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Increased Serum Levels of N-Telopeptides (NTx) of Bone Collagen in Postmenopausal Nigerian Women

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Abstract. Serum levels of cross-linked N-telopeptides (NTx) of bone collagen, alkaline phosphatase (ALP), and intact parathyroid hormone (PTH) were determined in 64 premenopausal (PRM) and 86 postmenopausal (PSM) women living in northern Nigeria. Serum NTx values were correlated with ALP activity ($r = 0.31–0.58$, $P < 0.01$) and PTH (0.32–0.35, $P < 0.01$)) in all of the subjects studied, and were also related to age (−0.47, $P < 0.001$) and body mass index (−0.45, $P < 0.001$) in PRM women. Menopause had the effect of increasing the circulating concentrations of NTx and ALP activity by 15% ($P = 0.001$) and 11% ($P = 0.02$), respectively; however, serum levels of PTH were not different between these two groups of women. Compared with Caucasian counterparts matched for age and body mass index, PSM Nigerian women had significantly increased circulating concentrations of NTx (21.7 versus 16.2 nmol BCE/liter, $P = 0.01$) and demonstrated a trend towards higher ALP activities and PTH levels. These results indicate that (1) discrete reference intervals should be defined for biochemical markers of bone metabolism in African populations, (2) Nigerian women have relatively higher rates of bone turnover, and (3) further investigation of the implications of increased serum NTx should be undertaken using physical methods such as dual X-ray absorptiometry (DXA) and bone ultrasound attenuation.

Key words: Africans — Bone markers — Bone collagen — Parathyroid hormone — Postmenopausal women — Serum NTx.

As is true for most women who inhabit the rural regions of sub-Saharan Africa, the bones of Nigerian women are subject to greater stress than those of their Caucasian counterparts for a number of reasons. First, the diets in the western Sahel contain low amounts of calcium, and several of the cereal staples of West Africa (e.g., millet and sorghum) contain large amounts of calcium chelators such as oxalates and phytates [1]. Second, briefly spaced pregnancies in the teenage years could deplete their calcium reserves [2]. Third, estrogen replacement therapy and other therapeutic interventions aimed at reducing bone loss are not widely available in many parts of Africa because of socioeconomic limitations. However, some of the cereal staples mentioned above also contain appreciable quantities of flavonoids, a class of compounds with antioxidant and estrogen-like properties which are cardioprotective and preserve bone mass [3].

Because populations of the western Sahel are exposed to a number of risk factors that affect the female skeleton, the rate of bone resorption may be increased in these women due to the pressure to maintain extracellular calcium levels. Radiographic methods for estimating bone mineral density (BMD), such as dual energy X-ray absorptiometry (DXA) and ultrasonography, are not widely available in many underdeveloped regions of the world; however, measurement of the cross-linked N-telopeptide of bone collagen (NTx) in urine or serum provides a reliable means for assessing the relative rate of bone resorption [4–7]. Furthermore, studies in the United States have shown that the level of NTx in the sera of postmenopausal women correlates significantly with DXA estimates of BMD in the femoral neck, vertebral spine, and total skeleton [8], and is a measurement applicable to populations who inhabit the western Sahel.

The goal of this study was to compare serum levels of several biochemical markers of bone turnover in premenopausal (PRM) Nigerian women with those of postmenopausal (PSM) women in the same region. An additional objective was to compare these data between Nigerian and North American women matched by age and body mass index (BMI). The rationale for making such comparisons was to inquire if there might be differences in the relative rates of bone turnover between African and Caucasian women, as assessed by serum indices of skeletal metabolism.

Materials and Methods

Selection of the Subjects

Sixty-four PRM and 86 PSM women were recruited from the outpatient clinics of the Obstetrics and Gynaecology Department of the University of Jos Teaching Hospital in Jos, Nigeria and the Gwange Primary Health Clinic in Maiduguri, Nigeria. Exclusion criteria included recent surgery or fracture, current pregnancy, malignancy, or immobility. Eighteen healthy, Caucasian, ambulatory PSM women recruited from the New Mexico Aging Process Study [8, 9] were precisely matched to the same number of PSM Nigerians by age and BMI. These 18 women were not receiving estrogen or thyroid replacement therapy or any other medications

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known to affect bone turnover, and their lumbar spine, femoral neck, and total body BMD were within 1 standard deviation (SD) of the mean defined for their age. Blood samples for the Nigerian and Caucasian populations were drawn between 8:00 and 10:00 a.m. (to avoid the diurnal variation expected in levels of serum bone markers) and allowed to clot. The serum was separated and frozen at −40°C and transported on dry ice until the biochemical analyses were performed in Albuquerque, New Mexico. Informed consent was obtained prior to enrollment of subjects in the study. The study was approved by the Ethics Review Committee at the Jos University Teaching Hospital and the University of Maiduguri Teaching Hospital, and by the Human Research Review Committee of the University of New Mexico School of Medicine.

Biochemical Analyses of Serum

Serum calcium levels and ALP activity were measured with the aid of a Kodak DT-60 analyzer (Johnson and Johnson Clinical Diagnostics, Rochester, NY). Serum calcium was measured with an Arsenazo III kinetic dye binding assay monitored at 680 nm and pH 5.6. The method for determining ALP activity was described by Bessey et al. and later modified by Bowers and McComb [10] based on the rate of nitrophenoxide production at pH 10.5, monitored at 400 nm. Prior to assay, aliquots of sera were incubated at 22°C for 18–24 hours to ensure full recovery of ALP activity [11]. The within assay coefficient of variation (CV) of the serum measurements was less than 5% and rigid analytical performance criteria were satisfied. Quality control materials were a gift of the Clinical Chemistry Division of the University of New Mexico Health Sciences Center Clinical Laboratory.

Serum NTx

Serum levels of NTx were measured using a microplate enzyme-linked immunosorbent assay (ELISA) in competitive inhibition format. The immunoassay uses a specific monoclonal antibody (mAb 1H11) that was developed for measuring NTx in human urine and the assay procedure was essentially the same as described previously [4]. The antibody mAb 1H11 was conjugated to horseradish peroxidase, and polystyrene microtiter plates were coated with NTx antigen by passive absorption. Test specimens and assay standards were diluted in buffer and combined with mAb 1H11-HRP in microtiter wells. Following incubation and subsequent washing with detergent solution, a hydrogen peroxide-tetramethylbenzidene buffer was added for color development. The color intensity was measured spectrophotometrically. Final assay results are reported as nanomoles of bone collagen equivalents (BCE)/liter. The assay had a ≤8% CV with a lower limit of detection of 1.0 nmol BCE/liter [6].

Parathyroid Hormone

Intact PTH was measured by a double antibody sandwich radioimmunometric assay (INCSTAR, Stillwater, MN). Radioactivity was determined using a United Packard RIASTAR 5400 (Downers Grove, IL) scintillation counter and the within-assay CV for the PTH determination was less than 5%.

Statistical Analysis

Descriptive statistics, correlation coefficients, and paired and two-sample *t*-tests were calculated using the Number Crunching Statistical Software program, version 6.0.22 (Kaysville, UT). The Mann-Whitney nonparametric two-sample *t*-test was used for comparisons of NTx and PTH levels and ALP activity.

Table 1a. Anthropometric characteristics of the Nigerian study populations

	Premenopausal (N = 64) (mean ± SD)	Postmenopausal (N = 86) (mean ± SD)	P-value
Age (years)	33.7 ± 8.6	54.3 ± 8.6	<0.0001
Height (m)	1.58 ± 0.09	1.55 ± 0.07	NS
Weight (kg)	61.8 ± 14.1	61.1 ± 11.8	NS
BMI (kg/m^2)	25.2 ± 5.2	25.6 ± 5.6	NS
Mid-arm circumference (cm)	29.3 ± 5.2	29.8 ± 4.7	NS
Triceps skinfold thickness	26.5 ± 10.9	24.8 ± 10.9	NS
Pregnancies	5 ± 3	6 ± 4	0.01

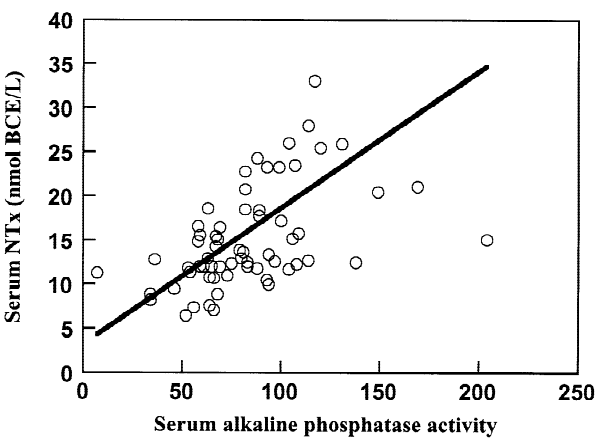

Fig. 1. Correlation of serum levels of NTx and ALP in 64 premenopausal Nigerian women (r = 0.58, P < 0.001).

Results

The Study Populations

All of the Nigerian and Caucasian women in the study were ambulatory, in good health, and not receiving any medications including estrogen or thyroid hormone replacement. None of the PRM Nigerian women were pregnant at the time their serum was collected. No significant differences were detected between PRM and PSM Nigerian women with regard to height, weight, BMI, or mid-arm circumference (Table 1a). The lumbar spine BMDs of the 18 Caucasian women were all within 1 SD of a defined age-adjusted reference value [12].

Correlation of Biochemical Markers of Bone Metabolism in Nigerian Women

In PRM Nigerian women, serum levels of NTx were directly correlated with ALP activity (r = 0.58, P < 0.001) (Fig. 1) and PTH concentration (r = 0.35, P < 0.05). Serum NTx in PSM Nigerians was correlated with ALP activity (r = 0.31, P < 0.01), PTH (r = 0.32, P < 0.05), and was also significantly and inversely related to BMI (r = −0.45, P < 0.001) (Fig. 2) and age (r = −0.47, P < 0.001) (Fig. 3).

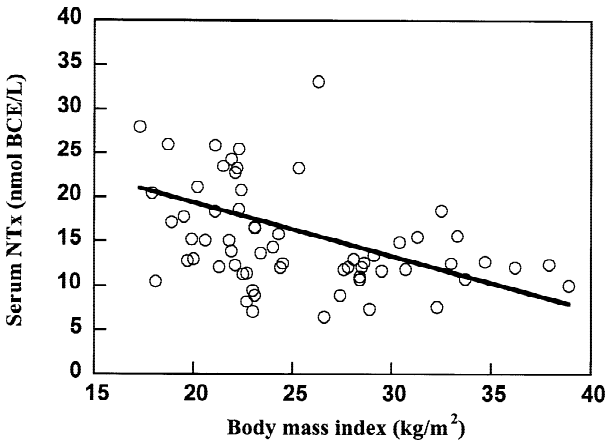

Fig. 2. Correlation of BMI and serum NTx levels in 64 premenopausal Nigerian women (r = −0.45, $P < 0.001$).

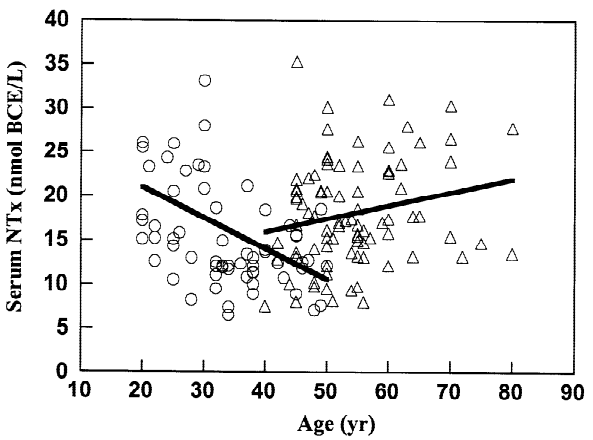

Fig. 3. Correlation of age with serum NTx concentration in 64 premenopausal (r = −0.47, $P < 0.001$, open circles) and 86 postmenopausal (not statistically significant, open triangles) Nigerian women.

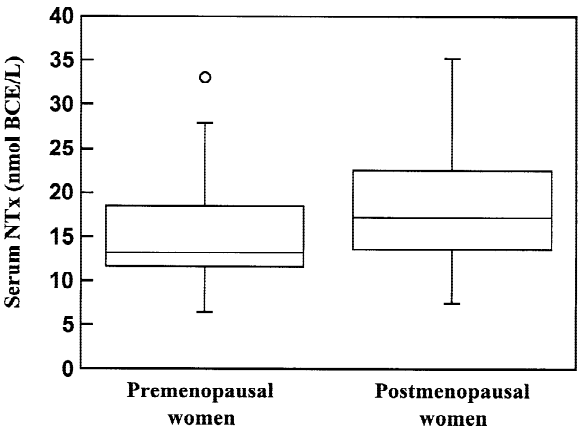

Fig. 4. Comparison of serum NTx levels in 64 premenopausal and 86 postmenopausal Nigerian women, ($P = 0.001$). Box limits demarcate the 25th and 75th percentile with the median represented as a solid horizontal line. Vertical lines extending from the box are maximum and minimum values that fall within the distribution. Circles represent outliers.

Table 1b. The effect of menopause on serum biochemical markers of bone metabolism in Nigerian women

Parameter	Premenopausal ($N = 64$) (mean)	Postmenopausal ($N = 86$) (mean)	P-value
NTx (nmol BCE/liter)	16.2	18.7	0.001
ALP (U/liter)	84	94	0.02
Calcium (mg/dl)	9.4	9.4	NS
PTH (pg/ml)	33.9	33.5	NS

ALP = alkaline phosphatase activity, NTx = cross-linked N-telopeptides of bone collagen expressed in bone collagen equivalents (BCE) per liter, PTH = intact parathyroid hormone
NS = P value >0.05.

Additionally, serum PTH values were directly related to ALP activity (r = 0.52, $P < 0.05$) and inversely correlated with calcium levels in the PRM group (r = −0.41, $P < 0.05$).

Effect of Menopause on Biochemical Markers of Bone Metabolism

Menopause had the effect of significantly increasing circulating concentrations of NTx (18.7 versus 16.2 nmol BCE/liter, $P = 0.001$) (Fig. 4, Table 1b) and ALP activity (94 versus 84 U/liter, $P = 0.02$, Table 1b). Levels of intact PTH and calcium in serum did not significantly differ between PRM and PSM Nigerian women (Table 1b).

Postmenopausal Nigerians and North Americans

To address the question of whether there were differences in bone turnover rates between Nigerian and North American women, we first precisely matched 18 PSM Caucasian women who were not receiving estrogen or thyroid replacement therapy to the same number of PSM Nigerian women according to age and BMI. Although there were no significant differences detected in the circulating level of calcium between these two groups, we did observe a statistically significant increase in serum NTx (21.7 versus 16.2 nmol BCE/liter, $P = 0.02$) (Fig. 5) and a trend towards increased ALP activity (103 versus 94 U/liter) and intact PTH levels (36.1 versus 32.2 pg/ml) in the PSM Nigerian women.

Discussion

The most interesting findings of the present study were the increased levels of PTH and NTx in the sera of PRM and PSM Nigerian women. Our data demonstrate that the serum concentration of NTx in both PRM and PSM women was directly correlated to the amount of circulating PTH and ALP activity, a finding that is consistent with our previous study of 202 Caucasian American PSM women living in New Mexico [8]. The relationships of NTx to ALP activity and PTH indicate that bone formation and resorption are coupled in healthy populations and support the contention

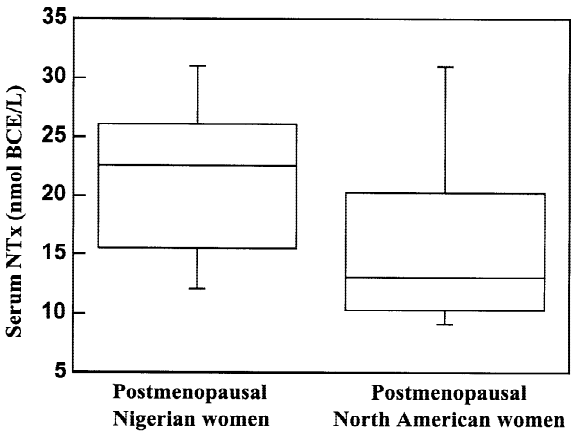

Fig. 5. Comparison of serum NTx in 18 Nigerian and 18 North American postmenopausal women matched for age and BMI ($P = 0.02$). Box limits demarcate the 25th and 75th percentile with the median represented as a solid horizontal line. Vertical lines extending from the box are maximum and minimum values that fall within the distribution.

that PTH is a direct mediator of osteoclast activity. Additionally, we have determined that the serum NTx value was inversely related to both BMI and age in PRM Nigerian women; however, these correlations were not statistically significant after menopause. Our study is unique in demonstrating an inverse relationship of BMI to a marker of bone resorption in PRM women.

Although increased metabolic activity of the skeletons of Nigerian women may be reflective of a relatively higher susceptibility to fracture, studies of African-American women indicate that they have lower fracture rates [13, 14], higher BMD [15, 16] higher peak bone mass, and lose bone at a slower rate than do Caucasians [17]. However, in a study conducted in Gambia by Aspray et al. [18], BMD was poorly preserved in elderly women in that country and was less than that of British women. However, most comparisons of African and African-American women have not taken into account differences in genetic, environmental, and dietary factors. Furthermore, there is no difference in architecture of bone between African-American and Caucasian women, as measured by bone ultrasound attenuation [17].

The finding of increased serum NTx levels in Nigerian women raises the possibility that during the formative stages of skeletal accretion, young Nigerian women in particular are exposed to a significant risk of osteopenia. To maintain extracellular calcium levels, Nigerian women may need to resorb more bone than Caucasians. In northern Nigeria, PSM women are prone to bone loss, especially those who have limited access to estrogen replacement therapy. The serum NTx level could be useful in studies whose purpose is to evaluate the effectiveness of replacement therapy using naturally occurring phytoestrogens (e.g., flavonoids), oral calcium supplementation, or exercise. Ultimately, the portable ultrasonographic methodologies we aim to use in future studies in Nigeria should answer the question of whether decreased BMD is a correlate of increased concentrations of biochemical markers of bone resorption.

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