*Original Article*

# Effects of dietary fiber on vascular calcification by repetitive diet-induced fluctuations in plasma phosphorus in early-stage chronic kidney disease rats

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**Vascular calcification progresses under hyperphosphatemia, and represents a risk factor for cardiovascular disease in chronic kidney disease (CKD) patients. We recently indicated that phosphorus (P) fluctuations also exacerbated vascular calcification in early-stage CKD rats. Dietary fiber intake is reportedly associated with cardiovascular risk. This study investigated the effects of dietary fiber on vascular calcification by repeated P fluctuations in early-stage CKD rats. Unilateral nephrectomy rats were used as an early-stage CKD model. For 36 days, a P fluctuation (LH) group was fed low-P (0.02% P) and high-P (1.2% P) diets alternating every 2 days, and a P fluctuation with dietary fiber intake (LH + F) group was fed low-P and high-P diets containing dietary fiber alternating every 2 days. The effect on vascular calcification was measured calcium content. Effects on uremic toxin were measured levels of indoxyl sulfate (IS) and investigated gut microbiota. The LH + F group showed significantly reduced vessel calcium content compared to the LH group. Further, dietary fiber inhibited increases in blood levels of IS after intake of high-P diet, and decreased uremic toxin-producing intestinal bacteria. Dietary fiber may help suppress progression of vascular calcification due to repeated P fluctuations in early-stage CKD rats by decreasing uremic toxin-producing intestinal bacteria.**

***Key Words*: hyperphosphatemia, vascular calcification, indoxyl sulfate, dietary fiber, gut microbiota**

Hyperphosphatemia is a trigger for vascular calcification in chronic kidney disease (CKD).[1,2] Progression of vascular calcification is a risk factor for cardiovascular disease (CVD), and represents a major cause of death among CKD patients and hemodialysis patients.[3] Prevention of hyperphosphatemia is therefore important to limit progression of vascular calcification among CKD patients. Recently, transient hyperphosphatemia has also been reported to affect vessels.[4–6] Transient plasma phosphorus (P) elevations are known to cause vascular endothelial dysfunction in healthy men.[4] Repeated, transient elevations in P increase inflammatory factors and oxidative stress, and cause vascular endothelial dysfunction in normal rats.[5] Vascular endothelial dysfunction has been reported to trigger atherosclerosis, and is an important risk factor for CVD.[7,8] Furthermore, our previous study demonstrated that vascular calcification in a rat model of early-stage CKD was exacerbated by repeated P fluctuations, despite having the same total P intake as a group receiving a normal P diet.[6] We therefore considered that it is important to avoid not only chronic hyperphosphatemia, but also P fluctuations due to diet.

P fluctuations occur in all individuals due to circadian rhythms.[9,10] In particular, P fluctuation is considered to often occur in CKD and dialysis patients. Many dialysis patients take P binders on a daily basis to improve hyperphosphatemia.[11] However, adherence to P binders is low, and many patients forget to take these agents.[12–14] As a result, P fluctuations occur when the patients forgets to take P binders. For these reasons, a focus on preventing repetitive P fluctuations is important in CKD patients.

Hyperphosphatemia is reportedly suppressed by soluble dietary fiber in 5/6 nephrectomy rats, as a model of advanced CKD.[15] In addition, we have previously demonstrated that intake of guar gum, as a type of soluble dietary fiber, improves vascular endothelial function in healthy men.[16] In addition, increased intake of dietary fiber retards decreases in estimated glomerular filtration rate (eGFR) and is negatively associated with cardiovascular risk in CKD patients.[17] Several studies have reported that dietary fiber may reduce serum concentrations of uremic toxins.[18,19] In a state of reduced renal function such as in CKD, excretion of the uremic toxin indoxyl sulfate (IS) decreases and IS thus accumulates in the body.[20–22]

IS has been reported to exacerbate production of inflammation and oxidative stress in multiple cell types,[23–26] and has been associated with vascular calcification.[27] In addition, IS is a harmful vascular toxin, and triggers induction of vascular calcification in CKD rats and patients.[28–31] IS is thus a risk factor for the progression of CVD, and managing IS is important for CKD patients.

Several studies have reported that unilateral nephrectomy rats show mild renal insufficiency.[32–34] We have previously used unilateral nephrectomy rats as an early-stage CKD model, and demonstrated that repeated P fluctuations exacerbated vascular calcification in these rats.[6] However, no reports appear to have examined effective diets to prevent progression of vascular calcification due to P fluctuations in early-stage CKD.

The present study investigated the effects of dietary fiber on the exacerbation of vascular calcification caused by repeated P fluctuations in rats with early-stage CKD.

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## Materials and Methods

**Animals and experimental design.** All study protocols were approved by the Ethics Committee of the University of Hyogo, School of Human Science and Environment. As a model of early-stage CKD, rats that had received unilateral nephrectomy were used. Eleven-week-old male Sprague-Dawley rats receiving unilateral nephrectomy at 10 weeks old were purchased from Japan SLC (Shizuoka, Japan). Rats were maintained on a 12-h light, 12-h dark cycle (09:00–21:00) and allowed free access to extra-pure water.

The experimental design is shown in Fig. 1. The experimental diets used were a high-P diet [1.2% P, 0.6% calcium (Ca)], a control-P diet (0.6% P, 0.6% Ca) and a low-P diet (0.02% P, 0.6% Ca) based on a commercial diet with casein as the protein source (AIN93-G; Oriental Yeast, Tokyo, Japan).[5,35] Before grouping, all rats ($n$ = 28) were fed MF (Oriental Yeast) for 1 week to allow acclimatization. At 12 weeks old, rats were divided into four groups, with each group fed a specific diet for 36 days. The HP group ($n$ = 7) was fed the high-P diet and the CP group ($n$ = 6) was fed the control-P diet. The P fluctuation group (LH group) ($n$ = 7) was alternately fed the low-P diet and the high-P diet, swapping every 2 days. The P fluctuation with dietary fiber intake group (LH + F group) ($n$ = 8) was alternately fed the low-P diet and the high-P diet containing 3% dietary fiber, swapping every 2 days. The dietary fiber used was partially hydrolyzed guar gum (PHGG) (Sunfiber; Taiyo-labo, Tokyo, Japan), a soluble dietary fiber. The amount of PHGG added to the diet was based on previous studies.[16] During the experimental period, rats were fed each diet under pair-feeding conditions at 11:00, and food intake was recorded daily. Blood samples were taken from the tail vein between 10:00 and 11:00 every 2 days until 32 days, although blood collection after that proved difficult due to blood vessel damage. Urine volume was recorded every 6 days. After 36 days, rats of all groups were administered anesthetic using isoflurane (Wako Pure Chemical Industries, Osaka, Japan) and laparotomized. Blood samples taken from the inferior vena cava, thoracoabdominal aorta, heart, spleen, and remaining kidney were collected for analysis.

**Biochemical parameters.** Biochemical parameters were measurement as described previously.[6] Briefly, plasma and urine levels of P, Ca, and creatinine (Cr) were measured using test kits (Wako Pure Chemical Industries). Plasma C-reactive protein (CRP) levels were measured using the Rat CRP ELISA Kit (Thermo Fisher Scientific, Tokyo, Japan). Plasma tumor necrosis factor (TNF)-α levels were measured using the Rat TNF-α Quantikine ELISA Kit (R&D Systems, Minneapolis, MN). Urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels were measured using the Highly Sensitive 8-OHdG Check ELISA kit (JaICA, Shizuoka, Japan).

**Measurement of vessel Ca content.** Ca content was measurement as previously described.[6] Briefly, the aorta was hydrolyzed in 6-M hydrochloric acid for 48 h. The calcium content in supernatant was measured using the Calcium-E test kit (Wako Pure Chemical Industries) and corrected for tissue weight.

**Measurement of IS levels.** IS was determined according to a previously described method[36] with some modification. Briefly, sample (or standard IS) solution was diluted by 10 volumes of acetonitrile containing 0.2 μg/ml IS-d4 (Toronto Research Chemicals, Toronto, Canada). After centrifugation, supernatant was further diluted 10-fold with water and 5 μl of sample was then injected using an ExionLC™ AD connected with a quadruple time-of-flight (Q-TOF) tandem mass spectrometer (X500R; Sciex, Framingham, MA). Separation was performed using a Hypersil GOLD column (1.9 μm, 2.1 × 100 mm; Thermo Fisher Scientific) with gradient elution at a flow rate of 0.4 ml/min. Solvent A was 0.1% formic acid in water and solvent B was acetonitrile. The gradient program was as follows: 0 min, 20% B; 1 min, 20% B; 3 min, 95% B; 4 min, 95% B; 5 min, 20% B; 10 min, 20% B. Quantification of IS was performed in TOF-MS mode with negative electrospray ionization as follows. IS, $[M–H]^-$ 212.0023 ± 0.02; IS-d4, $[M–H]^-$ 216.0269 ± 0.02 (internal standard). Ion spray voltage was set at –4,500 V, and the turbo spray temperature was set at 350°C.

**Analysis of gut microbiome.** DNA extraction from 200 mg of stool sample on day 36 was carried out using NucleoSpin® DNA Stool kit (MACHEREY-NAGEL GmbH & Co. KG, Duren, Germany), based on the instructions from the manufacturer. DNA concentration and quality of purified DNA were analyzed using a QUBit fluorometer (Thermo Fisher Scientific) and TapeStation (Agilent, Santa Clara, CA). A 16S library was constructed according to the 16S Metagenomic Sequencing Library Preparation protocol recommended by Illumina (San Diego, CA). Polymerase chain reaction (PCR) on a TaKaRa Cycler Dice Touch (TaKaRa, Kusatsu, Japan) was performed with 2 × KAPA HiFi HotStart ReadyMix (Roche, Basel, Switzerland) under the

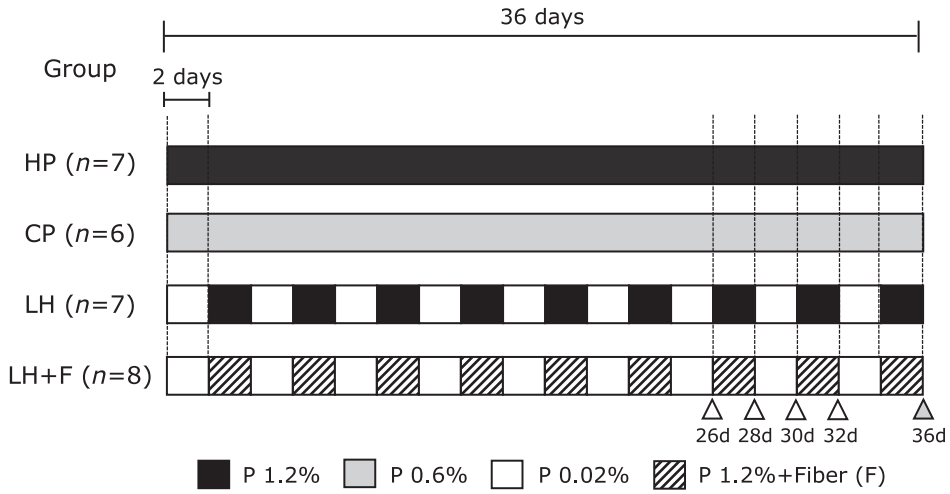


**Fig. 1.** Experimental design. The HP and CP groups were continuously fed the high-P (1.2%) diet and the control-P (0.6%) diet, respectively for 36 days. The LH group was alternately fed the low-P (0.02%) and high-P diet, swapping every 2 days for 36 days. The LH + F group was alternately fed the low-P (0.02%) and high-P diets containing 3% dietary fiber, swapping every 2 days for 36 days. White triangle denote measurement points of plasma indoxyl sulfate. Gray triangle denote measurement point of serum indoxyl sulfate.


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**Table 1.** Body weight and biochemical data at sacrifice day

| | HP[6] | CP[6] | LH[6] | LH + F |
|---|---|---|---|---|
| Total P intake (g) | 12.56 ± 0.18# | 6.43 ± 0.09 | 6.33 ± 0.16 | 5.84 ± 0.23 |
| Body weight (g) | 420.54 ± 7.28 | 427.30 ± 9.54 | 437.04 ± 5.78 | 420.59 ± 13.01 |
| Total P intake (g)/Body weight (100 g) | 2.99 ± 0.04# | 1.51 ± 0.05 | 1.45 ± 0.03 | 1.39 ± 0.05 |
| Plasma (mg/dl) | | | | |
| P | 5.76 ± 0.45 | 5.26 ± 0.34 | 5.52 ± 0.41 | 5.50 ± 0.25 |
| Ca | 9.21 ± 0.43 | 10.02 ± 0.50 | 9.55 ± 0.19 | 10.39 ± 0.21 |
| Cr | 0.63 ± 0.04 | 0.62 ± 0.03 | 0.64 ± 0.03 | 0.66 ± 0.04 |
| Urine (mg/day) | | | | |
| P | 95.07 ± 11.70# | 34.69 ± 6.41 | 87.13 ± 9.07* | 75.91 ± 8.39* |
| Ca | 0.84 ± 0.34 | 0.47 ± 0.14 | 0.52 ± 0.10 | 1.25 ± 0.60 |
| Cr | 9.76 ± 1.40 | 7.11 ± 1.33 | 8.98 ± 0.80 | 8.63 ± 0.88 |
| Renal weight (g) | 2.17 ± 0.12# | 1.75 ± 0.03 | 1.74 ± 0.05 | 1.61 ± 0.06 |
| Heart weight (g) | 1.13 ± 0.04 | 1.13 ± 0.02 | 1.04 ± 0.03 | 1.06 ± 0.03 |
| Spleen weight (g) | 0.86 ± 0.04 | 0.88 ± 0.03 | 0.82 ± 0.02 | 0.88 ± 0.05 |
| Plasma CRP (μg/ml) | 569.56 ± 38.03 | 537.85 ± 23.69 | 496.82 ± 24.15 | 479.35 ± 12.08 |
| Plasma TNF-α (pg/ml) | 1.79 ± 0.19 | 1.09 ± 0.32 | 1.12 ± 0.17 | 1.68 ± 0.14 |
| Urinary 8-OHdG (ng/day) | 73.66 ± 12.21# | 31.23 ± 5.57 | 84.83 ± 12.25* | 60.71 ± 11.68 |

HP, high-P diet group; CP, control-P diet group; LH, alternating low-P and high-P diet group; LH + F, alternating low-P and high-P contained dietary fiber diet group. Values are mean ± SE. Sharp denote statistical significance between the HP and the CP groups using Student's *t* test. #$p<0.05$. Asterisks denote statistical significance between the groups with the same phosphorus intake using Turkey-Kramer test. *$p<0.05$ vs CP.

following conditions: initial denaturation at 95°C for 3 min, followed by 25 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and ended with an extension step at 72°C for 5 min. DNA concentration and size distribution of ready libraries were analyzed with the QUBit fluorometer and TapeStation. PCR products were purified using AMPure XP magnetic beads (Beckman Coulter, Brea, CA) diluted into an equimolar concentration and pooled according to the unique barcode sequence, enabling multiplexing. Next, Illumina dual-index barcodes were added to pooled PCR products with the Nextera XT Index Kit (Illumina, San Diego, CA). Indexed PCR products were purified and pooled into equimolar concentrations prior to paired-end sequencing with a MiSeq Reagent Kit v3 (600-cycle; Illumina), following the directions from the manufacturer. For microbial sequence analysis, low-quality sequences were filtered and chimeric sequences were removed using USEARCH software (ver. 6.1.544). The QIIME ver. 1.9.1 pipeline was used with default parameters for identifying representative sequences for each operational taxonomic unit (OTU) generated from complete linkage clustering with 97% similarity and aligned to the GreenGenes 13_8 database. OTU tables with percentage relative abundances were further processed at different taxonomic levels. α-Diversity indices including Chao1, Shannon and Simpson and β-diversity calculations were performed and visualized with QIIME script core_diversity_analyses.py. β-Diversity calculations were visualized using principal coordinate analysis plots (PCoA), based on unweighted UniFrac.

**Statistical analysis.** Data are expressed as mean ± SE, median (interquartile range), and count (percentage) as appropriate. Differences between HP and CP groups were analyzed using Student's *t* test or the Mann-Whitney *U* test, as appropriate. Differences among CP, LH and LH + F groups were analyzed using the Tukey-Kramer or Steel-Dwass test, as appropriate. For all tests, two-tailed *p* values <0.05 were considered statistically significant.

## Results

### Early-stage CKD model rat data on day of sacrifice.

Total P intake per body weight in the HP group was approximately double that in the CP, while no significant differences were seen between LH and CP groups, as described in our previous study (Table 1).[6] In these three groups, plasma levels of P, Ca and Cr were not significantly different. Urinary P excretion at 24 h before sacrifice was significantly higher in the LH group than in the CP group, as was the HP group. Renal weight was significantly higher in the HP group than in the CP group, but no significant differences were seen between the LH and CP groups. Urinary 8-OHdG level, as a marker of oxidative stress, was significantly higher in the LH group than in the CP group, as was the HP group. These suggested oxidative stress level advanced in the LH group than in the CP, despite receiving the same total P intake.

To investigate the effects of dietary fiber intake on exacerbation of vascular calcification resulting from P fluctuations in this study, we focused on the LH + F group, which was alternately fed

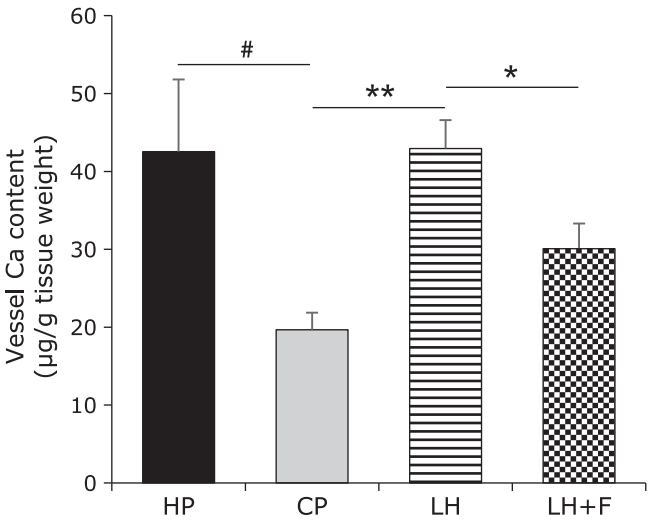


**Fig. 2.** Effects of dietary fiber on vascular calcification. Quantification of Ca content in the thoracoabdominal aorta. HP, high-P diet group; CP, control-P diet group; LH, alternating low-P and high-P diet group; LH + F, alternating low-P and high-P containing dietary fiber diet group. Values are given as mean ± SE. Sharp denotes statistical significance between the HP and CP groups using Mann-Whitney's *U* test. Asterisks denote statistical significance among the CP, LH and LH + F groups using the Tukey-Kramer test. #$p<0.05$, *$p<0.05$, **$p<0.01$.

low- and high-P diets containing 3% dietary fiber every 2 days. We therefore compared the LH + F group with the CP and LH groups that had no significant difference in total P intake per body weight. Plasma levels of P, Ca and Cr did not differ among CP, LH and LH + F groups. Urinary P excretion 24 h before sacrifice did not differ between LH and LH + F groups. Urinary 8-OHdG levels tended to be lower in the LH + F group than in the LH group.

**Effects of dietary fiber on vascular calcification.** To investigate the effects of dietary fiber on vascular calcification, Ca content in the aorta was measured (Fig. 2). Ca content was significantly higher in the HP group than in the CP group, and the LH group was also significantly higher than the CP group.[6] Interestingly, Ca content was significantly lower in the LH + F group than in the LH group. We therefore examined the following to investigate the mechanisms by which intake of dietary fiber suppressed progression of vascular calcification.

**Effects of dietary fiber on indoxyl sulfate.** To determine the effects of dietary fiber on uremic toxins, IS levels were measured (Fig. 3). Serum IS on the day of sacrifice was significantly higher in the HP group than in the CP group (Fig. 3A). Comparing CP, LH and LH + F groups, none of which showed any difference in total P intake, the LH group showed significantly higher serum IS than the CP group. Interestingly, the LH + F group showed significantly lower IS than the LH group, and similar levels to those of the CP group. Urinary IS excretion 24 h before sacrifice were dependent on serum IS in all groups. These results suggested that excretory function was maintained, and dietary fiber intake may be effective in suppressing IS production.

We examined the time course of plasma IS levels, because differences in P concentration of the preceding diet among the four groups could have affected IS production (Fig. 3B). From day 0 to 8, no significant differences were seen among all groups (data not shown). At all points from day 26 to 32, plasma IS levels were significantly higher in the HP group than in the CP group. There was no significant change over time of plasma IS in both the HP and CP groups, respectively. However, in the LH group, plasma IS increased after intake of the high-P diet and decreased after intake of the low-P diet. This indicated that plasma IS levels fluctuated according to the amount of P in the diet. Interestingly, the LH + F group suppressed the increase of plasma IS levels after intake of

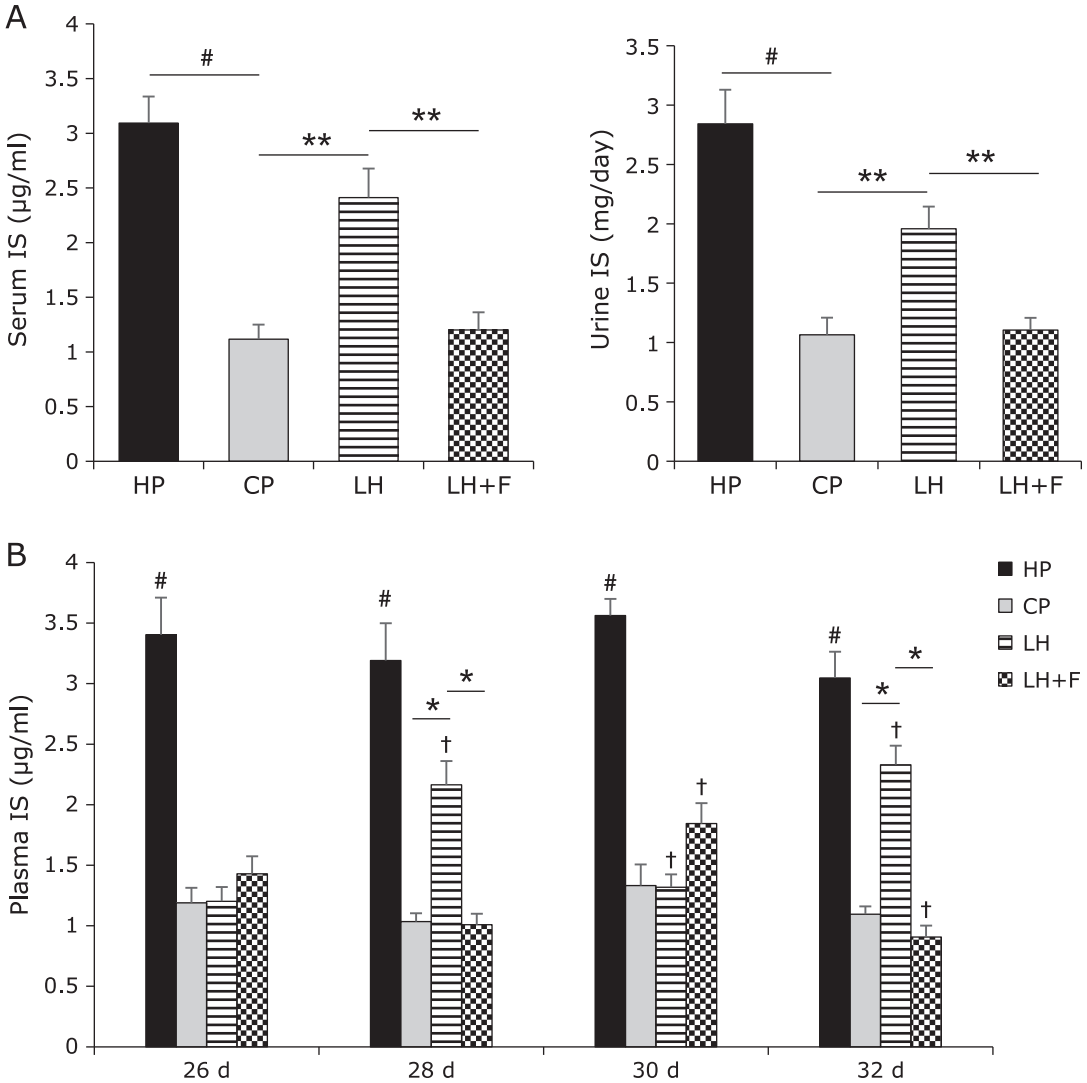


**Fig. 3.** Effects of dietary fiber on indoxyl sulfate (IS). (A) Serum IS and urinary IS at 36 days. (B) Change over time in plasma IS from 26 to 32 days. HP, high-P diet group; CP, control-P diet group; LH, alternating low-P and high-P diet group; LH + F, alternating low-P and high-P containing dietary fiber diet group. Sharp denotes a significant difference between the HP and CP groups using Student's *t* test. Asterisks denote statistical significance among the CP, LH and LH + F groups using the Tukey-Kramer test. Dagger denotes statistical significance before 2 days in the same group using the Tukey-Kramer test. $^{\#}p<0.01$, $^{*}p<0.05$, $^{**}p<0.01$, $^{\dagger}p<0.01$.


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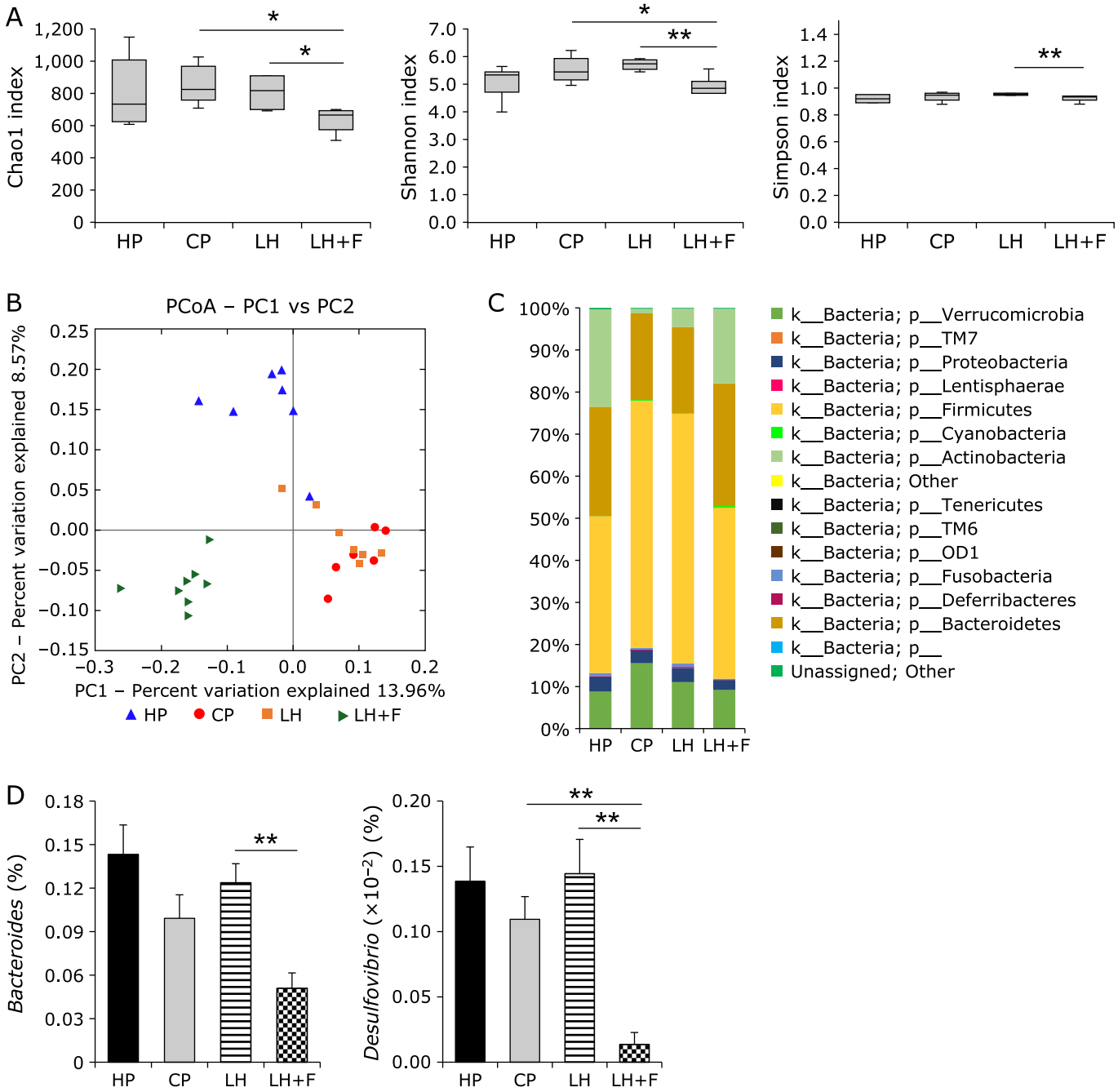


**Fig. 4.** Effects of dietary fiber on gut microbiota. (A) The α-diversity index of Chao1, Shannon and Simpson of gut microbiota. Boxplots indicate the smallest and largest values, 25th and 75th percentiles, medians and outliers. Asterisks denote statistical significance among the CP, LH and LH + F groups using the Steel-Dwass method. *$p$<0.05, **$p$<0.01. (B) Principal coordinates analysis based on unweighted UniFrac distances. (C) Relative abundance of gut microbiota at the phylum level. (D) Relative abundance of *Bacteroides* and *Desulfovibrio*, as IS-producing gut bacteria. Asterisks denote statistically significant difference among the CP, LH and LH + F groups using the Tukey-Kramer test. **$p$<0.01. HP, high-P diet group; CP, control-P diet group; LH, alternating low- and high-P diet group; LH + F, alternating low- and high-P diet containing dietary fiber group.

high-P diet to the same extent seen after intake of control-P diet.

**Influences of dietary fiber on the gut microbiome.** We investigated the effects of dietary fiber on gut microbiota, since IS produced by the gut microbiota (Fig. 4). The evenness and richness of gut microbiota in the four groups on day 36 were determined using the Chao1, Shannon and Simpson index (Fig. 4A). Chao1 and Shannon index were significantly lower in the LH + F group than in the CP and LH groups. Simpson index was significantly lower in the LH + F group than in the LH group. In addition, differences between groups were also found in principal coordinate analysis and in the composition of gut microbiome at the phylum level (Fig. 4B and C).

To determine the effects of dietary fiber on the gut microbiota reportedly involved in IS production (Fig. 4D). *Bacteroides* genus and *Desulfovibrio* genus tended to be present at high levels in the HP and LH groups, but were present at significantly lower levels in the LH + F group. These results suggested that continuing intake of dietary fiber only on a high-P diet may change the intestinal environment to reduce IS-producing gut bacteria.

## Discussion

We investigated the effects of dietary fiber on early-stage CKD rats, and showed dietary fiber intake inhibited progression of vascular calcification due to repeated P fluctuations. Further, we indicated that intake of dietary fiber decreased uremic toxin-producing gut microbiota, and suppressed increased levels of blood IS after intake of high-P diets.

The source of IS production is considered to be largely the intestinal bacteria,[37] and dietary fiber has been reported to suppress IS production.[18,38] The dietary fiber used was PHGG, a soluble dietary fiber. Changes in the intestinal environment are well known to affect soluble dietary fiber intake.[39] PHGG is easily fermented and decomposed by intestinal bacteria, and intestinal bacteria easily produce short-chain fatty acids (SCFAs), such as butyric acid.[40–43] SCFAs are considered to change intestinal pH and reduce production of putrefactive products in the intestine. The present study revealed significant reductions in *Bacteroides* and *Desulfovibrio* at the genus level. Several studies have reported theses bacteria as candidates for production of uremic toxins.[44–46] Intake of dietary fiber may thus have direct effects on production of uremic toxin-producing bacteria. From these results, although plasma IS levels increase after intake of high-P diet, intake of dietary fiber likely suppressed these. In addition, the repeated suppression of IS increase was considered to lead to inhibit the progression of vascular calcification due to P fluctuations. Our results also support findings from previous studies,[18,38] and suggested that intake of dietary fiber on a high-P diet may inhibit the vascular calcification induced by P fluctuations in early-stage CKD rats. Further studies are needed to clarify the mechanisms by which dietary fiber reduces IS-producing gut bacteria. Examination of the effects of dietary fiber intake on the production of uremic toxins other than IS is also necessary.

IS is made from tryptophan, an amino acid present in the diet, and is produced in the liver after conversion to indole by intestinal bacteria.[37] In the present study, no difference in the amount of protein in the diet was seen between groups. Nevertheless, plasma IS levels fluctuated depending on the amount of P in the diet. From this result, it is considered that not only amino acids, but also P may be involved in the production of IS under conditions of impaired renal function. Further, we considered that increases in blood IS led to P fluctuations significantly promoting increases in oxidative stress and progression of vascular calcification compared to the group with intake of a normal P diet. Although further researches are needed to clarify the mechanisms by which P is involved to the production of IS under conditions of reduced renal function, these results suggest the importance of managing P intake as well as protein in reducing IS production among CKD patients.

The influence of P has been also shown to difference depend on the form of P.[47,48] P intake from the diet is classified as organic P from natural foods, or inorganic P from food additives. The rate of absorption of inorganic P is high than organic P,[49,50] and we have previously reported inorganic P has a stronger influence on vascular endothelium function than organic P in healthy men.[47] In addition, organic P is classified as animal-derived P and plant-derived P. In healthy subjects, the decrease in serum P levels have been reported to be lower after the intake of the milk, an animal product, than after the intake of the soymilk, a plant product.[48] This result may be because the bioavailability of animal-derived P is higher than that of plant-derived P in human. Further researches are needed to clarify differences in effect of dietary fiber and/or in production of IS depending on the P form.

Previous studies have shown that 5% wakame significantly decreased plasma P levels in 5/6 nephrectomy rats.[15] The wakame powder used in that study contained 2.66% dietary fiber.[51] Another study that showed effects on intestinal absorption of iron in anemic growing rats used 7.5% PHGG.[52] We therefore added 3% dietary fiber to the diet in this study. However, this amount of dietary fiber is considered too high for humans. Further studies are thus needed to clarify suitable dosages for CKD and dialysis patients. In addition, investigations using not only PHGG but also other types of dietary fiber are needed to determine fiber types affecting the results of this study.

Our studies used diets with extreme P concentrations, to cause clear fluctuations in plasma P levels. Further research is needed to clarify the effects of dietary fiber on P fluctuations from normal P to high P. Fluctuations in P often occur in CKD and dialysis patients, and could represent a risk factor for CVD.[5,6,9] The present results will thus likely prove useful for devising diets to suppress progression of CVD in CKD and dialysis patients.

In conclusion, we demonstrated that intake of dietary fiber concomitant with intake of a high-P diet may suppress progression of vascular calcification due to repeated P fluctuations through decreases in the intestinal bacteria that produce uremic toxins. The present results may provide evidence for the intake of dietary fiber on a high-P diet as a useful approach to preventing vascular calcification due to repeated P fluctuations and may lead to prevention of cardiovascular decease in early-stage CKD patients.


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## Abbreviations

| | |
|---|---|
| Ca | calcium |
| CKD | chronic kidney disease |
| Cr | creatinine |
| CVD | cardiovascular disease |
| IS | indoxyl sulfate |
| 8-OHdG | 8-hydroxy-2'-deoxyguanosine |
| P | phosphorus |
| PHGG | partially hydrolyzed guar gum |
| SCFAs | short-chain fatty acids |

## Conflict of Interest

No potential conflicts of interest were disclosed.

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