High-fat diets provoke phosphorus absorption from the small intestine in rats

Running head: Effect of high-fat diet on phosphorus absorption

Authors: Keisuke Kawamoto, MS[1], Masae Sakuma, Ph.D[1, 2], Sarasa Tanaka, Ph.D[3], Masashi Masuda, Ph.D[4], Mari Nakao-Muraoka, Ph.D[4], Yuki Niida, MS[4], Yurino Nakamatsu, BS[1], Mikiko Ito, Ph.D[3], Yutaka Taketani, Ph.D[4], Hidekazu Arai, Ph.D[1]

Affiliations:

[1] Laboratory of Clinical Nutrition and Management, Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, Shizuoka, Japan

[2] Department of Human and Nutrition, School of Life Studies, Sugiyama Jogakuen University, Nagoya, Japan

[3] School of Human Science and Environment, University of Hyogo, Himeji, Japan

[4] Department of Clinical Nutrition and Food Management, Institute of Biomedical Sciences, University of Tokushima, Tokushima, Japan

Role of authors: MS, YT and HA contributed to the conceptualization; KK and MS contributed to data curation; KK and YN contributed to formal analysis; MS contributed funding acquisition; KK, MS, ST, MM, MN, and YN contributed to investigation; MS contributed to the project administration; ST, MM, MI, and YT contributed to the resources; MS contributed to supervision; KK and MS contributed to visualization; KK contributed to writing original draft; MS and HA contributed to writing review & editing.

20 The final version of the manuscript was approved by all authors.

21 **Word count:** 4,778

22 **Number of Figures/Tables:** 4/2

23 **Corresponding author:** Masae Sakuma. Ph.D.

24 **Address:** Department of Human Nutrition, School of Life Studies, Sugiyama Jogakuen

25 University, 17-3 Hoshigaoka Motomachi, Chikusa-ku, Nagoya 464-8662, Japan

26 **Telephone and Facsimile number:** 81-52-781-4390

27 **E-mail address:** sakuma@sugiyama-u.ac.jp[1]

[1] **Abbreviations:** BA, total bile acid; CKD, chronic kidney disease; Control, control diet; CVD, cardiovascular disease; F-Ca, fecal calcium concentration; FGF23, fibroblast growth factor 23; F-Pi, fecal phosphorus concentration; HF, high-fat diet; HF-SFA, high saturated–fat diet; IAP, intestinal alkaline phosphatase; IFN-γ, interferon-γ; MCP-1, monocyte chemotactic protein-1; NaPi-2a, type-IIa sodium-phosphate cotransporter; NaPi-2b, type-IIb sodium-phosphate cotransporter; NaPi-2c, type-IIc sodium-phosphate cotransporter; Pit-1, type-III sodium-phosphate cotransporter; PTH, parathyroid hormone; S-1,25(OH)2D, serum 1,25-dihydroxyvitamin D (1,25[OH]2D) levels; SBA, secondary bile acid; S-Ca, serum calcium levels; S-iFGF23, serum intact FGF23 levels; S-Pi, serum phosphorus levels; S-PTH, serum intact PTH levels, TNF-α, tumor necrosis factor-α; U-Ca, urine calcium concentration; U-Pi, urine phosphorus concentration.

Abstract

Objective: Dietary carbohydrate/fat ratio may affect phosphorus metabolism because both calcium and phosphorus are regulated by similar metabolic mechanisms, and a high-fat diet (HF) induces deleterious effects on the absorption of dietary calcium. We hypothesized that the HF induces an increase in phosphorus absorption; therefore, this study aimed to evaluate the effects of differences in the quantity and quality of dietary fat on phosphorus metabolism over the short and long term.

Research Methods & Procedures: Eighteen 8-week-old Sprague–Dawley male rats were fed an isocaloric diet containing varied carbohydrate/fat energy ratio and sources of fat (control diet [Control], HF, and high saturated-fat diet [HF-SFA]). At 3 days and 7 weeks after the allocation and initiation of the test diets, feces and urine were collected and used for phosphorus and calcium measurement.

Results: The fecal phosphorous concentration (F-Pi) was lower in the HF-SFA group than in the other two groups; however, the urine phosphorus concentration (U-Pi) was significantly higher in the HF-SFA group than the other two groups when the rats were fed over the short ($p<0.01$) and long term ($p<0.01$ vs Control group, $p<0.05$ vs HF group). There were no significant differences in type-IIa sodium-phosphate cotransporter (NaPi-2a) and type-IIc sodium-phosphate cotransporter (NaPi-2c) mRNA expression, which are renal phosphate transport-related genes; however, the expression of type-IIb

47 sodium-phosphate cotransporter (NaPi-2b) and type-III sodium-phosphate cotransporter
48 (Pit-1) mRNA in the duodenum was higher in the HF and HF-SFA groups than in the
49 Control group ($p<0.05$), although there were no significant differences in these in the
50 jejunum.
51 **Conclusions:** Our results indicate that HF, particularly HF-SFA, increases intestinal
52 phosphate absorption compared with Control.

53

54 **Keywords:** serum phosphorus levels, calcium, dietary fat, saturated fat, duodenum

Background

Hyperphosphatemia leads to vascular smooth-muscle calcification, endothelial dysfunction [1, 2], and an increased risk of cardiovascular events and death in patients undergoing dialysis and those with chronic kidney disease (CKD) [3-6]. In addition, previous studies [7-9] have reported that in individuals with normal renal function, the risk of cardiovascular diseases (CVDs) and mortality increases with increasing serum phosphorus levels; thus, it suggests that the control of serum phosphorus levels is important in patients with impaired renal function and individuals with normal renal function.

Phosphorus homeostasis is primarily regulated by its absorption in the small intestine and by renal reabsorption and excretion. Excessive phosphorus intake stimulates the parathyroid glands to secrete parathyroid hormone (PTH) and bones to secrete fibroblast growth factor 23 (FGF23) [10-12]. PTH promotes urinary phosphorus excretion by decreasing the expression of the type-IIa and IIc sodium-phosphate cotransporters (NaPi-2a, NaPi-2c) in the renal proximal tubule [13]. FGF23 reduces serum phosphate levels by decreasing the expression of NaPi-2a and NaPi-2c in the renal proximal tubule and inhibiting vitamin D activation by impairing the production of 1α-hydroxylase and elevating the production of 24-hydroxylase [14-19]. This results in inhibitory effects on phosphorus absorption in the small intestine and renal phosphorus reabsorption.

The Western-type diet, which is rich in animal products, such as meat and dairy, has become more globally widespread. Moreover, together with highly developed food-preparation techniques and food convenience, the use of processed food has also become more widespread. Processed food uses many food additives, including phosphorus, which is grouped into organic and inorganic. Organic phosphorus is divided into plant and animal phosphorus. Animal phosphorus is more highly absorbed than plant phosphorus [20], and inorganic phosphorus is more highly absorbed than organic phosphorus [21, 22]; therefore, increasing the intake of animal products and processed foods will cause excessive consumption of well-absorbed phosphorus. In addition, the Western-type diet is rich in fats, especially saturated fat. It has been reported in epidemiological studies [23, 24] that a high-fat diet (HF) is associated with osteoporosis. Another study on animals [25] has demonstrated that HF results in deleterious effects on the absorption of dietary calcium; therefore, the dietary carbohydrate-to-fat ratio might affect phosphorus metabolism because calcium and phosphorus are regulated by similar metabolic mechanism. However, there are few reports on this and the detailed mechanisms are unclear.

Thus, the purpose of this study was to evaluate the effects of the differences in the quantity and quality of dietary fat on phosphorus metabolism over periods of short- and long-term ingestion.

MATERIALS AND METHODS

Animals

Eighteen male Sprague–Dawley rats 8 weeks old (Japan SLC Inc, Shizuoka, Japan) were housed in individual cages and maintained on a 12-h artificial light/ dark cycle throughout the study. Before the study, the rats were initially fed standard powder diets (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) for 10 d to adapt to their surroundings and conditions. The rats were allowed free access to water.

Diets

The compositions of the test diets are shown in Table 1. Three types diets were prepared depending on the carbohydrate/fat energy ratio and source of fat as follows: control diet (Control): 75/10, soy bean oil; HF: 45/40, soy bean oil; high saturated-fat diet (HF-SFA): 45/40, soy bean oil + lard. The protein energy ratios of the diets were kept constant for all groups (14.9%). The phosphorus, calcium, and vitamin D/energy ratios were matched in all diets. The Control was based on the nutrient composition of AIN-93M, which is the standard diet for rat and mice. The HF and HF-SFA were defined as a diet with a higher fat energy ratio than the Control diet.

Experimental design

The rats were weighed and assigned to one of the three dietary groups— Control, HF, or HF-SFA—with six rats in each group All groups were offered 80 kcal/d diet with pair

feeding and water *ad libitum* for 8 weeks. Food intakes were weighed daily and body weights were measured weekly during the experimental period.

The rats were allocated and fed one of the three diets for 3 d after being placed in a cage based on diet type and the feces and urine were collected to measure the short-term in/out balance. After 7 weeks, the feces and urine were collected in a similar manner to measure the long-term in/out balance. The collected feces and urine samples were used to measure the fecal/urine phosphorus and calcium, respectively.

At the end of the long-term period (after 8 weeks), all rats were fasted for 12 h and then dissected. Under pentobarbital sodium anesthesia, 4 mL of blood samples were taken from the jugular vein. The rats were sacrificed and kidney and gut mucosa samples were harvested. Blood samples were centrifuged at 12000 rpm and 4ºC for 5 min. The obtained serum samples were dispensed into storage containers and stored at −80ºC until analysis of serum phosphorus levels (S-Pi), serum calcium levels (S-Ca), serum intact PTH levels (S-PTH), serum 1,25-dihydroxyvitamin D levels (S-1,25$(OH)_2$D), and serum intact FGF23 levels (S-iFGF23). This study was approved by the University of Shizuoka (Shizuoka, Japan) Animal Care and Use Committee and developed according to the institution's guidelines for the care and use of laboratory animals.

Feces data

Feces were dried at 110ºC for 12 h and micropulverized. The samples were then ashed at 250ºC for 3 h, at 350ºC for 3 h, and at 550ºC for 24 h. The samples were then dissolved in 25 mL 1% hydrochloric acid (HCl). F-Pi was measured using the vanadomolybdate method and F-Ca was measured using the calcium–cresol complexone method. The rates of excretion to food intake in the feces are given as percentages.

Urine and blood data

Urine phosphorus concentration (U-Pi) and S-Pi were measured using the Phospha C-Test Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Urine calcium concentration (U-Ca) and S-Ca were measured using the Calcium E-Test Wako (Wako). S-PTH was measured using the enzyme-linked immunosorbent assay (ELISA) kit (Quidel, San Clemente, CA, USA). S-1,25$(OH)_2$D was measured using the radioimmune assay (RIA) method (FUJIREBIO Inc., Tokyo, Japan). S-iFGF23 was measured using the ELISA method (KAINOS Laboratories, Inc., Tokyo, Japan). The rate of excretion to food intake in the urine is shown in percentages.

RNA extraction and cDNA synthesis

Total RNA was extracted using TRIzol reagent according to the manufacturer's instructions. Extracted 1 μg equivalent RNA, 4 μL 5× PrimeScript RT Master Mix (Perfect

Real Time; TaKaRa Bio Inc., Shiga, Japan), and RNase Free dH_2O (TaKaRa) were reacted in a 20 μL, and cDNA was synthesized.

Real-time quantitative reverse transcription polymerase chain reaction

The expression of each gene was determined using real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) with the Thermal Cycler Dice Real Time System (TaKaRa); the relative fold change in gene expression indicated the ratio from the Control group. One microliter of synthesized cDNA was used as a template and subjected to an amplification reaction using specific primers for each gene (Table 2); it was confirmed using the analysis of the melting curve that a single PCR product was obtained. The specificity of the amplified products was further confirmed using electrophoresis with 3% ethidium bromide gel, and the expression level of each gene was corrected by 18S ribosomal RNA.

Statistical analyses

The data are shown as the mean ± SE. After confirming the normality of each group, comparisons across groups were conducted by one-way analysis of variance for a normal distribution and by the Kruskal–Wallis test for an abnormal distribution. The Tukey's multiple comparison test was subsequently conducted, and a p value < 0.05 was regarded as statistically significant. All statistical analyses were performed using the SPSS ver. 22 (IBM Corp., Armonk, NY, USA).

RESULTS

Food consumption and body weight

There were no significant differences in food consumption in each group during the study period. Initial body weight in Control, HF and HF-SFA group was 307.4 ± 2.8, 307.5 ± 2.8 and 307.3 ± 2.4 g, respectively. At the end of the study, the body weight in each group was as follows: Control group, 422.3 ± 5.9 g; HF group, 445.7 ± 6.3 g; and HF-SFA group, 433.8 ± 4.6 g. There were no significant differences in initial body weight among the groups. At the end of the study, the body weight of the those in the HF group was significantly higher than that of those in the Control group ($p < 0.05$).

Short-term phosphorus and calcium balance

After 3 d, F-Pi was low in the HF-SFA group compared with that in the HF group ($p = 0.057$). U-Pi was significantly higher in the HF-SFA group than in the other two groups ($p < 0.01$). F-Ca in the HF-SFA group was significantly higher than that in the Control group ($p < 0.05$) and tended to be higher than that in the HF group ($p = 0.091$). U-Ca was significantly lower in the HF and HF-SFA groups than in the Control group ($p < 0.05$) (Fig. 1).

Long-term phosphorus and calcium balance

After 7 weeks, the rate of excretion to food intake in the feces and urine is presented as percentages. F-Pi in the HF-SFA group was significantly lower than that in the Control

group ($p < 0.05$). U-Pi in the HF-SFA group was significantly higher than that in the other two groups ($p < 0.01$ vs Control group, $p < 0.05$ vs HF group). There were no significant differences in F-Ca among the groups. U-Ca in the HF and HF-SFA groups was significantly lower than that in the Control group ($p < 0.01$ vs HF group, $p < 0.05$ vs HF-SFA group) (Fig. 2).

Biochemical examination of blood

The serum parameters are provided in Fig. 3. There were no significant differences in S-Pi among the groups. S-Ca in the HF and HF-SFA groups were significantly lower than that in the Control group ($p < 0.05$). There were no significant differences in S-1,25$(OH)_2$D and S-PTH among the groups, but S-1,25$(OH)_2$D and S-PTH had a tendency be high in the high-fat groups, especially in the HF-SFA group, than in the Control group. S-iFGF23 in the HF and HF-SFA groups was significantly higher than that in the Control group ($p < 0.05$).

Gene expression involved in phosphorus transport in the kidney and intestine

There were no significant differences in mRNA expression levels of NaPi-2a and NaPi-2c among the groups (data not shown).

The expression of phosphorus transport-related genes NaPi-2b and Pit-1 in the duodenum and jejunum are shown in Fig. 4. Duodenal NaPi-2b mRNA expression in the Control group was significantly lower than that in the HF group ($p < 0.05$) and tended to

206 be lower than that in the HF-SFA group. The expression level of Pit-1 mRNA in the
207 duodenum of the HF and HF-SFA groups was significantly higher than that in the Control
208 group ($p < 0.05$). On the other hand, there were no significant differences among the
209 groups in NaPi-2b and Pit-1 mRNA expression in the jejunum. There were no significant
210 differences in mRNA expression levels of intestinal alkaline phosphatase (IAP) among the
211 groups.

Discussion

Elevated serum phosphorus levels are known to promote vascular calcification, arterial sclerosis, and cardiovascular diseases [1, 2]. These evidences were observed not only in patients with CKD [3-6] but also in individuals with normal renal function [7-9]. Therefore, it is recommended that serum phosphorus levels should be maintained within the appropriate range. Moreover, regulation of serum phosphorus may be affected by the dietary fat. The aim of this study was to examine the effects of the quantity and quality of different dietary fats on phosphate metabolism. F-Pi was lower in the HF-SFA group than in the other two groups; however, U-Pi was significantly higher in the HF-SFA group than in the other two groups (Figs. 1, 2). Phosphorus homeostasis is regulated by its absorption in the small intestine, by migration between blood and tissues, and by renal reabsorption (excretion in the urine). Feces contain phosphorus that has not been absorbed in the intestine and that is secreted into the digestive juice. In the urine, the amount of phosphorus excreted is equivalent to the apparent absorption [26]. An increase in U-Pi has been reported to reflect an increase in intestinal phosphate absorption [27, 28]; therefore, increase in the apparent phosphate absorption was observed in groups fed a HF, particularly the HF-SFA group. Some other factors might be involved in these results.

First, it is presumed that fat inhibits calcium absorption. It was suggested that the HF can attenuate intestinal calcium absorption because of the formation of calcium

soap—water-insoluble calcium salts of fatty acids [29]. Furthermore, Xiao et al. [25] have indicated that calcium absorption is significantly reduced and calcium transport-related gene expression is downregulated in mice fed HF. In this study, a significantly reduction in U-Ca in two HF groups was observed. Urine calcium excretion is almost equivalent amount of apparent calcium absorption [30]. In our study, it is suggested the following; there was no significant difference in fecal calcium excretion rate, but amount of calcium absorption in HF and HF-SFA groups were less than that of Control group, resulting in low S-Ca in HF and HF-SFA group. Calcium absorption is an important determinant for phosphorus absorption because calcium and phosphorus form calcium phosphate and insoluble calcium–phosphorous complexes, and are then excreted into the feces [31]. Our data in which HF increased phosphorus absorption might be explained by the following mechanism: formation of calcium soaps from fatty acids and calcium reduced the amount of phosphorus that binds to calcium; therefore, absorption of free phosphorus could then be easily increased. A previous study [32] has shown that rats fed a low-carbohydrate diet/HF show increases in the apparent rate of phosphate absorption, which is in agreement with the results of our study. In addition, differences in the digestibility of fatty acids might be involved. The digestibility of saturated fatty acids is relatively lower than that of other fatty acids [33-35]. It was suggested that the HF-SFA group, which consumed lard as a major source of fat, decreased its absorption of fat and increased the amount of free phosphorus

in the intestine compared with those in the HF group, which consumed soy bean oil as a major source of fat.

The next possible factor for these results is the enhancement of intestinal permeability. Intestinal phosphate transport could be classified into either transcellular or paracellular [36, 37]. A tight junction is a major determinant in intestinal permeability [38]. Suzuki et al. [39] have reported that HF increases intestinal permeability by suppressing the expression of tight junction proteins in non-obese rats. HF elevates blood tumor necrosis factor-α (TNF-α) and monocyte chemoattractant protein-1 (MCP-1) [40], and induces the dysfunction of the intestinal epithelial barrier in the synergy between interferon-γ (IFN-γ) and TNF-α [41]. Moreover, a previous mice study [42] has reported that HFs (soybean oil, lard, or a combination of these) increase total bile acid (BA) and total secondary BA concentrations in the cecum compared with those in the control diet. Most BAs appear to have the ability to induce intestinal hyperpermeability [39, 43-46]; therefore, increased sodium-independent transport resulting from increased intestinal permeability that is accompanied by the enhancement of inflammatory responses and bile secretion might be involved, in part, in the increase in intestinal phosphate absorption in the HF groups.

To investigate, in particular, the mechanism by which intestinal phosphate absorption increases after consuming HF, we examined the phosphorus transport-related

gene expression in the kidney and intestine at the end of the study. There were no significant differences in NaPi-2a and NaPi-2c mRNA expressions, which are renal phosphate transport-related genes; however, NaPi-2b and Pit-1–mRNA expressions in the duodenum were higher in the HF and HF-SFA groups than in the Control group, although there were no significant differences in the expression levels in the jejunum (Figs. 4A–D). The factor that increases duodenal NaPi-2b and Pit-1 mRNA expression in the HF groups appears to change with S-1,25$(OH)_2$D and S-iPTH. A previous mouse study [25] has demonstrated that a HF decreases apparent calcium absorption and serum calcium levels and elevates serum 1,25$(OH)_2$D and iPTH levels. Our study has observed the same tendency. 1,25$(OH)_2$D administration has also been reported to upregulate intestinal NaPi-2b expression and increase phosphate absorption [47, 48]. In addition, Brown et al. [49] have shown that orally administrating vitamin D analog to mice every 2 d for 8 d upregulates NaPi-2b expression in the duodenum and jejunum and increases intestinal phosphate absorption. It is believed from the present study that long-term consumption of HF elevates S-1,25$(OH)_2$D and S-iPTH from impaired calcium absorption, and, as a result, upregulates duodenal NaPi-2b expression and intestinal phosphate absorption. Moreover, NaPi-2b transport activity increases in basicity because the NaPi-2b substrate is a divalent phosphoric acid (HPO_4^{2-}) rather than a monovalent phosphoric acid ($H_2PO_4^-$) [50-52]. The perfusion into the mucosal tissue in the proximal jejunum of the rat with 2 mmol/L sodium

deoxycholate solution, one of the secondary BAs, was reported to significantly increase the pH of the mucosal surface [53]. In addition, increasing amino acids, fatty acids, and BAs in the lumen after meals promotes bicarbonate secretion in the duodenum [54]; therefore, it is possible that intestinal NaPi-2b is activated through the increased pH of the intestinal lumina resulting from BA and bicarbonate secretion after ingesting HF.

S-iFGF23 in the HF and HF-SFA groups was significantly higher than that in the Control group; however, there were no significant differences in S-Pi among the groups, in this study. FGF23 is one of the hormones that maintain the homeostasis of serum phosphorus levels. Excessive phosphorus intake stimulates secretion of FGF23 [10-12], and reduces serum phosphate levels by decreasing phosphorus absorption in the small intestine and reabsorption in the kidney [14-19]. HF and HF-SFA groups had higher intestinal phosphorus absorption than Control group, moreover, FGF23 secretion increased. As a result, it is suggested that there was no difference in S-Pi among the groups. Although S-iFGF23 in the HF and HF-SFA groups was significantly higher than that in the Control group, the expression of phosphorus transport-related genes in the duodenum was significantly higher in the HF and HF-SFA group than that in the Control group. This warrants the need for further studies. It has been reported that chronic hyper S-iFGF23 induced FGF23 resistance [55], which might be involved.

There were no significant differences in IAP mRNA expression among the groups

(Figs. 4E, F). IAP plays an important role in intestinal phosphorus absorption by hydrolyzing phosphate esters into inorganic phosphorus and alcohol. Long-chain and medium-chain triglycerides increase IAP expression and/or activity [56-59]. Moreover, $1,25(OH)_2D$ has been reported to increase IAP activity and expression in the experiments using Caco-2 cells [60]; therefore, IAP activity in the HF-SFA group might be increased over that in the other groups, even if IAP expression were similar among the groups.

Conclusion

Our results indicate that HF, especially a HF-SFA, increases intestinal phosphate absorption over that with a Control. This phenomenon was considered to be related to increases in free phosphorus and decreases in calcium absorption (Figure 5); this may trigger increase in the serum phosphorus levels. It was suggested that not only the phosphorus intake but also the carbohydrate/fat ratio and the quality of the dietary fat are important for the control of serum phosphorus levels and helpful in preventing the onset of cardiovascular events.

Funding

This work was supported by a Grant-in-Aid for Young Scientists (B) 26750046 from the Japan Society for the Promotion of Science (awarded to MS).

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327 **Decleration of interest:** none

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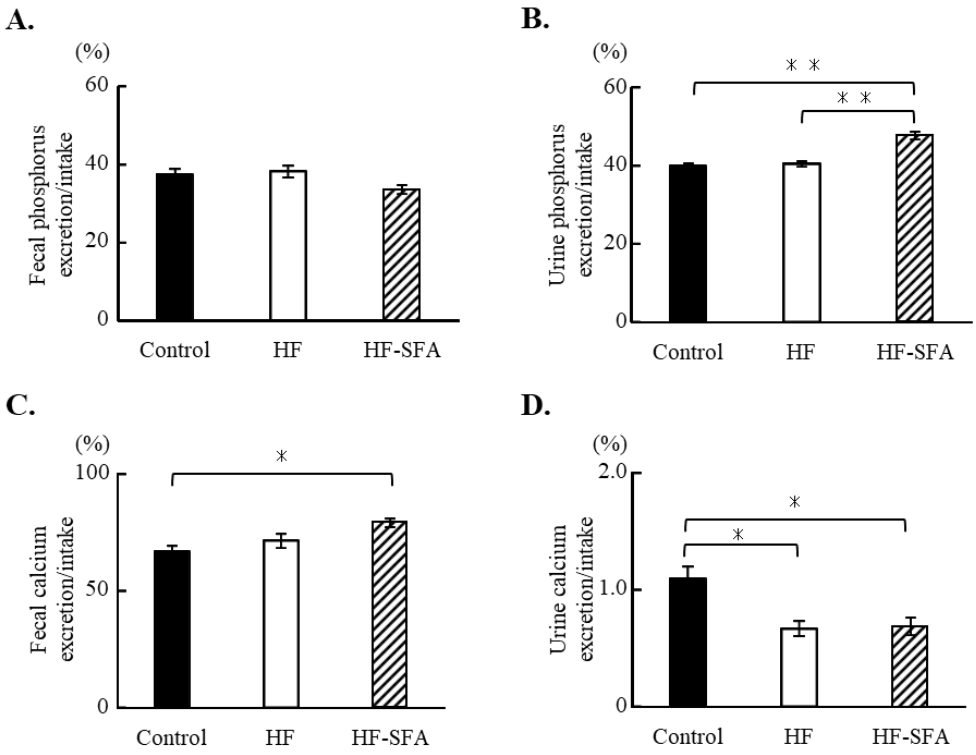

Figure 1. Short-term balances of phosphorus and calcium excretion rates measured in the feces and urine of rats fed different ratios of carbohydrates and fats. (A) Fecal phosphorus excretion rate, (B) urine phosphorus excretion rate, (C) fecal calcium excretion rate, (D) urine calcium excretion rate. Notes: Control: control diet, HF: high-fat diet, HF-SFA: high saturated-fat diet. Black bar: Control, white bar: HF, hatched bar: HF-SFA. Values are the mean ± SE. **significant difference among groups at $p < 0.01$; * significant difference among the groups at $p < 0.05$

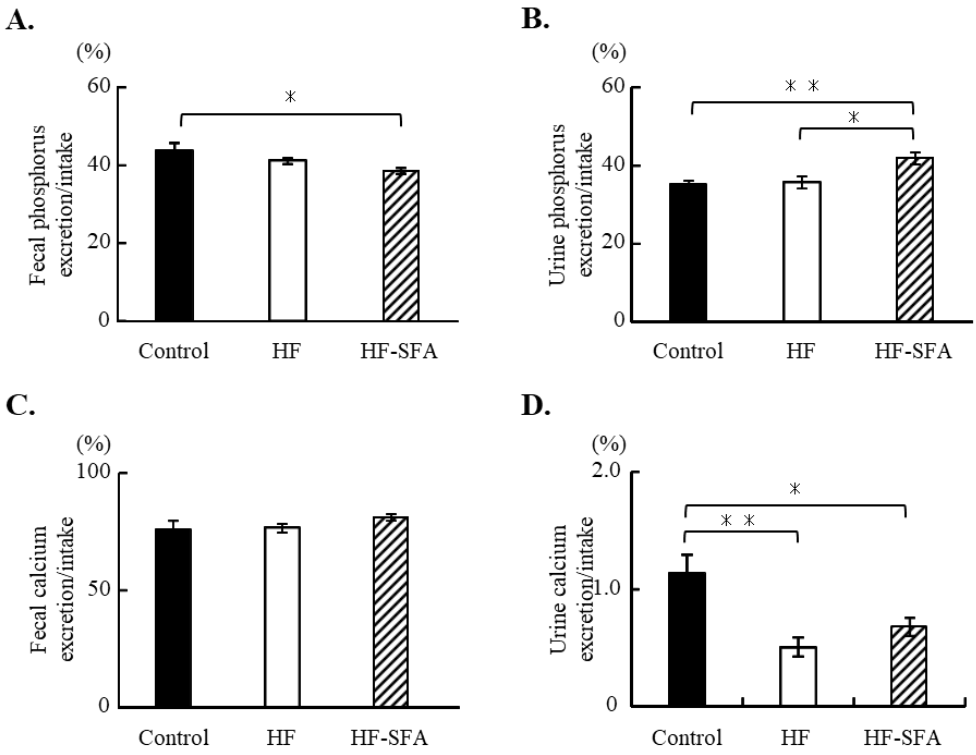

Figure 2. Long-term balances of phosphorus and calcium excretion rate measured in the feces and urine of rats fed different ratios of carbohydrates and fats for 7 weeks. (A) Fecal phosphorus excretion rate, (B) urine phosphorus excretion rate, (C) fecal calcium excretion rate, (D) urine calcium excretion rate. Notes: Control: control diet, HF: high-fat diet, HF-SFA: high saturated-fat diet. Black bar: Control, white bar: HF, hatched bar: HF-SFA. Values are the mean ± SE. **Significant difference among the groups at $p < 0.01$; *significant difference among the groups at $p < 0.05$.

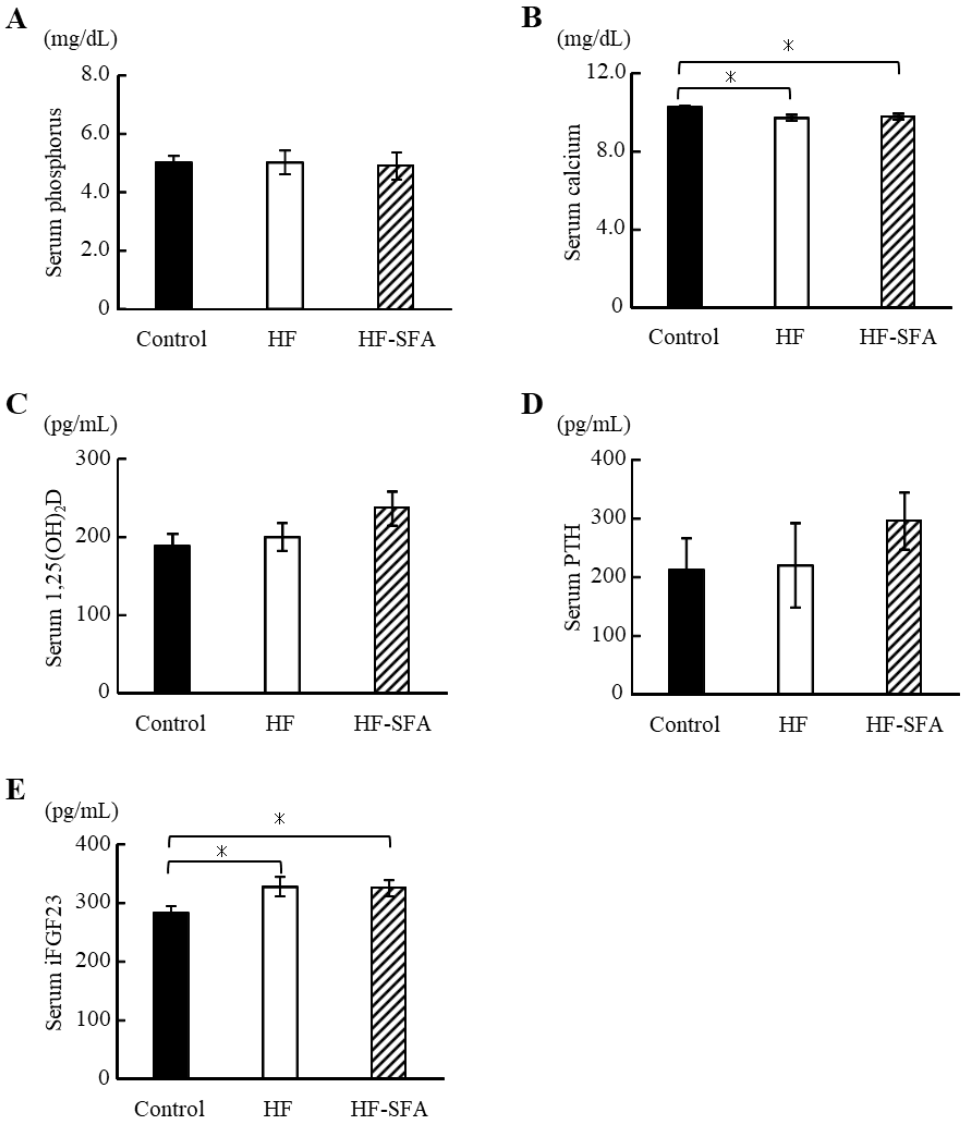

Figure 3. Serum phosphorus, calcium, 1,25-dihydroxyvitamin D (1,25[OH]$_2$D), parathyroid hormone (PTH), and intact fibroblast growth factor 23 (iFGF23) levels in rats fed different ratios of carbohydrates and fats for 8 weeks. (A) Serum phosphorus levels, (B) serum calcium levels, (C) serum 1,25(OH)$_2$D levels, (D) serum PTH levels, and (E) serum iFGF23 levels. Notes: Control: control diet, HF: high-fat diet, HF-SFA: high saturated-fat diet. Black bar: Control, white bar: HF, hatched bar: HF-SFA. Values are the mean ± SE. *Denotes significant difference among the groups at $p < 0.05$.

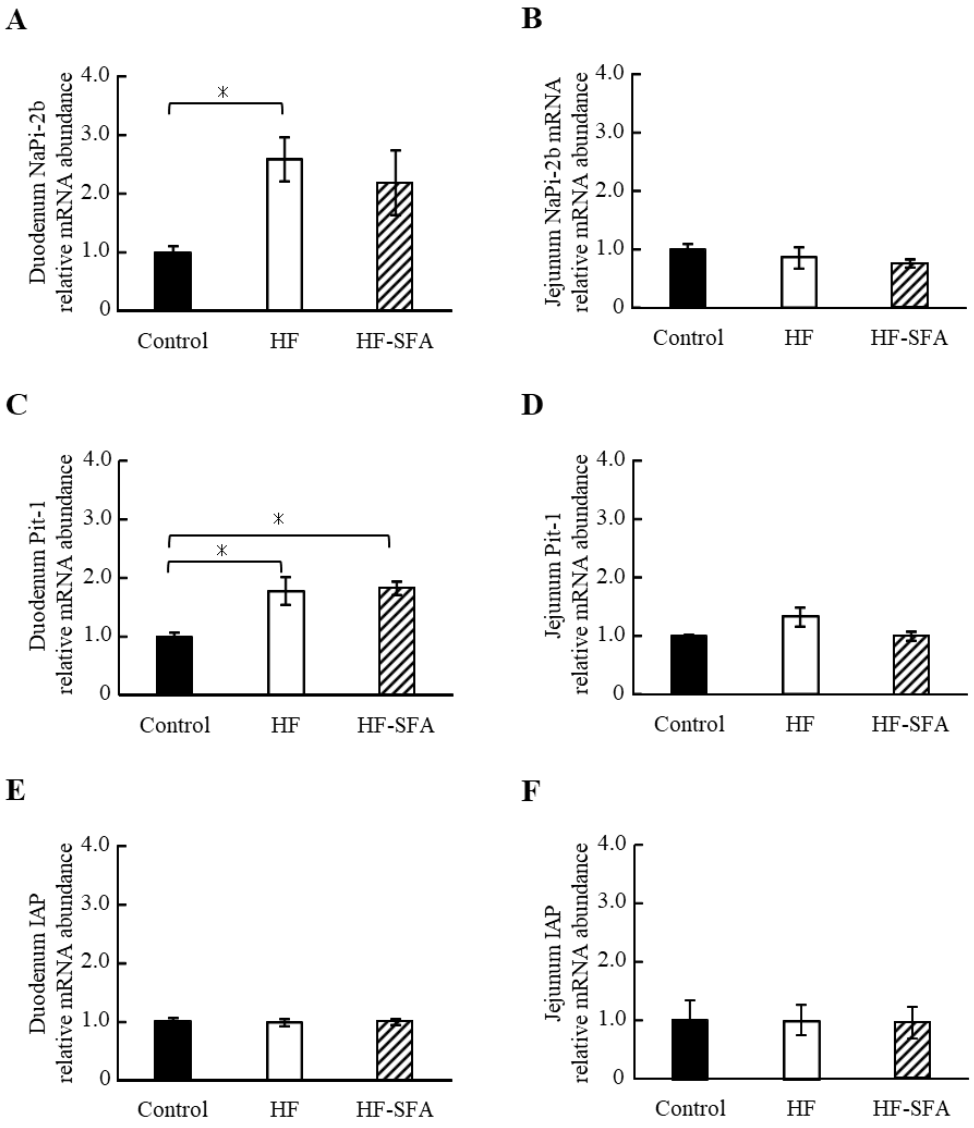

Figure 4. Gene expression in the duodenum and jejunum of rats fed different ratios of carbohydrates and fats for 8 weeks. (A) Duodenum NaPi-2b mRNA expression, (B) jejunum NaPi-2b mRNA expression, (C) duodenum Pit-1 mRNA expression, (D) jejunum Pit-1 mRNA expression, (E) duodenum IAP mRNA expression, (F) jejunum IAP mRNA expression. Notes: Control: control diet, HF: high-fat diet, HF-SFA: high saturated-fat diet. Black bar: Control, white bar: HF, hatched bar: HF-SFA. Values are the mean ± SE. *Significant difference among the groups at $p < 0.05$.

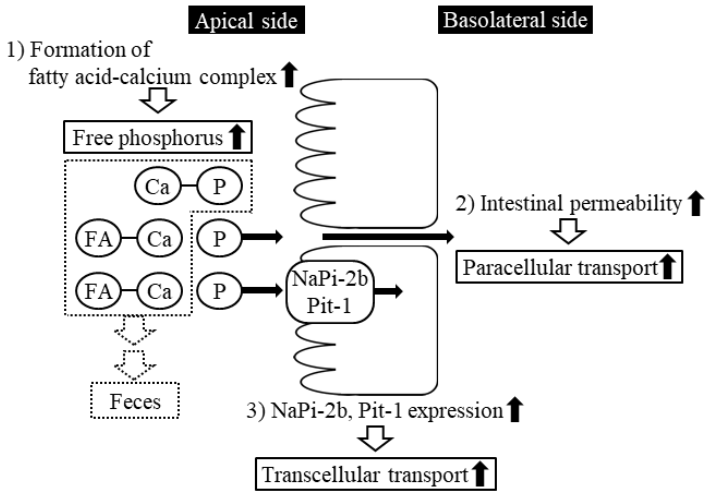

Figure 5. Schema of the effects of HF on phosphorus metabolism. Notes: P: phosphorus, Ca: calcium, FA: fatty acid.

Table 1. Composition of experimental diets

Group	Control	HF	HF-SFA
Protein (% of Energy)	**14.9**	**14.9**	**14.9**
Milk casein (g)	14.2	17.1	17.1
L-Cystein (g)	0.18	0.18	0.18
Fat (% of Energy)	**10**	**40**	**40**
Soybean oil (g)	4.26	20.60	4.26
Lard (g)	0	0	16.34
Carbohydrate (% of Energy)	**75.1**	**45.1**	**45.1**
Corn Starch (g)	45.4262	30.4213	30.4213
α-Corn Starch (g)	15.1	10	10
Sucrose (g)	10	10	10
Fiber (cellulose) (g)	5	5	5
Mineral mixture (g)	2.765679	2.765679	2.765679
Vitamin mixture (g)	0.025875	0.025875	0.025875
KH_2PO_4 (g)	1.0073	1.3945	1.3945
$CaCO_3$ (g)	0.0757	0.348	0.348
Choline bitartrate (g)	0.25	0.25	0.25
Tert-butylhydroquinone (g)	0.0008	0.0008	0.0008
Total (g)	100	100	100
Energy: 80kcal (g/day)	**20.79**	**17.25**	**17.25**
P (mg/80kcal)	110.32	110.32	110.32
Ca (mg/80kcal)	110.32	110.33	110.33
Vitamin mixture (g/80kcal)	2.0789	2.0789	2.0789

Control: control diet, HF: high-fat diet, HF-SFA: high-fat diet (SFA).

Table 2. Primer used for real-time quantitative RT-PCR

Gene		Sequence (5'→3')	Product length (bp)
NaPi-2a	F	tcctcgtcaagatgctcaac	111
	R	caaagtagcctgtgacccaa	
NaPi-2c	F	gttccaccccaggcttagag	125
	R	gaggaagccgctgaccac	
NaPi-2b	F	tgggggcaggcatgaccttca	146
	R	gtggtggtgccaatgttggag	
Pit-1	F	cccatcagcacaacacattg	124
	R	tagggacggtgacaaaccag	
IAP	F	tcagcagacccctccctggc	128
	R	taagccgtgcccgcatggtg	

NaPi-2a: the type IIa sodium-phosphate cotransporter, NaPi-2c: the type IIc sodium-phosphate cotransporter, NaPi-2b: the type IIb sodium-phosphate cotransporter, Pit-1: the type III sodium-phosphate cotransporter 1, IAP: intestinal alkaline phosphatase.
F: forward, R: reverse