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Urinary Excretion of Type I Collagen Cross-Linked N-Telopeptides, Bone Mass and Related Lifestyle in Middle-Aged Women

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The relationship between past and present lifestyle and urinary excretion of type I collagen cross-linked N-telopeptides (NTx) was studied in 61 Japanese females aged 34-59, with a view toward using NTx excretion rates as a predictor of future osteoporosis. Bone mineral density (BMD) of the lumbar spine, the speed of sound (SOS) and broadband ultrasound attenuation (BUA) of the os calcis, urinary NTx, serum osteocalcin (BGP) and bone-specific alkaline phosphatase (BAP) were measured. Stiffness index (stiffness) was calculated from SOS and BUA. The subjects were asked whether they took regular exercise in their childhood and teen years (in elementary, junior-high, senior-high school and college), the past (20-40 years of age) and present adulthood. Regular calcium intake, smoking habits, alcohol and other beverage consumption and milk consumption were also covered in the questionnaire. The mean NTx values of premenopausal and postmenopausal group were 22.2 and 56.0 nM bone collagen equivalents (BCE)/mM urinary creatinine (Cr), respectively. The group which did not exercise regularly between the ages of 20 and 40 had a higher mean NTx value (40.9 nMBCE/mMCr) than the group which did exercise regularly (22.7 nMBCE/mMCr). In multiple regression analyses, age, stiffness and exercise in past adulthood could explain 43.5% of the NTx variance. For prevention of bone metabolic increases around menopause, habitual exercise in early adulthood seems to be effective.

Key words: N-telopeptides, bone turnover, osteoporosis, menopause, lifestyle

Osteoporosis and osteoporosis-related fractures have a great influence on morbidity and disability of the elderly. The risk factors for developing osteoporosis and osteoporosis-related fractures have been well publicized in medical literature (1-3). As for women, menopause is a great risk factor for developing osteoporosis. The rate of bone loss is accelerated after menopause because of high turnover of bone metabolism induced by a decline of estrogen (1-4). In order to prevent postmenopausal osteoporosis, it is important for perimenopausal women to know their rate of bone resorption, so that treatment can be initiated before any major loss of bone mass. In clinical studies, biochemical markers of bone metabolism have been used to assess various therapies, such as medical treatment of osteoporosis and parathyroidectomy (5-7). Recently, these markers have come under scrutiny as possible predictors of osteoporosis and related fractures (4, 8). Type I collagen cross-linked N-telopeptides (NTx), a new biochemical marker, is a degradation product of type I collagen which is a main component of bone, and is excreted in urine without further degradation. It has been reported to be a specific and sensitive bone resorption marker (5, 9-11). An increase in urinary excretion of NTx indicates high bone resorption. To know bone metabolism in order to prevent osteoporosis, the measurement of NTx is considered to be very useful in the perimenopausal period. Postmenopausal increase of urinary NTx and negative correlation between NTx and bone mass have been reported in many studies (12-15), but whether the elements of lifestyle which affects bone mass (like regular exercise and calcium consumption) also affect urinary NTx excretion is still not clear. Gernero *et al.* (16) suggested that the contribution

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of genetic factors to postmenopausal bone turnover and bone loss was probably small. The aim of our study was to see how lifestyle factors (diet, exercise and physical activity) relate to NTx excretion (which is thought to reflect bone resorption).

Subjects and Methods

Subjects. The subjects consisted of 61 Japanese females from 34–59 years of age who visited a general hospital in Okayama city for a general check-up and a voluntary screening for osteoporosis. None of the subjects were receiving any treatment for conditions known to affect bone metabolism such as hyper- and hypothyroidism, diabetes millitus, renal failure, osteoporosis, and estrogen therapy within the previous year. Subjects were divided into premenopausal, menoxenia, and postmenopausal groups by menstrual condition over the previous year. Groups consisted of 28, 6, and 24 individuals, respectively. In order that our subjects would better understand the aims and potential benefits of this study, we described it in detail and received informed consent from all who participated.

Questionnaires. A self-adminished questionnaire, including the subjects' medical history, use of medication, family history of osteoporosis, menstrual and reproductive history, physical activity and diet, was distributed to each subject. The subjects were asked whether they took regular exercise in their childhood and teen years (in elementary, junior-high, senior-high school and college), the past (20–40 years of age) and present adulthood. Habitual exercise was defined as taking exercise for more than once per week and 30 min or longer each time over one year. The exercise intensities were converted into METs (metabolic equivalents) which represent multiples above the resting metabolic rate (17). As for physical activity, daily sitting, standing and walking time during working, commuting and doing housework were inquired about. Regular calcium intake, smoking habits, alcohol and other beverages consumption and milk consumption were also covered in the questionnaire. As milk is considered to be the most common and important source of dietary calcium, we provided three possible categories for milk consumption (everyday, often, and seldom) and asked subjects to rate their milk consumption according to these categories both for past (before 30 years of age) and present. We also asked them to rate their milk consumption both for past and present by an open question (number of glasses per day or per week). Past and present calcium intake originating from milk was calculated by multiplying the frequency of milk consumption by the calcium content of the specified portion. The subjects were also asked how often on average they had consumed other calcium-rich foods (dairy products, small fish, seaweed, soybeans), and four response categories ranging from "seldom (none to once/week)", "sometimes (2 to 4 times/week)", "almost everyday (5 to 6 times/week)", to "everyday", could be chosen. All questionnaire data were reconfirmed on site by a trained public health nurse, and participants were asked to complete any missing items.

Measurement. For bone mass measurement, dual-energy X-ray absorptiometry and quantitative ultrasound instrument were used. Bone mineral density (BMD) of the lumbar spine (L) was measured by dual-energy X-ray absorptiometry (QDR-2000, Hollogic Co., MA, USA) in L2–4. Speed of sound (SOS) and broadband ultrasound attenuation (BUA) of the os calcis were measured using a quantitative ultrasound instrument (Achilles, Lunar Co., WI, USA). Stiffness index (stiffness) of bone was calculated from SOS and BUA values. Anthropometric measurements (height, weight, percentage body fat, waist and hip) were carried out for each subject. Percentage body fat was measured by bioelectrical impedance analysis using a TBF-202 machine (Tanita Co., Tokyo). Body mass index (BMI) and waist-hip ratio (WHR) were calculated as shown below.

$$\mathrm{BMI} = \frac{\text{weight (kg)}}{(\text{height (m)})^2}$$

$$\mathrm{WHR} = \frac{\text{waist (cm)}}{\text{hip (cm)}}$$

Biochemical markers. An overnight fasting serum and urine sample were collected from each subject in the morning for the purpose of measuring bone formation markers, serum osteocalcin (BGP) and bone-specific alkaline phosphatase (BAP) and a bone resorption marker (urinary NTx). Serum BGP and BAP were measured using two-site immunoradiometric assay (18) and enzyme-immunoassay (19), respectively. The former is specific for intact human BGP and is measured with a BGP-IRMA kit (Mitsubishi Chemical Co., Tokyo), and the latter is measured with monoclonal antibody anti-BAP (Metra Biosystems, Inc., CA, USA). The normal ranges of serum BGP and BAP values for women in the general population are 3.1 to 9.2 (mean $\pm$ 2 SD) ng/ml

and 9.6 to 35.4 (mean ± 1.96 SD) IU/l, respectively. As for bone resorption marker, enzyme-linked immunosorbent assay of urinary NTx was performed with an Osteomark kit (Mochida Pharmaceutical Co., Ltd., Tokyo), which uses a monoclonal antibody to the N-telopeptide of the helix molecules that forms cross-links in type I collagen isolated from human urine (7). The data of NTx were corrected by urinary creatinine concentration (Cr) measured at the same time. Normal urinary NTx level was ranged from 8.3 to 69.9 nM bone collagen equivalents (BCE)/mMCr in premenopausal women (19–56 years) and 14.0 to 99.5 nMBCE/mMCr in postmenopausal women (39–80 years) (the normal range for healthy individuals is from 2.5 to 97.5 percentile). All assays were carried out by Mitsubishi Kagaku Bio-Clinical Laboratories, Inc. (Tokyo).

Statistics. The mean and standard deviations (SDs) of the variables for all subjects and each categorized group were calculated for numerical variables. Significance of the differences between the two groups was examined by Student's t-test for unpaired data, as well as by the χ^2 test for independence to exclude confounding factors. Difference among the three groups regarding menstrual status was analyzed by one-way analysis of variance (ANOVA). When an F value was significantly high, Tukey's test (honestly significant test) was carried out to know where the statistically significant difference exists ($P < 0.05$). Pearson's correlation coefficients were calculated to explore the relationship between NTx and other variables. Stepwise multiple linear regression analysis was also conducted to evaluate the relationship between lifestyle factors (physical activities, exercise and diet) and urinary levels of NTx, in which the independent variables were age, bone mass (BMD or stiffness), BMI, percentage body fat, WHR, age at menarche, daily hours of standing, sitting, walking, exercise at present, exercise in early adulthood, and past and present daily milk-originated calcium intake. Significant variables at the 0.15 level were entered into the model. Interactions and higher ordered terms were investigated. The coefficient of determination (R^2) is a measure of the proportion of the variance of the dependent variables that is accounted for by the independent variables. The change in R^2 with certain variables entering into the model was also analyzed. This was done to evaluate the extent to which lifestyle factors influence NTx excretion. All statistical analyses were performed using the PC-SAS statistical program (ver 6.11).

Results

The characteristics of subjects are summarized in Table 1. Postmenopausal women were significantly older by 10.2 years, shorter of stature by 4.0 cm and older at menarche by 1.4 years than premenopausal ones. The characteristics of the menoxenia group were not signifi-

Table 1 Characteristics of subjects (mean ± SD)

	All (n = 61)	PRE (n = 28)	Menoxenia (n = 6)	POST (n = 24)
Age (years)	46.9 ± 6.4	42.6 ± 4.8	44.7 ± 4.8	52.8 ± 3.3**
Height (cm)	155.7 ± 5.4	157.5 ± 5.3	155.9 ± 4.9	153.5 ± 5.1*
Weight (kg)	53.1 ± 9.3	52.8 ± 7.3	50.9 ± 7.5	53.8 ± 12.2
BMI (kg/m²)	21.9 ± 3.9	21.3 ± 2.5	20.9 ± 2.4	22.9 ± 5.4
%BF (%)	26.3 ± 6.9	25.9 ± 5.2	24.4 ± 4.8	27.2 ± 9.1
Menarche (years)	13.0 ± 1.4	12.4 ± 1.1	12.8 ± 0.8	13.8 ± 1.6**
Yr since M (years)	—	—	—	6.1 ± 7.0
Reproductive period (years)	—	—	—	32.9 ± 7.1

PRE: Premenopausal group; Menoxenia: Menoxenia group; POST: Postmenopausal group; BMI: Body mass index; %BF: Percentage body fat; Yr since M: Years since menopause.
*$P < 0.01$; **$P < 0.001$ *vs* PRE.

Table 2 The bone mass and biochemical markers in premenopausal, menoxenia, and postmenopausal groups (mean ± SD)

	PRE (n = 28)	Menoxenia (n = 6)	POST (n = 24)
BMD (g/cm²)	1.03 ± 0.12	0.99 ± 0.17	0.90 ± 0.17[b]
T-score (%)	101.9 ± 11.7	97.8 ± 17.3	89.0 ± 17.0[b]
Z-score (%)	103.0 ± 11.2	100.6 ± 18.1	99.1 ± 17.9
Stiffness	82.9 ± 10.0	80.2 ± 6.9	71.6 ± 11.4[c]
SOS (m/s)	1541.7 ± 22.5	1531.8 ± 19.7	1516.1 ± 22.7[c]
BUA (dB/MHz)	107.1 ± 7.6	107.0 ± 5.2	100.8 ± 9.1[a]
BGP (ng/ml)	5.0 ± 1.5	6.8 ± 2.7	9.0 ± 3.6[c]
BAP (IU/l)	17.3 ± 5.4	19.2 ± 7.2	25.7 ± 8.0[c]
NTx (nMBCE/mMCr)	22.2 ± 11.0	31.7 ± 21.7	56.0 ± 23.5[c]

BMD: Bone mineral density; Stiffness: Stiffness index; SOS: Speed of sound; BUA: Broadband ultrasound attenuation; BGP: Osteocalcin; BAP: Bone-specific alkaline phosphatase; NTx: Type I collagen cross-linked N-telopeptides; PRE; Menoxenia; POST: See legend to Table 1. *a*: $P < 0.05$; *b*: $P < 0.01$; *c*: $P < 0.001$ PRE *vs* POST.

cantly different from those of the premenopausal group. Table 2 shows the mean ± SD of the bone indices and biochemical markers. T-score (%) is the subjects' BMD scores expressed as a percentage of the mean BMD score for 20-year-old females in the general population and Z-score (%) is the subjects' BMD scores expressed as a percentage of the mean BMD scores for females of the same age in the general population. The mean T-score of postmenopausal women (89.0 %) was lower than that of premenopausal women (101.9 %), but the mean Z-score between pre- and postmenopausal women was not significantly different. In the postmenopausal group, the bone indices (BMD, stiffness) demonstrated significantly low values, and the mean BMD and stiffness were 87.4 % and 86.4 % of those of premenopausal group. The mean NTx values of premenopausal, menoxenia and postmenopausal group were 22.2, 31.7 and 56.0 nMBCE/mMCr, respectively (Table 2). The mean values of BGP and BAP of postmenopausal women showed significantly high values as compared with those of the premenopausal women. The menoxenia group appeared to have lower bone mass and higher values of biochemical markers than the premenopausal group, although the difference was not statistically significant.

Table 3 shows the correlations among age, bone mass (BMD, stiffness) and biochemical markers. The correlation coefficient between NTx and age was high ($r = 0.57$). The NTx values plotted against age are shown in Fig. 1. While in the premenopausal women, an age-related increase in NTx level was observed (Fig. 2), in the postmenopausal women, it was not ($r = -0.049$, $P = 0.83$). Neither years since menopause nor the reproductive period were correlated with NTx values (Fig. 3). There was a weak but significant correlation between NTx and the age at menarche ($r = 0.340$, $P = 0.008$).

With BMD and stiffness, NTx correlated inversely (Table 3). The correlation coefficients were -0.408 ($P = 0.002$) and -0.455 ($P < 0.001$). After adjusting for age, there was a statistically significant correlation of NTx with stiffness ($r = -0.345$, $P = 0.011$), but not with BMD ($r = -0.242$, $P = 0.078$). BGP and BAP were also correlated with BMD and stiffness, but the correlation was weaker than those between NTx and bone mass. Correlation between NTx and BGP or BAP was markedly high ($r = 0.756$, 0.751). BMI and WHR had no significant correlation with NTx.

Frequency of calcium-rich food intake had no significant influence on urinary NTx excretion nor on bone mass, although a tendency toward high NTx value was observed in women with low frequency of milk intake in the past (before 30 years of age).

The effect of exercise on the urinary NTx excretion was observed only in the past adulthood ($P = 0.001$), not in school days and at present. The non-exercise group in past adulthood appeared to have a higher NTx value (mean: 40.9 nMBCE/mMCr) than the exercise group (mean: 22.7 nMBCE/mMCr) (Fig. 4). Correlation coefficient between weekly hours of exercise in past adulthood and NTx was -0.266 ($P = 0.042$). Types of exercise varied (volleyball, dance, tennis, table tennis, swimming, walking, *etc.*). The exercise intensity of each subject was estimated to be approximately 3 to 5 METs (17). No combined effect of menstrual status and exercise in past adulthood on NTx value was observed by the χ^2 test ($P < 0.05$) (data not given).

Table 4 gives the multiple regression model of the NTx variance accounted for by age, bone mass, and weekly hours of exercise in past adulthood. BMD and stiffness are shown to be highly correlated, which will affect the partial regression coefficients of other variables if they are put together within one multiple regression model as independent variables. Therefore, BMD and

Table 3 Correlations among age, biochemical markers and bone mass

	(n)	Age	BMD	Stiffness	BGP	BAP	NTx
Age	(61)						
BMD	(60)	-0.432^c					
Stiffness	(60)	-0.348^b	0.572^c				
BGP	(59)	0.509^c	-0.300^a	-0.373^b			
BAP	(59)	0.492^c	-0.355^b	-0.438^c	0.742^c		
NTx	(59)	0.570^c	-0.408^b	-0.455^c	0.756^c	0.751^c	

BAP: Bone-specific alkaline phosphatase; BMD; Stiffness; BGP; NTx: See legend to Table 2. a: $P < 0.05$; b: $P < 0.01$; c: $P < 0.001$.

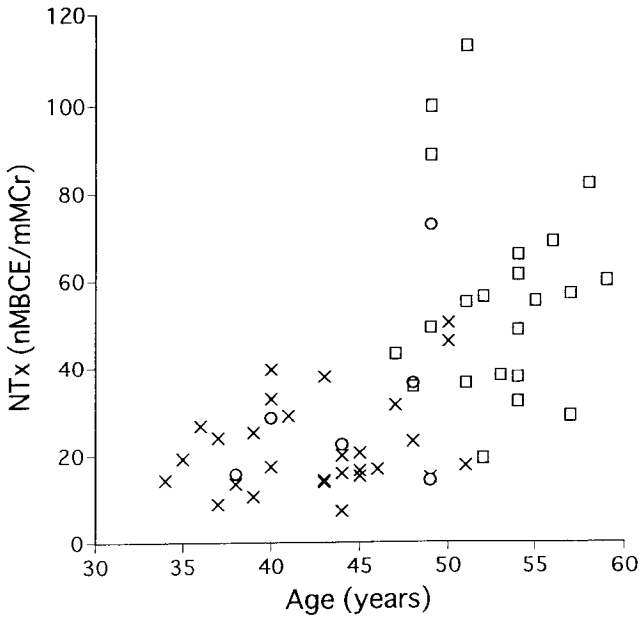

Fig. 1 Plots of urinary NTx *vs* age for 56 subjects. Premenopausal (×), menoxenia (○), and postmenopausal (□) groups consisted of 28, 6 and 24 women, respectively.

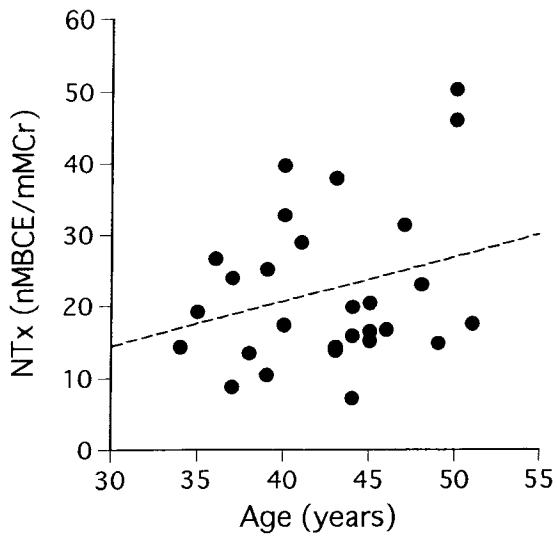

Fig. 2 Linear regression of urinary NTx *vs* age for premenopausal women (n = 28). Correlation coefficient was 0.27 ($P = 0.16$). The regression equation is $y = -4.455 + 0.626x$.

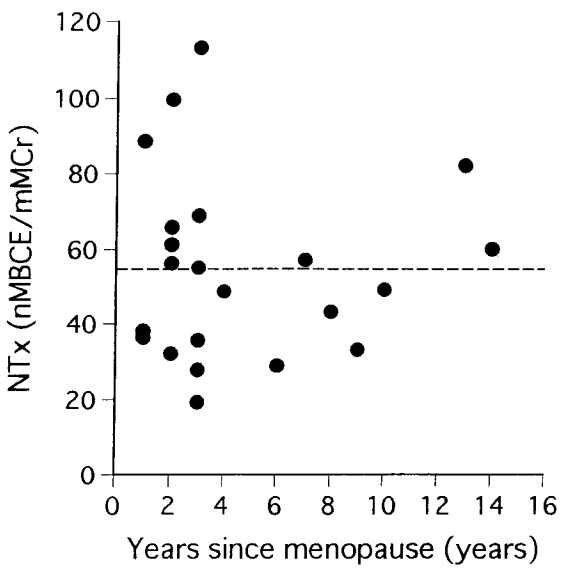

Fig. 3 Linear regression of urinary excretion of NTx *vs* years since menopause in 22 postmenopausal women ($r = -0.09$, $P = 0.68$).

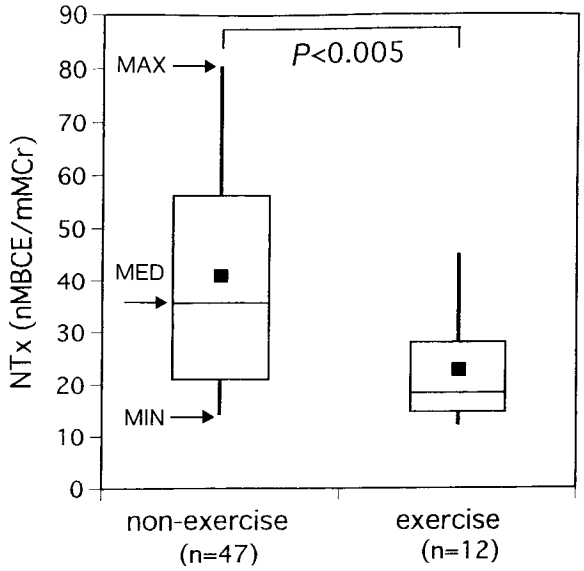

Fig. 4 Effect of exercise in past adulthood (20 40 years of age) on the urinary NTx excretion. Urinary NTx levels of the non-exercise and exercise group were 40.9 and 22.7 nMBCE/mMCr (mean), respectively. Data are expressed as median (MED), minimum (MIN) and maximum (MAX). ■ represents the mean value.

Table 4 Multiple regression models for NTx

Independent variables	Partial regression coefficient	*P*	Multiple correlation coefficient
Age (years)	1.788	0.000	
BMD (L2-4) (g/cm^2)	−29.048	0.139	
Exercise in past adulthood (h/week)	−2.276	0.149	0.620
Intercept	−16.107	0.630	
Age (years)	1.695	0.000	
Stiffness (the os calsis)	−0.613	0.012	
Exercise in past adulthood (h/week)	−2.399	0.105	0.659
Intercept	7.980	0.794	

NTx; BMD; Stiffness: See legend to Table 2.

stiffness were analyzed separately in two multiple regression models to assess their contributions to the level of NTx. When stiffness was entered as an independent variable, the proportion of variance increased to 43.5 %. In contrast, when BMD was entered as an independent variable, the proportion of variance was 38.4 %. Present weekly hours of exercise, past or present daily calcium intake from milk, years since menopause and the reproductive period had only a small impact on the NTx variability.

Discussion

Several researchers have reported that the rate of bone resorption obviously increases during the period from premenopause to 3 years postmenopause (8, 12, 14, 15, 20). Some longitudinal studies have indicated that the rise in bone resorption and formation occurs even before the premenopausal period (21). Although this study was cross-sectional, we did not observe an increase in NTx levels (a marker of bone resorption) in proportion to the lapse of years after the menopause. In this study, there was a positive correlation between age and NTx values from the period around 35 years of age to the onset of the menopause. This indicates that a rise in bone resorption begins along with a gradual decrease in estrogen levels in the years prior to menopause. Furthermore, we observed wide variation among individuals in NTx excretion levels and bone mass. This phenomenon may be explained by the fact that the sensitivity of the estrogen for the bone is much higher than that for the gonad.

Estrogen promotes the synthesis of transforming growth factor β and insulin-like growth factor I working in the osteoblast, and it has the action of maintaining the bone matrix (22, 23). The lack of estrogen stimulates the production of interleukin (IL)-1 and IL-6, and these in turn activate osteoclasts (24). Although wide variability is observed in NTx excretion after menopause, it seems to be based on genetic differences in the estrogen receptor on the osteoblast. It has been reported that genetic factors account for about 75 % of an individual's BMD, the remaining 25 % being accounted for by environmental factors (25, 26).

With aging, the rate of osteogenesis falls and the numbers of osteoblasts decrease, while at the same time malfunctions of the osteoblasts increase. Physical activity during youth has been reported to be important for achieving peak bone mass (1, 27-29). It has been suggested that exercise is a physiological stimulus to the elevation of plasma estradiol (30). On the other hand, strenuous exercise is well known to induce amenorrhea and can lead to the development of osteoporosis (1). In our study, a multiple regression analysis showed that regular exercise between the ages of 20 and 40 had a significant influence on NTx excretion. In lowering NTx excretion, it appears that regular exercise of moderate intensity may have a positive effect on the perimenopausal bone metabolism.

One way to protect against bone mass decrease at menopause is to use NTx levels in urine to monitor the rates of bone metabolism. In the cases where high NTx excretion is observed, an estrogen replacement therapy may be recommended. A detailed examination of the relationship between estrogen level and NTx excretion will be done in the future.

Recently, urinary type I C-telopeptide breakdown product (CTx) has been studied as a new bone formation marker. The measurement of NTx and CTx can be performed on an untreated urine sample, which is non-invasive for the subjects and makes it easy to collect samples. Both urinary NTx and CTx is said to be a good predictor of perimenopausal bone loss and hip fracture in the elderly (4, 11).

The variation in NTx was explained more by stiffness, age and exercise in past adulthood than by BMD, age and past exercise as shown in Table 4. These findings suggest that an elevation of bone resorption is reflected more

sensitively by stiffness of the calcaneus than by BMD of the lumbar spine. As the os calcis contains higher percentage of cancellous bone than the lumbar spines, the change of bone resorption seems to express earlier.

In conclusion, we found an age-related increase in NTx excretion levels in premenopausal women but not in postmenopausal women. We believe that differences in individual levels of NTx excretion are caused by differences in individual lifestyles. In particular, our results suggest that habitual exercise in early adulthood is likely to be effective in preventing bone metabolic increase in the years around menopause.

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