Japanese Citrus Fruit (Sudachi) Juice Is Associated with Increased Bioavailability of Calcium from Whole Small Fish and Suppressed Bone Resorption in Rats

Yoshitaka Nit1, Kazuhiro Fukuta1, Kentaro Sakai2 and Shigeru Yamamoto3

1 Food Technology Division, Tokushima Prefectural Industrial Technology Center, Tokushima 770–8021, Japan
2 Department of Nutrition and Health Promotion, Faculty of Human Life Science, Hiroshima Jogakuin University, Hiroshima 732–0063, Japan
3 Department of Nutrition, School of Medicine, The University of Tokushima, Tokushima 770–8503, Japan

(Received October 11, 2003)

Summary Shirasuboshi (boiled and semi-dried whitebait) is a processed fish food that contains abundant calcium. It is eaten whole and commonly consumed in Japan. In this study, the effect of sudachi (Citrus sudachi) juice on calcium, magnesium and phosphorus bioavailability, and bone metabolism in rats was examined. After 14 d of diets low in calcium and phosphorus, male Sprague-Dawley rats were fed shirasuboshi diets containing dried shirasuboshi powder treated with 20% (S20) or 40% (S40) sudachi juice, or distilled water (C) (0.5% Ca; 0.3% P) for 14 d. The apparent absorptions and retentions of calcium, magnesium and phosphorus from shirasuboshi were determined. Bone formation was calculated by measuring serum osteocalcin, and bone resorption by measuring urinary pyridinoline and deoxypyridinoline. The apparent absorption and retention of calcium and magnesium in the S20 group were significantly higher than in the C and S40 groups. Although serum osteocalcin was not affected by the addition of sudachi juice, the urinary pyridinoline and deoxypyridinoline concentrations in the S40 group were significantly lower than in the C and S20 groups. Our results indicate that sudachi juice added to shirasuboshi was associated with increased calcium bioavailability and suppressed bone resorption in rats.

Key Words calcium-absorption, citrus fruit, fish, bone resorption, rats

The importance of adequate calcium intake for the development and maintenance of peak bone mass is well established (1). Insufficient calcium intake increases bone resorption (1, 2), decreases bone mass (3) and increases the risk of osteoporosis after menopause (4). A sufficient calcium intake is therefore required to prevent bone loss. For these reasons, promoting diets high in calcium and exploring new ways of increasing the bioavailability of dietary calcium are very important.

Shirasuboshi (boiled and semi-dried whitebait) is a processed fish food that is eaten whole and commonly consumed in Japan. Although whole small fish with bones are potentially a good source of dietary calcium, bone calcium is unavailable for absorption due to its incorporation in insoluble hydroxyapatite crystals (5). There is very little information on the availability of calcium from the bones of small fish (5, 6), and methods to efficiently increase calcium availability from small fish have not been fully examined.

It is thought that calcium must be ionized in order to be absorbed in the intestine (7). Several reports have dealt with the significant effects of citric acid and citric acid salts on the bioavailability of calcium (8, 9). Mehansho et al. (10) demonstrated that CCM (a combination of calcium carbonate, citric acid and malic acid) in orange juice enhances the bioavailability of calcium. These results suggest that citric acid is very effective in solubilizing dietary calcium.

Sudachi (Citrus sudachi) is a major citrus fruit grown in Tokushima Prefecture, which is located on Shikoku Island in Japan. There is a dietary habit of squeezing sudachi juice on shirasuboshi in this area. The major organic acid component of sudachi juice is citric acid. Previously, we found that sudachi juice solubilizes calcium from shirasuboshi in vitro (11). Furthermore, we observed solubilization of calcium by sudachi juice following in vitro peptic and peptic-pancreatic digestions of shirasuboshi (11). These results suggest that sudachi juice treatment may enhance the bioavailability of calcium from shirasuboshi. The present study was therefore designed to examine the effect of sudachi juice on calcium, magnesium and phosphorus bioavailability, and bone metabolism in young male rats fed shirasuboshi diets.

MATERIALS AND METHODS

Preparation of dried shirasuboshi powder: Shirasuboshi were purchased from Yoshimi Kaisan Co., Ltd. (Komatsu-shima, Tokushima, Japan). The content of water in the shirasuboshi was 38.7 g/100 g. Sudachi juice (pH 2.2) was purchased from Tokushima City Agricultural Cooperatives (Tokushima, Japan). The concentrations of citric acid and malic acid in the sudachi juice were 60 mg/mL and 3.5 mg/mL, respectively. To 100 g of
Table 1. Composition of experimental diets.

| Ingredients                      | Low Ca and P diet | Dietary group (g/kg diet) | C | S20 | S40 |
|----------------------------------|-------------------|---------------------------|---|-----|-----|
| β-Cornstarch                     | 397.236           | 322.086                   |   |     |     |
| Casein                           | 200               | 284                       | 255| 287 |
| Dried shirasuboshi powder        | —                 | —                         | — | —   | —   |
| α-Cornstarch                     | 132               | 132                       | 132| 132 | 132 |
| Sucrose                          | 100               | 100                       | 100| 100 | 100 |
| Soybean oil                      | 70                | 54                        | 53 | 52  |
| Cellulose                        | 50                | 50                        | 50 | 50  |
| Mineral mixture (Ca, P-free)     | 35                | 35                        | 35 | 35  |
| Vitamin mixture                  | 10                | 10                        | 10 | 10  |
| t-Cystine                        | 3                 | 2                         | 2  | 2   |
| Choline bitartrate               | 2.5               | 2.5                       | 2.5| 2.5 |
| t-Butylhydroquinone              | 0.014             | 0.014                     | 0.014| 0.014|
| CaCO₃                            | 0.25              | 7.5                       | 7.3| 7.3 |
| KH₂PO₄                           | —                 | 0.9                       | 0.4| 0.4 |
| Chemical analysis                |                   |                           |    |     |     |
| Calcium                          | 2.5               | 117                       | 115| 114 |
| Magnesium                        | 20.3              | 42.3                      | 54.3| 39.3|
| Phosphorus                       | 17.3              | 106                       | 112| 102 |

1. C, the diet containing dried shirasuboshi powder treated with distilled water; S20, the diet containing dried shirasuboshi powder treated with 20% sudachi juice; S40, the diet containing dried shirasuboshi powder treated with 40% sudachi juice.
2. Containing (g/100g): protein, 63.6; fat, 5.2; carbohydrate, 0.2; (mg/100g): calcium, 750; magnesium, 190; phosphorus, 950.
3. Prepared according to AIN-93G formulation except for calcium and phosphorus.

Fresh shirasuboshi, 20 or 40 mL of sudachi juice was added to make the experimental diets. Distilled water was added to an equivalent amount of sudachi juice to make the control diet. The shirasuboshi treated with sudachi juice or distilled water was then dried for 12 h at 80°C. Dried shirasuboshi was ground to a powder using a food cutter (DLC-XG; Conair Corp., Stamford, CN, USA).

Animals and diets. Male Sprague-Dawley rats, 3 wk of age (Japan SLC Co., Ltd., Hamamatsu, Shizuoka, Japan) were preliminarily maintained on a stock diet (MF; Oriental Yeast Co., Ltd., Tokyo, Japan) for 7 d, and the rats were then fed ad libitum a low-calcium (0.01%), low-phosphorus (0.15%) diet for 14 d (Table 1). The rats were then divided into three groups of six rats each. The three groups were fed ad libitum the shirasuboshi diets containing dried shirasuboshi powder treated with 20% (S20) or 40% (S40) sudachi juice, or distilled water (C) (0.5% Ca; 0.3% P) for 14 d (Table 1). The diets were based on the AIN-93G formulation (12).

The dried shirasuboshi powder was the main source of protein, calcium and phosphorus in each diet, and the total level of fat was adjusted to 7% (w/w) with soybean oil. The contents of energy, protein, fat, fiber, calcium and phosphorus were constant in each diet. The rats were housed individually in stainless steel cages at room temperature (24°C) and 55% humidity with a 12 h light-dark cycle. All rats were given distilled water ad libitum. The animals were maintained in accordance with the Guideline for the Care and Use of Laboratory Animals at the University of Tokushima.

Metabolism studies. During the periods of 11–14 d and 25–28 d, the rats were placed in metabolic cages (Try Tec Co., Ltd., Osaka, Japan). The food intake was measured, and feces and urine were collected separately for each period for the determination of apparent absorption and retention. The 0.1% carmine was added to each diet as a marker, and the feces were collected until the marker was all excreted. Feces collected were dried for 24 h at 105°C, weighed, and ground with a blender. Contaminants, including hairs, were removed by filtering through an 80-mesh stainless steel sieve.

Urine was collected in a glass flask containing 1 N hydrochloric acid, and filtered with No. 7 filter paper (Toyo Roshi Co., Ltd., Tokyo, Japan).

Analytical methods. At the end of the study period, the rats were anesthetized by ethyl ether and killed, and blood samples and femurs were collected. Serum was aliquoted after centrifugation (1,500×g) for 15 min at 4°C and stored at −40°C until analysis. Feces, right femurs and food samples were collected, and then ashed for 24 h at 550°C in a muffle furnace (FO300; Yamato Scientific Co., Ltd., Tokyo, Japan). The temperature was gradually raised until 550°C. The ashed samples were dissolved in 1% hydrochloric acid.

Calcium and magnesium concentrations were determined by atomic absorption spectrophotometry (Z-
Effect of Citrus Fruit on Calcium Absorption

Table 2. Body weight gain, food intake and food efficiency in rats fed low Ca and P diet or dried shirasuboshi diet.

(A) Low Ca and P diet

|                          |                         |            |
|--------------------------|-------------------------|------------|
| Body weight gain (g/14 d) | 80.4±10.4               |            |
| Food intake (g/14 d)     | 200.4±16.1              |            |
| Food efficiency (%)      | 40.0±3.2                |            |

(B) Dried shirasuboshi diets

|                      | Dietary group |
|----------------------|---------------|
|                      | C             | S20          | S40          |
| Body weight gain (g/14 d) | 100.3±19.6    | 86.4±10.0    | 100.5±19.4  |
| Food intake (g/14 d)     | 290.3±17.2    | 266.0±23.6   | 280.7±24.5  |
| Food efficiency (%)      | 34.4±4.8      | 31.8±4.0     | 35.8±5.9    |

Values are means±SD, n=18 in (A) and n=6 per group in (B).

8100; Hitachi, Ltd., Tokyo, Japan) with strontium added to the sample at a final concentration of 3,000 mg/L. Phosphorus was determined by a colorimetric method using ammonium molybdate (13). The apparent absorption and retention of calcium, magnesium and phosphorus were calculated by the following formulae: Apparent absorption (%) = [(intake − fecal excretion)/(intake)] × 100, and apparent retention (%) = [(intake − fecal excretion − urinary excretion)/(intake)] × 100.

Citric acid and malic acid in sudachi juice were measured by high-performance liquid chromatography (Organic Acids Analytic System; Japan Spectroscopic Co., Ltd., Tokyo, Japan) (14).

Biochemical analysis. The concentrations of calcium, magnesium and inorganic phosphorus in serum were measured by a colorimetric method using assay kits for each mineral (Calcium C, Magnesium B and Phosphor C; Wako Pure Chemical Industries, Ltd., Osaka, Japan).

The concentrations of calcitonin and osteocalcin in serum were measured by enzyme immunoassay (Peninsula Laboratories, Inc., San Carlos, CA, USA and Biomedical Technologies Inc., Stoughton, MA, USA). Urinary pyridinoline and deoxypyridinoline were measured according to the method of Fujimoto et al. (15). The urine samples were hydrolyzed in 6 mol/L sodium chloride for 18 h at 107°C in a heat block (DTU-2B; TAITEC Co., Koshigaya, Saitama, Japan).

Assessment of bone length, bone mass and bone mineral densities. The lengths of the right femurs from the proximal neck to the distal condylar surfaces were measured with a digital caliper. The right femurs were dried for 24 h at 105°C and ashed at 550°C in a muffle furnace (FO300; Yamato Scientific Co., Ltd.) and the dry weights and ash weights were determined.

The left femoral diaphysis was scanned by peripheral quantitative computed tomography (pQCT) (XCT Research SA+; Stratec Medizintechnik GmbH, Pforzheim, Germany) with 120×120×460 μm voxel size, and total and cortical bone mineral contents (BMC) and densities (BMD) were analyzed as well as strength strain index (SSI), which is an indicator of whole bone strength (16–18).

In vitro solubilization of calcium from dried shirasuboshi powder. To 20 mL of Ultra Pure Water (Milli-Q SO; Millipore Corp., Tokyo, Japan), 1 g of dried shirasuboshi powder was added and incubated on a shaker for 1 h at 37°C. Samples were centrifuged (18,000×g) for 10 min at 4°C and the amount of calcium in the supernatant was determined by atomic absorption spectrophotometry (Z-8100; Hitachi, Ltd.). The amount of solubilized calcium was expressed as a percentage of the total calcium in the dried shirasuboshi powder.

Statistical analysis. All data are presented as mean values±standard deviation. Statistical analyses were performed using StatView 5.0 (SAS Institute, Cary, NC, USA). Fisher’s protected least significant difference (PLSD) test was done after one-way ANOVA to examine the effect of diet, and was considered statistically significant at p<0.05.

RESULTS

Body weight gain, food intake and food efficiency

There were no significant differences in body weight gain, food intake or food efficiency between rats fed the low-calcium, low-phosphorus diet or the shirasuboshi diets (Table 2).

Apparent absorption and retention of calcium, magnesium and phosphorus

The apparent absorptions and retentions of calcium, magnesium and phosphorus in the rats fed the low-cal-
Apparent absorptions and retentions of calcium, magnesium and phosphorus in the rats fed the shirasuboshi diets are shown in Figs. 1 and 2. The apparent absorption of calcium in the S20 group was significantly higher than in the C and S40 groups, and the apparent retention of calcium in the S20 group was significantly higher than in the C group. The apparent absorption and retention of magnesium in the S20 group were significantly higher than in the C and S40 groups.

**Serum concentrations of calcium, magnesium and phosphorus**

There were no significant differences in serum calcium or inorganic phosphorus concentrations among the dietary groups (Table 4). However, the serum magnesium concentration in the S40 group was significantly lower than the S20 group.

**Biochemical markers for bone remodeling**

The serum calcitonin concentration in the S40 group was significantly higher than in the C group (Table 5). The serum osteocalcin, which is a marker for bone formation, was not affected by the addition of sudachi juice to the diet. In addition, the urinary pyridinoline and deoxypyridinoline, which are markers for bone resorption, were significantly lower in the S40 group than in the C and S20 groups.

**Bone and ash weight, and length and mineral contents of the right femur**

Bone and ash weights, lengths, and mineral contents of the right femur were not significantly different among the dietary groups (Table 6).

**pQCT measurements of the left femur**

Neither total nor cortical BMC and BMD, nor stress strain index of the left femur were statistically different among the dietary groups (Table 7).

**The effect of sudachi juice on the solubilization of calcium from dried shirasuboshi powder**

The amount of solubilized calcium from the dried shirasuboshi powder was measured in vitro. The amount of solubilized calcium was significantly higher in the dried shirasuboshi powder treated with 20% or 40% sudachi juice compared with distilled water, and significantly more calcium was solubilized by 40% sudachi juice compared to 20% (Fig. 3).
Table 6. Right femoral measurements of bone weight, length and mineral contents.

|                | C          | S20         | S40         |
|----------------|------------|-------------|-------------|
| Wet weight (g) | 0.50±0.04  | 0.49±0.04   | 0.51±0.05   |
| Dry weight (g) | 0.29±0.01  | 0.27±0.03   | 0.29±0.03   |
| Ash weight (g) | 0.16±0.01  | 0.16±0.02   | 0.16±0.02   |
| Length (mm)    | 29.8±0.8   | 29.1±1.1    | 29.8±0.7    |
| Long diameter (mm) | 4.1±0.2 | 4.0±0.1     | 4.0±0.1     |
| Short diameter (mm) | 3.2±0.1 | 3.2±0.1     | 3.1±0.1     |
| Calcium (mg/g of dry weight) | 193.3±6.5 | 186.5±7.4   | 181.0±2.0   |
| Phosphorus (mg/g of dry weight) | 88.3±2.1  | 87.8±1.4    | 88.2±0.7    |
| Magnesium (mg/g of dry weight) | 4.88±0.3  | 4.98±0.2    | 4.85±0.3    |

Values are means±SD, n=6 per group.

Table 7. Left femoral measurements with peripheral quantitative computed tomography (pQCT).

|                | C          | S20         | S40         |
|----------------|------------|-------------|-------------|
| Total BMC* (mg/mm) | 5.29±0.25  | 4.96±0.61   | 4.98±0.40   |
| Total BMD* (mg/cm³) | 569.9±38.5 | 569.5±52.9  | 571.2±23.1  |
| Cortical BMC (mg/mm) | 4.48±0.24  | 4.35±0.39   | 4.28±0.36   |
| Cortical BMD (mg/cm³) | 1076.8±22.3 | 1087±10.6  | 1085.8±9.9  |
| SSI*           | 4.82±0.23  | 4.60±0.54   | 4.51±0.57   |

Values are means±SD, n=6 per group.
* BMC, bone mineral contents; BMD, bone mineral densities; SSI, strength strain index.

Fig. 3. Effect of sudachi juice on in vitro solubilization of calcium from dried shirasuboshi powder treated with 20% or 40% sudachi juice, or distilled water. Values are means±SD, n=6 per group. Values were considered significantly different at *** p<0.001.

DISCUSSION

It is apparent that when calcium intake is adequate, differences in bioavailability, as from increased solubilization, play no or only a minor role in the amount of calcium that is absorbed or deposited in the skeleton (7). We therefore performed the comparison between each diet under the condition for raising the calcium requirement. In this study, we examined the calcium, magnesium and phosphorus bioavailability from experimental diets for 14 d, after rats were fed a calcium- and phosphorus-restricted diet for 14 d. Experimentally, low calcium feeding has been accepted as a method of increasing bone resorption (19).

We observed significantly higher apparent absorption of calcium and magnesium in the S20 group than in either the C or the S40 groups. Calcium absorption is influenced by the type of calcium salt and by other food components. Nicar and Pak (20) suggest that the high solubility of calcium citrate is responsible for an increased absorption of calcium. When an appropriate mixture of calcium hydroxide and citric acid are suspended in water, a metastably supersaturated solution of calcium citrate is formed, keeping calcium in a soluble form at a much higher concentration than is possible from a solid preparation of tricalcium dicitrate (21). In the intestine, calcium citrate or a mixture of calcium citrate and calcium malate has been shown to enhance calcium absorption, compared to calcium carbonate (8). Moreover, citrate may increase calcium bioavailability because soluble calcium-citrate complexes are formed instead of insoluble calcium salts such as carbonate or phosphate. In several studies examining the effect of fruit juice on calcium absorption, Newell and Miller (22) reported the beneficial influence of orange juice on children's growth, and Mehansho et al. (10) indicated that orange juice enhanced the bioavailability...
of calcium and iron.

Although 40% sudachi juice results in much greater calcium solubilization than water or 20% sudachi juice, this was not reflected in the apparent calcium absorption or other indices. For this reason, we think that the calcium absorption observed in this study cannot be explained by calcium solubilization alone. Although the absorption of soluble organic calcium complexes is controversial, it is unlikely that the soluble calcium-citrate complexes formed by addition of sudachi juice at a level of 40% would have a negative effect on absorption. There may be other unidentified factors related to sudachi juice that explain the poor calcium absorption in the S40 group compared to the S20 group.

There may also exist an optimal ratio of citric acid to solubilized calcium for maximum calcium absorption. Pak and colleagues found the most efficient calcium absorption was obtained with a calcium to citrate molar ratio of 1.25, based on a range of molar ratios from 0.67 to 1.5 (21). Our data show that a ratio of 20% sudachi juice to shirasuboshi in the diet increased apparent absorption and retention the most.

Intestinal calcium absorption involves two processes: transcellular, metabolically driven transport and a paracellular, passive process (23). Favus and Pak (24) demonstrated that soluble calcium complexes are absorbed in the duodenum by a passive paracellular process, and ionic calcium is easily absorbed in the small intestine and the colon. For most minerals including calcium, the paracellular pathway appears to be the predominant route of entry from lumen to blood. The amount of mineral absorbed by the paracellular route is determined by how much is solubilized in the intestinal lumen (23).

The results of our experiment suggest that the solubilization of magnesium and phosphorus is increased by 20% sudachi juice. In rats, the calcium and phosphorus content of the diet influences magnesium homeostasis (25). Brink et al. (26) speculated that magnesium solubility was a determinant of magnesium absorption. Magnesium is extremely important in skeletal metabolism and there is a growing appreciation that magnesium deficiency may be a cause of osteoporosis (27).

Calcitonin lowers serum calcium by decreasing bone resorption in osteoclastic cells, and it is therefore a potent inhibitor of osteoclastic resorption. Serum calcitonin was significantly increased in rats in the S40 group, suggesting that bone resorption was suppressed in osteoclasts. Biochemical markers of bone metabolism have also been used to determine qualitative changes in bone turnover (28). We measured serum osteocalcin, which is released from osteoblasts as the biochemical marker of bone formation. We also measured urinary pyridinoline and deoxypyridinoline as the biochemical markers of bone resorption. These markers are products of the post-translational modification of collagen, and their urinary excretion has been used as a specific index of bone resorption (29). Our results indicate that although there is no apparent effect of a sudachi-treated shirasuboshi diet on bone formation, there is an effect on resorption. We postulate that this is due to the presence of organic acids such as citric acid in sudachi juice, but further studies are required to determine if other components are involved.

In the S20 group calcium absorption was significantly enhanced, yet bone resorption was not suppressed. On the other hand, in the S40 group, bone resorption was significantly suppressed, but calcium absorption was not enhanced. The component of sudachi juice, especially the quantity of citric acid, greatly differs in the diets with S20 and S40 groups. In the diet with S40 group, the citric acid from dried shirasuboshi powder treated with sudachi juice is included further than the diet with S20 group. Lacour et al. (8) reported that the urinary excretion of the calcium is increased in the presence of high amounts of citric acid. However, it may be a condition that bone metabolism in the S40 group was balanced according to bone resorption effect by sudachi juice in this study. Although the apparent absorption and retention of calcium were significantly increased by adding sudachi juice to shirasuboshi, bone mineral content and bone mineral density in rats were not influenced. This may be due to the short duration of the experiment. If the experimental period were to be prolonged, enhanced bone metabolism due to the intake of sudachi-treated shirasuboshi might become apparent.

Mühlbauer and Li (30) reported that several common vegetables in the human diet alter the bone metabolism in rats. It is therefore plausible that citrus fruits such as sudachi may affect bone metabolism as well. We have shown that the intake of sudachi-treated shirasuboshi is associated with suppressed bone resorption in rats. A similar effect may be expected from other citrus fruit juices, if this effect is due to the citric acid in sudachi juice. If such an effect also happens in humans, adding sudachi juice to shirasuboshi meals could be an effective way to decrease the risk of osteoporosis. These results suggest that a dietary habit of squeezing sudachi juice on shirasuboshi affects the mineral absorption from whole small fish as the traditional Japanese diet style.

Acknowledgments

The authors thank Ms. Kaori Yamamoto (ELK Corporation, Tokyo, Japan) for her help with pQCT analysis, Ms. Kimiko Nii for her technical assistance, and Dr. Jonathan Siekmann for help with the manuscript. This study was supported by a grant from the Regional Science Promoter Program of the Japan Science and Technology Corporation.

REFERENCES

1) Creedon A, Cashman KD. 2001. The effect of calcium intake on bone composition and bone resorption in the young growing rat. Br J Nutr 86: 453–459.
2) Cashman KD. 2002. Calcium intake, calcium bioavailability and bone health. Br J Nutr 87 (Suppl 2): S169–S177.
3) Persson P, Gagnemo-Persson R, Hakanson R. 1993. The effect of high or low dietary calcium on bone and cal-
Effect of Citrus Fruit on Calcium Absorption

4) Morris HA, Need AG, Horowitz M, O'Loughlin PD, Nordin BE. 1991. Calcium absorption in normal and osteoporotic postmenopausal women. Calcif Tissue Int 49: 240–243.

5) Hansen M, Thilsted SH, Sandström B, Kongsbak K, Larsen T, Jensen M, Sørensen SS. 1998. Calcium absorption from small soft-boned fish. J Trace Elem Med 12: 148–154.

6) Larsen T, Thilsted SH, Kongsbak K, Hansen M. 2000. Whole small fish as a rich calcium source. Br J Nutr 83: 191–196.

7) Bronner F, Pansu D. 1999. Nutritional aspects of calcium absorption. J Nutr 129: 9–12.

8) Lacour B, Tardivel S, Driëcke T. 1997. Stimulation by citric acid of calcium and phosphorus bioavailability in rats fed a calcium-rich diet. Miner Electrolyte Metab 23: 79–87.

9) Harvey JA, Kenney P, Poindexter J, Pak CYC. 1990. Superior calcium absorption from calcium citrate than calcium carbonate using external forearm counting. J Am Coll Nutr 9: 583–587.

10) Mehansho H, Kanerva RL, Hudepohl GR, Smith KT. 1989. Calcium bioavailability and iron-calcium interaction in orange juice. J Am Coll Nutr 8: 61–68.

11) Nil Y, Sakai K, Yamamoto S. 2001. Effects of citrus fruit juices on solubilization of calcium from shirasuboshi (boiled and semi-dried whitebait). Ann Nutr Metab 45 (Suppl 1): 386–387.

12) Reeves PG. 1997. Components of the AIN-93 diets as improvements in the AIN-76A diet. J Nutr 127: 8385–8415.

13) Fiske CH, Subbarow Y. 1925. The colorimetric determination of phosphorus. J Biol Chem 66: 375–400.

14) Nakajima M, Ozawa Y, Tamura Z. 1976. A highly efficient carboxylic acid analyzer and its application. J Chromatogr 123: 129–138.

15) Fujimoto S, Kudo T, Tanaka H, Miura M, Seino Y. 1995. Urinary pyridinoline and deoxypyridinoline in healthy children and in children with growth hormone deficiency. J Clin Endocrinol Metab 80: 1922–1928.

16) Ferretti JL, Capozza RF, Zanchetta JR. 1996. Mechanical validation of a tomographic pQCT index for noninvasive estimation of rat femur bending strength. Bone 18: 97–102.

17) Schiessl H, Ferretti JL, Tysarczyk-Niemeyer G, Willecke J. 1996. Noninvasive bone strength index as analyzed by peripheral quantitative computed tomography pQCT. In: Paediatric osteology: New developments in diagnostics and therapy (Schönau E, ed), p 141–146. Elsevier Science BV, Amsterdam, The Netherlands.

18) Ferretti JL. 1999. Peripheral quantitative computed tomography pQCT for evaluating structural and mechanical properties of small bone. In: Mechanical Testing of Bone and the Bone-Implant Interface (An YH, Draughn RA, eds) p 385–406. CRC Press, Boca Raton, FL.

19) Sissons HA, Kelman GJ, Marotti G. 1984. Mechanisms of bone resorption in calcium-deficient rats. Calcif Tissue Int 36: 711–721.

20) Nicar MJ, Pak CYC. 1985. Calcium bioavailability from calcium carbonate and calcium citrate. J Clin Endocrinol Metab 61: 391–393.

21) Pak CYC, Harvey JA, Hsu MC. 1987. Enhanced calcium bioavailability from a solubilized form of calcium citrate. J Clin Endocrinol Metab 65: 801–805.

22) Newell F, Miller EW. 1923. Effect of adding orange juice to the diets of underweight. J Home Econ 15: 241–248.

23) Bronner F. 1998. Calcium absorption—A paradigm for mineral absorption. J Nutr 128: 917–920.

24) Favaus MJ, Pak C. 2001. Evidence for absorption of ionic calcium and soluble calcium complexes by duodenum and jejunum in the rat. Am J Ther 8: 425–431.

25) Steinert PD, Forrer R, Kneissel M, Gasser JA, Thomsen JS, Moskilde L, Riond JL. 2001. Influence of a low calcium and phosphorus diet on the anabolic effect of human parathyroid hormone 1–38 in female rats. Bone 29: 344–351.

26) Brink RJ, Beynen AC, Dekker PR, van Beresteijn EC, van der Meer R. 1992. Interaction of calcium and phosphate decrease ileal magnesium solubility and apparent magnesium absorption in rats. J Nutr 122: 580–586.

27) New SA, Robins SP, Campbell MK, Martin JC, Garton MJ, Bolton-Smith C, Grubb DA, Lee SJ, Reid DM. 2000. Dietary influences on bone mass and bone metabolism: Further evidence of a positive link between fruit and vegetable consumption and bone health? Am J Clin Nutr 71: 142–151.

28) Weaver CM. 1998. Use of calcium tracers and biomarkers to determine calcium kinetics and bone turnover. Bone 22: 1038–1048.

29) Egger CD, Mühlbauer RC, Flex R, Delmas PD, Marks SC, Fleisch, H. 1994. Evaluation of urinary pyridinolin crosslink excretion as a marker of bone resorption in the rat. J Bone Miner Res 9: 1211–1219.

30) Mühlbauer RC, Li E. 1999. Effect of vegetables on bone metabolism. Nature (Lond) 401: 343–344.