

# Low calcium intake is associated with increased bone resorption in postmenopausal Japanese women: Yokogoshi Study

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## Abstract

**Objective:** Low Ca intake is common among Japanese women, but its effect on bone metabolism has not been fully elucidated. The aim of the present study was to determine the relationship between Ca intake and serum markers of bone turnover in postmenopausal Japanese women.

**Design:** A cross-sectional study.

**Setting:** A community setting.

**Subjects:** Subjects were 595 home-dwelling postmenopausal Japanese women. Ca intake was assessed by a validated FFQ. Serum type I collagen cross-linked N-telopeptides (NTX) and osteocalcin were measured as markers of bone turnover. The relationships between demographic characteristics, lifestyles, serum Ca, vitamin D and intact serum parathyroid hormone and bone turnover were also assessed.

**Results:** The average age of the subjects was 64.5 (SD 5.8) years and the mean Ca intake was 527 (SD 160) mg/d. Ca intake was significantly associated with serum NTX ( $P=0.0104$ ), but not with serum osteocalcin. Mean serum NTX concentration in the lowest quartile of Ca intake ( $<417$  mg/d) was significantly higher than in the fourth, referent quartile. Among these Japanese postmenopausal women, very low Ca intake (less than  $\sim 400$  mg/d) was associated with increased bone resorption but not bone formation.

**Conclusions:** Increased bone resorption may be one mechanism by which this Ca-depleted population normalizes bone metabolism and prevents osteoporosis.

## Keywords

Bone metabolism  
Bone resorption  
Calcium intake  
Postmenopause

Low Ca intake is recognized as a risk factor for osteoporosis and osteoporotic fractures in postmenopausal women. Daily Ca intake of at least 1200 mg is recommended for postmenopausal women<sup>(1,2)</sup>. However, Ca intake among Japanese women and other East Asian populations is typically well below present recommendations. The National Nutrition Survey of Japan (2003) showed that the average daily Ca intake of peri- and postmenopausal Japanese women was only 562 mg<sup>(3)</sup>. This is lower than present

clinical guidelines and also lower than that of people in many European and North American countries. Nevertheless, the incidence of hip fractures in Japan has been estimated to be less than half that in the USA<sup>(4)</sup>, suggesting that the prevalence of osteoporosis in Japan is also lower. This paradox may partly be explained by the fact that populations consuming Ca-depleted diets exhibit physiological adaptations that maintain normal bone metabolism. However, the relationship between Ca intake and bone

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metabolism in populations with low Ca intake has not been well studied.

Previous studies have demonstrated that a higher rate of bone turnover leads to bone loss, disruption of trabecular networks and reduced connectivity, and that bone turnover markers indicating bone resorption predict subsequent osteoporotic fractures independent of bone mineral density<sup>(5)</sup>. In fact, high bone remodelling may be a primary cause of osteoporotic bone fragility<sup>(6)</sup>. Markers of bone turnover can thus be used to assess the relationship between Ca intake and clinically significant aspects of bone metabolism. The aim of the present study was to determine the relationship between Ca intake and two serum markers of bone turnover – serum type I collagen cross-linked N-telopeptides (NTX)<sup>(7,8)</sup> and osteocalcin<sup>(9)</sup> – among postmenopausal Japanese women with low Ca intake.

## Subjects and methods

### Subjects

On 31 March 2006, we targeted all 1310 women aged 55–74 years who lived in the town of Yokogoshi, Japan. Of these women, 667 women agreed to participate and underwent measurement of serum bone turnover markers in the baseline investigation of the Yokogoshi Study, a community-based, epidemiological study on bone health for postmenopausal women<sup>(10)</sup>. The medical examination was conducted in November 2005. All subjects were non-institutionalized, ambulatory and independent. The following women who had medical histories that may have affected bone metabolism were excluded from analysis: (i) twelve women with a history of bilateral oophorectomy; (ii) seven women who had undergone corticosteroid therapy; and (iii) fifty-three women treated with bisphosphonates, selective oestrogen receptor modulators, active vitamin D analogues, vitamin K (menatetrenone), oestrogen or calcitonin for suspected osteoporosis. Ultimately, 595 of 667 women were analysed. Written informed consent was received from all subjects. The study protocol was approved by the Ethics Committee of Niigata University School of Medicine. Further details of the Yokogoshi Study have been published elsewhere<sup>(10)</sup>.

### Biochemical measurements

A fasting blood specimen was drawn during the daytime, at least 6 h following the last ingestion of any food or drink. Each specimen was immediately maintained at 4°C and the serum was obtained within the day of collection by centrifugation at 1613g for 10 min. The specimen was subsequently stored at –80°C prior to biochemical analysis. Serum NTX concentration, a marker of bone resorption, was determined by ELISA (Osteomark NTX Serum; Ostex International, Inc., Seattle, WA, USA; reference value: 10.7–24.0 nmol BCE/l), which had an inter-assay CV of 2.8%. Serum osteocalcin concentration, a marker of bone

formation, was determined by an immunoradiometric assay (Mitsubishi Kagaku Medical, Inc., Tokyo, Japan; reference value: 3.1–12.7 ng/ml) with an inter-assay CV of 6.6%. Serum vitamin D concentration, measured as 25-hydroxy-vitamin D (25(OH)D), was determined by RIA (DiaSorin, Stillwater, MN, USA) with an inter-assay CV of 9.9%. Serum intact parathyroid hormone (PTH) concentration was measured with a two-site immunoradiometric assay (Nichols Institute Diagnostics, San Clemente, CA, USA), which has an inter-assay CV of 1.5%.

### Other measurements

Age, medical history, reproductive history, current medication list and lifestyle information were obtained from all patients. Current Ca intake was assessed with a previously validated FFQ for the Japanese diet<sup>(11)</sup>, with the correlation coefficient between values measured by this method and the conventional 3 d diet record being 0.668. Physical activity levels were assessed based on whether subjects engaged in the following activities at least once weekly: (i) light exercise, such as gate ball (or croquet), taking walks, etc., as light activity; and (ii) moderate exercise, such as farm work, gardening, etc., as moderate activity. Body height and weight of the subjects in light underwear were measured to the nearest 1 mm and 100 g, respectively. BMI was calculated by dividing body weight (kg) by the square of body height (m<sup>2</sup>).

### Statistical analysis

All continuous variables were assessed for normality. Serum NTX and intact PTH concentrations were skewed to higher values, and thus they were transformed logarithmically when conducting statistical tests. Categorical variables, such as 'light exercise' and 'moderate exercise', were coded as 0 for 'no' and 1 for 'yes'. Pearson's product moment correlation coefficients were calculated to evaluate an association between two continuous variables. Student's *t* test was used to test associations between physical activity measures and the two serum bone turnover markers. A stepwise method of multiple regression analysis was used to explore independent variables associated with outcome variables. Candidate predictor variables were significant variables obtained by the bivariate analyses. Analysis of covariance with Dunnett's multiple comparisons was used to compare one reference mean value with other mean values. Computations were performed using the SAS statistical software package release 8.02 (SAS Institute Inc., Cary, NC, USA). A *P* value less than 0.05 was considered statistically significant.

## Results

Characteristics of the subjects are shown in Table 1. Regarding physical activity, 569 (95.6%) subjects engaged in light activity and 293 (49.2%) engaged in moderate activity. Serum markers of bone turnover may have an intra-day fluctuation. Nevertheless, there was no

significant difference in mean log-transformed serum NTX concentration between subjects who underwent blood collection in the morning and in the afternoon ( $P=0.3234$ ). The mean serum osteocalcin concentration among samples collected in the afternoon (10.3 mg/ml) was significantly higher ( $P=0.0486$ ) than among those collected in the morning (9.6 mg/ml).

Correlation coefficients between predictor variables and log-transformed serum NTX or osteocalcin concentration are shown in Table 2. Age, weight, BMI, Ca intake and log-transformed serum intact PTH concentration were significantly correlated with log-transformed

serum NTX concentration. Years since menopause, weight, BMI and log-transformed serum intact PTH concentration were significantly correlated with serum osteocalcin concentration. None of the physical activity measures was associated with the log-transformed serum NTX or osteocalcin concentration. Additionally, a correlation coefficient between Ca intake and log-transformed serum intact PTH concentration was of borderline significance ( $r=-0.072$ ,  $P=0.0776$ ).

Results of the stepwise multiple regression analysis are shown in Table 3. Age, weight, Ca intake and log-transformed serum intact PTH concentration were independently associated with log-transformed serum NTX concentration. BMI and log-transformed serum intact PTH concentration were independently associated with serum osteocalcin concentration.

Table 4 shows mean values of serum NTX and osteocalcin concentration according to quartile relative to the highest quartile of Ca intake. The lowest quartile of Ca intake had significantly lower log-transformed serum NTX concentrations. There was no significant association, however, between Ca intake and serum osteocalcin concentration.

## Discussion

Previous research has shown that increased bone resorption markers are associated with increased fracture risk

**Table 1** Demographic and laboratory characteristics of the study subjects: home-dwelling postmenopausal Japanese women ( $n=595$ ), Yokogoshi Study, 2006

	<i>n</i>	Mean	SD
Age (years)	595	64.5	5.8
Years since menopause	578	14.5	6.9
Height (cm)	595	150.7	5.5
Weight (kg)	595	53.1	8.4
BMI (kg/m <sup>2</sup> )	595	23.4	3.5
Total Ca intake* (mg/d)	595	527	160
Serum 25(OH)D (nmol/l)	595	55.5	14.6
Serum intact PTH (pmol/l)	595	4.25	1.40
Serum NTX (nmol BCE/l)	595	21.0	6.5
Serum osteocalcin (ng/ml)	595	9.94	3.95

25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; NTX, type I collagen cross-linked N-telopeptides.

\*Ca intake from dietary sources was 518 (SD 146) mg/d.

**Table 2** Correlation coefficients ( $r$ ) between selected variables and serum NTX\* and osteocalcin concentrations: home-dwelling postmenopausal Japanese women ( $n=595$ ), Yokogoshi Study, 2006

Variable	Serum NTX*		Serum osteocalcin	
	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value
Age (years)	0.081	0.0482	-0.019	0.6376
Years since menopause	0.004	0.9318	-0.084	0.0442
Height (cm)	-0.023	0.5703	-0.018	0.6574
Weight (kg)	-0.113	0.0060	-0.115	0.0049
BMI (kg/m <sup>2</sup> )	-0.105	0.0102	-0.115	0.0051
Ca intake (mg/d)	-0.096	0.0194	-0.020	0.6200
Serum 25(OH)D (nmol/l)	0.049	0.2355	-0.052	0.2038
Serum intact PTH* (pmol/l)	0.089	0.0302	0.100	0.0149

NTX, type I collagen cross-linked N-telopeptides; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone.

\*Logarithmically transformed.

**Table 3** Results of a stepwise multiple linear regression analysis with log-transformed serum NTX and osteocalcin concentrations as outcomes: home-dwelling postmenopausal Japanese women ( $n=595$ ), Yokogoshi Study, 2006

Independent variable	Regression coefficient ( $\beta$ )	SE	<i>P</i> value
Serum NTX*			
Age (years)	0.00407	0.00193	0.0353
Weight (kg)	-0.00374	0.00132	0.0048
Ca intake (mg/d)	-0.000179	0.0000695	0.0104
Serum intact PTH* (pmol/l)	0.07806	0.0342	0.0230
Serum osteocalcin			
BMI (kg/m <sup>2</sup> )	-0.148	0.047	0.0017
Serum intact PTH* (pmol/l)	1.03	0.51	0.0457
Time of blood collection (0, morning; 1, afternoon)	0.726	0.327	0.0269

NTX, type I collagen cross-linked N-telopeptides; PTH, parathyroid hormone.

\*Logarithmically transformed.

**Table 4** Mean values of serum NTX concentration at each quartile of calcium intake: home-dwelling postmenopausal Japanese women (*n* 595), Yokogoshi Study, 2006

	Serum NTX* (nmol BCE/l)	
	Mean	95 % CI
Ca intake (mg/d)		
Q1 (<417)	22.6†	21.2, 24.0
Q2 (≥417, <525)	20.0	19.1, 20.9
Q3 (≥525, <619)	21.3	20.3, 22.3
Q4 (≥619)	20.2	19.4, 21.1 (reference)
	Adjusted <i>P</i> for trend = 0.0135	

NTX, type I collagen cross-linked N-telopeptides; Q1, first quartile; Q2, second quartile; Q3, third quartile; Q4, fourth quartile.

\*Logarithmically transformed.

†Mean value was significantly different from that of reference group (analysis of covariance and Dunnett's multiple comparisons, with age, weight and log-transformed serum parathyroid hormone as covariates): *P* < 0.05.

independent of bone mineral density<sup>(5)</sup>. Therefore, the correlates of bone resorption are relevant to assessing the aetiology of osteoporotic fractures. In the present study, we measured serum NTX concentrations as a bone resorption marker. This approach is more robust because serum-based markers of bone turnover show less variability than urine-based markers<sup>(12)</sup> and serum NTX measurements can assess bone resorption with decreased intra-subject variability<sup>(7)</sup>.

The present study demonstrated that Ca intake was inversely associated with serum NTX concentration. This finding has been reported previously by a small study<sup>(13)</sup> and is consistent with previous work showing that Ca supplementation reduces bone resorption<sup>(14–16)</sup>. Increased bone resorption by low Ca intake is hypothesized to be mediated through hyperparathyroidism induced by low Ca intake<sup>(16,17)</sup>. Our data, however, failed to demonstrate a significant association between Ca intake and intact PTH concentration, but rather showed that serum NTX concentration was associated with Ca intake independent of PTH. This suggests that a different pathophysiological mechanism may be responsible for the effects of low Ca intake on bone metabolism.

An additional key finding of the present study was that mean NTX concentration in the lowest quartile of Ca intake (<417 mg/d) was significantly lower than that in the highest, reference quartile (≥619 mg/d). Interestingly, this finding is in accordance with an epidemiological study demonstrating that the lowest quartile of Ca intake in peri- and postmenopausal Japanese women has a significantly increased risk of vertebral fracture than the highest, reference quartile<sup>(18)</sup>. Taken together, these data suggest that the lowest levels of Ca intake may be a particularly serious problem in this population.

Average daily Ca intake of most subjects in the present study was only 527 mg, lower than that recommended for peri- and postmenopausal women by the Ministry of Health and Welfare of Japan<sup>(19)</sup>. This is consistent with the National Nutrition Survey<sup>(3)</sup>, which reported that Ca intake of Japanese persons aged 50–69 years was 568 mg/d

on average, much lower than that of people in many European and North American countries. Nevertheless, only a quarter of women with very low Ca intake exhibited increased bone resorption in the present study. Japanese people historically have had a low-Ca diet<sup>(20)</sup> and therefore they may be physiologically adapted to low Ca through increased Ca absorption.

Although serum osteocalcin concentration is a major predictor of bone mineral density of the elderly<sup>(10,21)</sup>, the strength of the association between serum osteocalcin concentration and fracture occurrence shows considerable variation<sup>(5)</sup>. In the present study, Ca intake was not associated with serum osteocalcin concentration.

The present study also showed that low body weight or BMI is associated with high bone resorption and formation markers. The inverse association between body weight (or BMI) and bone turnover markers has been reported by others<sup>(22–24)</sup>. A number of studies have also shown that low body weight is a major predictor of bone mineral density and bone loss of the elderly<sup>(21,25,26)</sup> and that this association may be mediated by increased bone turnover.

Exercise is known to affect bone turnover markers<sup>(27)</sup>. However, the present study did not find physical activity to be associated with bone turnover markers. One major reason for this may be that we obtained qualitative, rather than quantitative, data for physical activity, because assessment of physical activity in the elderly is difficult. Physical activity is one of the important determinants of bone health, and future studies should clarify this association in elderly populations.

One limitation of our study is that we measured only serum NTX and osteocalcin concentrations, which reflect only certain aspects of bone turnover or bone quality. Another limitation is that an FFQ is not an ideal method to evaluate Ca intake, although the FFQ used in our study was validated with improved accuracy over other FFQ. These limitations weaken the association of Ca intake with serum NTX concentration seen here.

In summary, the present study showed that very low Ca intake (less than ~400 mg/d) is associated with increased bone resorption in peri- and postmenopausal Japanese women. These results suggest that Ca supplementation programmes should focus on those women at highest risk. On the other hand, a population approach is also of interest because Ca intake in most adults in Japan is lower than current recommendations. Future studies are needed to develop interventions that effectively address low Ca intake among postmenopausal women.

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