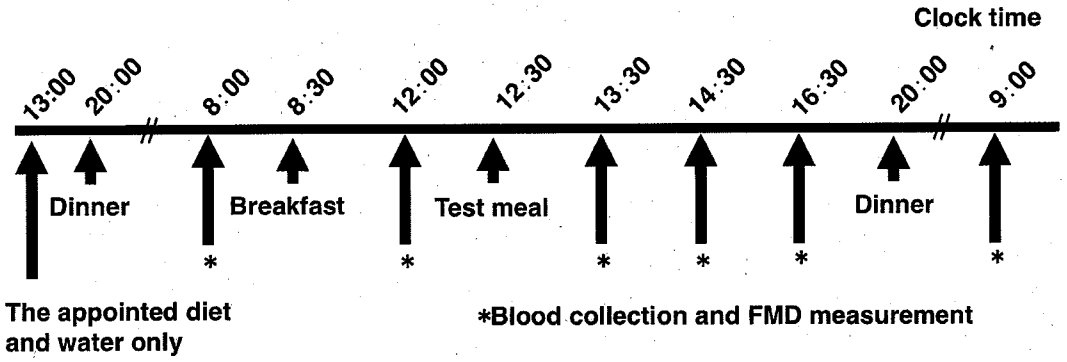
455

456 **Figure 3.** Effects of high dietary P intake on areas under the curve for
457 post-prandial changes in serum P (A) and intact PTH levels (B) over 4h after
458 ingestion of the test meal. Data are mean±S.E.M. for 16 subjects. $^{**}P<0.01$
459 for differences among the meals.

460

461 **Figure 4.** Effects of high dietary P intake (open diamond, P400 meal; open
462 square, P800 meal; open triangle, P1200 meal) on %FMD (B) before and
463 after ingestion of test meals. Data are mean±S.E.M. for 16 subjects.
464
465 **Figure 5.** Univariate association analysis of the ratios of changes (%) in
466 serum P and %FMD from pre-prandial measurements to those made 4h after
467 ingestion of the test meals. All variables were centralized according to the
468 median value for each individual. Each symbol is used as in Figure 2.
469 Spearman's correlation coefficient (r_s) and its P-value for r_s=0 are presented
470 in the association.
471

472 **Figure 1**



473

474

475

476

477

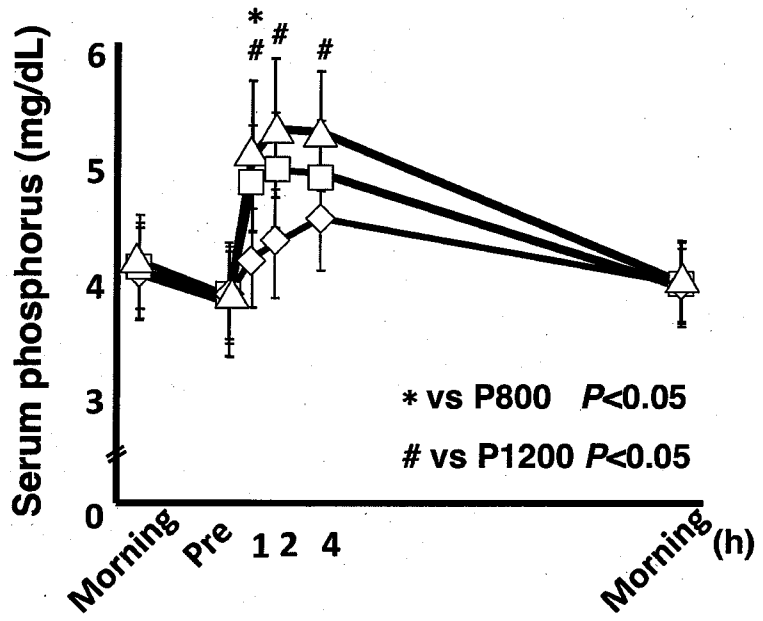
478

479

480

481

Figure 2



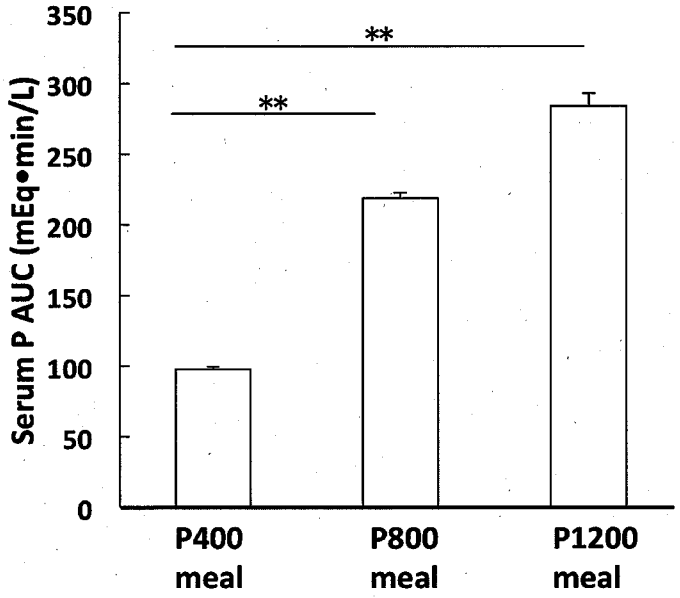
482

483

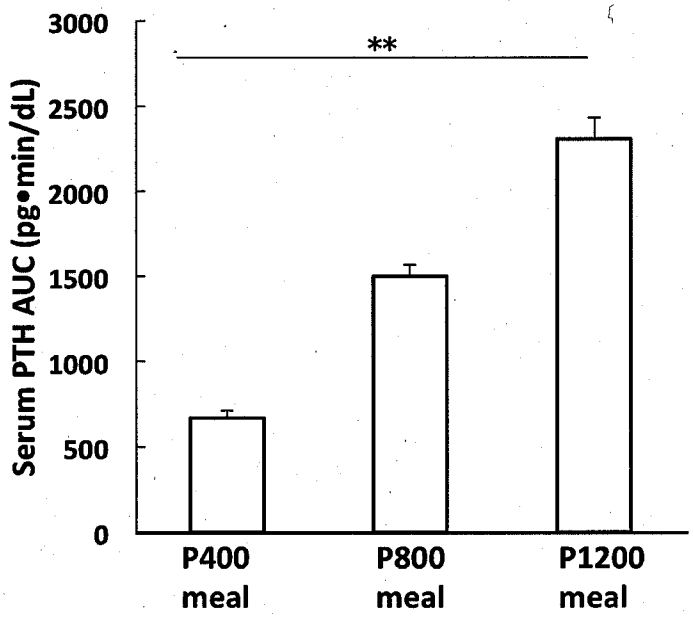
484

Figure 3

A


Serum P AUC (mEq•min/L)
350
300
250
200
150
100
50
0
**
**
P400 meal
P800 meal
P1200 meal

B

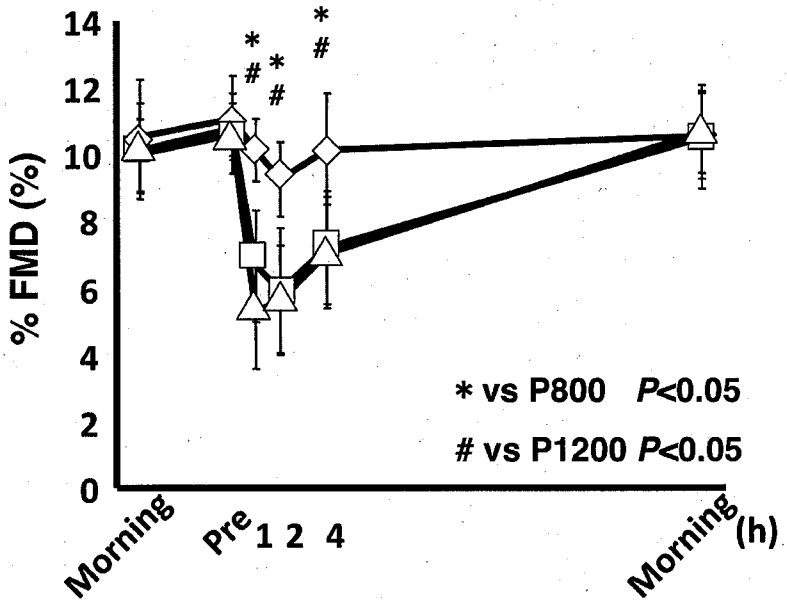

Serum PTH AUC (pg•min/dL)
3000
2500
2000
1500
1000
500
0
**
P400 meal
P800 meal
P1200 meal

488

489

490

Figure 4



491

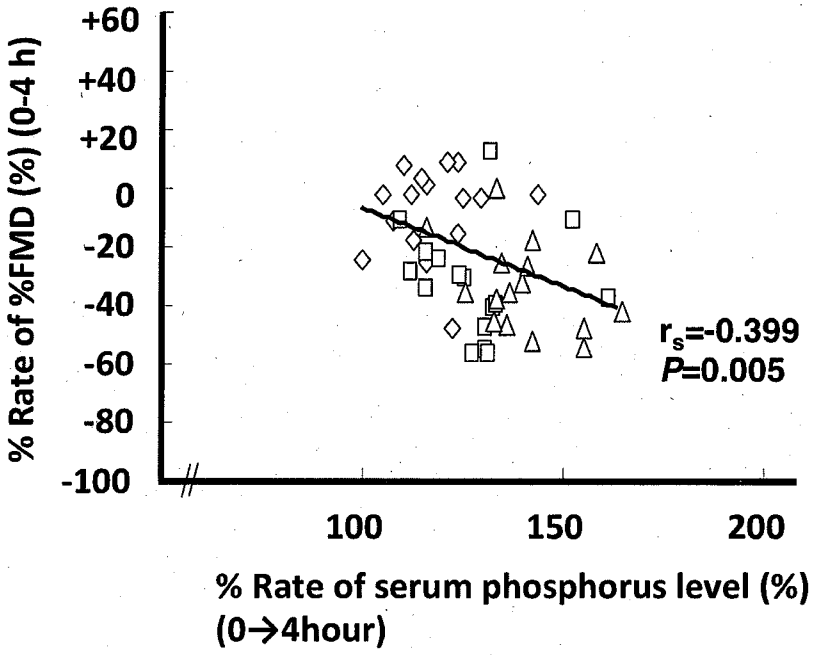
492

493

494

495

Figure 5


+60
+40
+20
0
-20
-40
-60
-80
-100
% Rate of %FMD (%) (0-4 h)
100
150
200
% Rate of serum phosphorus level (%) (0→4hour)
r_s=-0.399
P=0.005

496