Alkaline phosphatase (ALP, EC 3.1.3.1) is an enzyme containing zinc which hydrolyzes monophosphate esters into phosphoric acid and alcohol at a high optimum pH (pH 8–10). In humans, there are at least four distinct but related ALP types: tissue non-specific (TNSALP, liver/bone/kidney), intestinal, placental, and germ cell types (1–3). Based on studies of hypophosphatasia (HPP), which is a systemic bone disease caused by the presence of either one or two pathologic mutations in ALPL that encodes TNSALP, TNSALP was suggested to be indispensable for skeletal mineralization. In this study, we explored the possibility that dietary nutrients contribute to regulate serum bone-specific ALP (BAP) activity. Serum biochemical parameters, such as serum ALP, BAP, osteocalcin, and fibroblast growth factor 23 (FGF23), were measured in healthy young subjects (n=193). Dietary nutrient intakes were measured based on 3-d food records before the day of blood examinations. The presence of a carrier of the deletion of T at nucleotide 1559 (c.1559delT), which has been reported to be the most frequent in Japanese HPP, was not detected in any subject. By the analysis of BAP activity and other biochemical parameters or dietary nutrient intakes, we obtained significant correlations between BAP activity and serum phosphorus (r=−0.165, p=0.022), calcium intake (mg/1,000 kcal/d) (r=−0.186, p=0.010), or phosphorus intake (mg/1,000 kcal/d) (r=−0.226, p=0.002). Further study on the regulation of BAP activity and calcium and/or phosphorus homeostasis will provide useful data for improving skeletal health.

**Key Words** bone-specific alkaline phosphatase, hypophosphatasia, dietary nutrient intake, serum phosphorus, fibroblast growth factor 23

**Summary** Alkaline phosphatase (ALP) hydrolyzes a variety of monophosphate esters into phosphoric acid and alcohol at a high optimum pH (pH 8–10). Human ALPs are classified into four types: tissue non-specific (TNSALP, liver/bone/kidney), intestinal, placental, and germ cell types. Based on studies of hypophosphatasia (HPP), which is a systemic bone disease caused by the presence of either one or two pathologic mutations in ALPL that encodes TNSALP, TNSALP was suggested to be indispensable for skeletal mineralization. In this study, we explored the possibility that dietary nutrients contribute to regulate serum bone-specific ALP (BAP) activity. Serum biochemical parameters, such as serum ALP, BAP, osteocalcin, and fibroblast growth factor 23 (FGF23), were measured in healthy young subjects (n=193). Dietary nutrient intakes were measured based on 3-d food records before the day of blood examinations. The presence of a carrier of the deletion of T at nucleotide 1559 (c.1559delT), which has been reported to be the most frequent in Japanese HPP, was not detected in any subject. By the analysis of BAP activity and other biochemical parameters or dietary nutrient intakes, we obtained significant correlations between BAP activity and serum phosphorus (r=−0.165, p=0.022), calcium intake (mg/1,000 kcal/d) (r=−0.186, p=0.010), or phosphorus intake (mg/1,000 kcal/d) (r=−0.226, p=0.002). Further study on the regulation of BAP activity and calcium and/or phosphorus homeostasis will provide useful data for improving skeletal health.
with the administration of vitamin B₆ is effective for convulsions, and low calcium-containing milk is recommended for hypercalcemia in HPP (14).

In the present study, we aimed to clarify the association between bone-specific alkaline phosphatase (BAP) activity and serum biochemical parameters or dietary nutrients to obtain basic information for the planning of desirable nutritional management for bone health.

Furthermore, we examined the carrier frequency of the common mutation c.1559delT in ALPL, which is one of the most frequent ALPL mutations in the lethal form of HPP in Japanese (15).

Materials and Methods
Subjects. Young subjects living in Tokyo, Japan, were recruited. Participants were excluded if they had metabolic disease. The study population consisted of 97 healthy Japanese males and 96 females. All subjects were unrelated volunteers and were aged 22.1±1.8 (mean±standard deviation (SD)), with a height of 164.9±8.9 cm, weight of 57.2±9.2 kg, and body mass index (BMI) of 21.0±2.3 kg/m².

In accordance with the Helsinki Declaration on human studies, the study protocol was approved by the ethical committee of Japan Women’s University, and written informed consent was obtained from all study subjects.

Measurements. Fasting blood samples were obtained and sera were kept frozen at −80°C until measurement. A bone formation marker, serum BAP, was determined by enzyme immunoassay (Mitsubishi Kagaku Bio Clinical Laboratories Inc., Tokyo, Japan), which can detect only serum BAP activity in total ALP. ALP activity was determined employing the method of Bessey et al. (16). Calcium was measured employing the o-cresol-phthalein complexone color development method (17), and inorganic phosphorus was determined using the method of p-methylamino phenol reduction (18). Serum-intact osteocalcin [bone gamma-carboxyglutamic acid (Gla) protein: BGP], which is one of the secreted osteoblast-specific proteins and is associated with the mineralized matrix of bone tissue (19), was measured by immunoradiometric assay (Mitsubishi Kagaku Bio Clinical Laboratories Inc.). Fibroblast growth factor (FGF23) was measured employing the FGF23-enzyme-linked immunosorbent assay (Kainos Laboratories, Inc., Tokyo, Japan).

Dietary nutrient intakes were measured based on 3-d food records taken up to the day before blood collection. Trained personnel reviewed the food records, and the nutrient content was determined with the use of Eiyokun software (Kenpaku-sha, Japan).

All subjects were analyzed for ALPL mutation (a deletion of T at nucleotide position 1559; c.1559delT) in ALPL, which has been reported to be the most frequent in Japanese HPP. The mutation c.1559delT was not detected in any subjects of this study, and no subjects were heterozygous carriers of c.1559delT. Recently, it was reported that the 1559delT carrier frequency is 1/480 (95% confidence interval, 1/1,562–1/2,848), and −1 in 900,000 individuals have the perinatal lethal form of HPP caused by a homozygous 1559delT mutation in Japanese (15). Therefore, we considered that more subjects must be accumulated in order to reveal the frequency.

In all subjects (n=193), the mean (±SD) levels of serum BAP and ALP activity were 26.9±7.8 and 193±48 U/L, respectively. The levels of serum calcium, phosphorus, osteocalcin, and FGF23 were 9.7±0.4 mg/dL, 3.6±0.5 mg/dL, 7.9±3.1 ng/mL, and 43±13 pg/mL, respectively. As the results of 3-d food records, the mean (±SD) dietary energy, calcium, phosphorus, vitamin D, and vitamin B₆ intakes of the subjects were calculated as 2,078±555 (kcal/d), 556±223 (mg/d), 1,059±302 (mg/d), 5.8±4.8 (µg/d), and 3.10±11.08 (mg/d), respectively.

We analyzed the relations between BAP activity and serum parameters or nutrient intakes. As shown in Table 1, serum BAP activity was significantly correlated with the concentration of serum phosphorus (r=−0.165, p=0.022), osteocalcin (r=0.339, p<0.001) and FGF23 (r=0.191, p=0.008), but not with serum calcium. Serum BAP activity was also correlated with the calcium (mg/1,000 kcal/d) (r=−0.186, p=0.010), and phosphorus (mg/1,000 kcal/d) (r=−0.226, p=0.002).
We suggest that the difference in dietary phosphorus intake influenced the level of BAP activity or serum phosphorus. Actually, the concentration of serum phosphorus was significantly correlated with the dietary phosphorus intake (mg/1,000 kcal/d) (Table 2) (r=0.183, p=0.011), but there were no significant correlations between serum phosphorus and the calcium, vitamin D, or vitamin B₆ intake.

Previously, we reported that phosphate starvation increased BAP activity and regulated its expression in mouse stromal ST2 cells (20, 21). In mineralizing tissues, BAP may regulate extracellular PPI concentrations by hydrolyzing PPI, which is an inhibitor of the formation of hydroxyapatite crystals (22). These findings suggest that the extracellular concentration of phosphate is one of the factors which modulate BAP activity.

Furthermore, there were significant negative correlations between the serum level of BAP and serum phosphorus or phosphorus intake. Kemi et al. reported that high phosphorus intake adversely affects bone metabolism by decreasing the marker of bone formation, BAP activity, in healthy young females (23). In the present study, a significant positive correlation was also observed between BAP and the concentration of osteocalcin or FGF23 (Table 1). FGF23 is a 32-kDa peptide secreted by osteocytes involved in the control of phosphorus homeostasis (24). Although there are limitations due to the small sample size and results of single correlation analysis, our results indicated that the dietary assessment of phosphorus intake is needed to evaluate bone metabolism and phosphorus homeostasis.

Concerning the relationship between serum BAP activity and calcium intake, Fardellone et al. reported that calcium supplementation resulted in a significant reduction of BAP activity and bone-resorption markers in postmenopausal women (25). In the present study, we demonstrated that serum BAP activity was significantly and negatively correlated with calcium intake (Table 1).

In conclusion, our results demonstrated that there were significant correlations between BAP activity and serum phosphorus, calcium intake, or phosphorus intake. Further investigations of the effect of adequate nutritional management on the regulation of BAP activity and calcium and/or phosphorus homeostasis will provide useful data for improving skeletal health.

Table 2. Correlations between serum phosphorus and dietary nutrient intakes.

| Nutrient      | r values | p-values |
|---------------|----------|----------|
| Calcium (mg/1,000 kcal/d) | 0.063 | 0.380 |
| Phosphorus (mg/1,000 kcal/d) | 0.183 | 0.011* |
| Vitamin D (µg/1,000 kcal/d) | -0.038 | 0.598 |
| Vitamin B₆ (mg/1,000 kcal/d) | 0.012 | 0.868 |

*p<0.05.

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