Changes in markers of bone turnover following urbanisation of black South African women

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Objectives. To investigate dietary changes occurring with urbanisation of black South African women of different ages and how these changes influence biochemical markers of bone turnover.

Design. Biochemical markers of bone turnover and resorption were determined in a subset of subjects living in the North West province of South Africa (one rural and one urban area). Women aged 15 - 25 years and 55 - 65 years were included. Food intake was measured using food frequency questionnaires. Biochemical markers were correlated with relevant changes in dietary intake that occurred with urbanisation.

Results. Major findings included a significant increase in intake of animal protein, combined with very low calcium intake (< 400 mg/day). Bone turnover decreased with urbanisation as measured using osteocalcin, and bone resorption increased with urbanisation as measured with N-telopeptides from type 1 collagen (NTx), while bone formation stayed constant. These findings were prominent in the group of active growing girls (aged 15 - 25 years). Significant negative correlations were found between NTx and body mass index (BMI), indicating the protective effect of higher BMI on bone mass. Urinary calcium was significantly positively correlated with dietary protein, calcium/protein ratio and with fibre intake.

Conclusions. The changes in biochemical markers were clearer in the younger group of women and changes in diet with urbanisation seemed to have an impact on this group. Changes in bone accretion at adolescence can compromise bone strength during menopause and ageing as peak bone mass should be attained at the end of adolescence and up to age 30 years.

Osteoporosis is a condition of reduced bone mass leading to skeletal fragility — the clinical outcome is usually a fracture. Studies of involutional bone loss have mostly been limited to white women as they have the highest incidence of osteoporotic fractures. In South Africa, too, osteoporosis and fractures occur more frequently in whites than blacks. The notion that black women are relatively protected from osteoporosis has been overemphasised — older black women may increasingly represent a population at risk for osteoporosis because of urbanisation, which leads to low physical activity and changes in diet.[1]

It is known that peak bone mass, which is attained in early adult life, is dependent primarily on genetic factors, but is also influenced considerably by nutrition during adolescence and by physical activity. Genetic factors are obviously present, since there are differences in peak bone mass in various ethnic groups. For example, blacks have a greater bone mass than Caucasians, who in turn have greater peak bone mass than Asians.[2,3]

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Previous findings show decreased 25-hydroxyvitamin D and urinary calcium in normal black subjects compared with white subjects,[4] and increased 1,25 dihydroxyvitamin D_3 levels in black subjects. These findings may suggest upregulation of vitamin D receptors and a more efficient level of calcium absorption in blacks.[4] Rural blacks obtain minimal calcium from their diet compared with the National Institutes of Health (NIH) recommended daily allowance (RDA) of 1 000 mg/day. Despite the low calcium intake (< 200 mg/day) and multiple pregnancies in the rural groups, osteopenia does not manifest. It is therefore possible that black subjects upregulate calcium absorption, obtaining maximal absorption of the low quantity of calcium from the diet, and using this calcium to the utmost. A combination of upregulated calcium absorption and physical activity might explain the higher bone mineral content usually found in blacks.[5] Support for this theory was also found in a study by Abrams *et al*.[6] who found calcium absorption to be greater in black girls aged 7 - 14 years compared with white girls.

Indices of bone turnover as reflected by biochemical markers of bone formation and resorption have been shown to be lower,[2] similar,[7] and higher[8] for black women compared with Caucasian women in the USA before as well as during the menopause. Serum levels of 25-$(OH)_2D_3$ and urinary calcium were also shown to be lower in this group.[3] 1,25-$(OH)_2D_3$ as well as parathyroid hormone (PTH) levels have been shown to be higher in black women especially during the menopause.[3] The high levels of PTH indicate a skeletal resistance to PTH and lower urinary calcium may be due to the high PTH levels.[3]

Histomorphometric evidence suggests that black South African adults may have a higher bone turnover that whites.[8] The relatively high turnover rates minimise the volume of bone damaged by fatigue and stress fractures, and may contribute to better bone quality and hence lower fracture rates in blacks.[8]

Recent research has shown an increasing incidence of stress-induced illnesses in blacks associated with urbanisation and changes in environment. High stress levels are associated with increases in stress hormones such as cortisol and prolactin.[9,10] Long-term exposure of the body to high levels of cortisol, as measured in the abovementioned studies,[9,10] may induce changes in bone turnover. Other Western diseases such as hypertension and diabetes mellitus are also associated with changes in bone mass. Measurement of markers of bone turnover may be an early way of detecting changes in bone mass that may only be detected by measuring bone density when mass has been reduced by more than 10%.

The main overall objective of this study was to do a broad screening of markers for bone formation and degradation in girls and older women from a rural and an urban community in the North West province of South Africa. Correlations were drawn between dietary calcium, nutritional status, body mass index (BMI) and the bone markers. Results may help to predict the prevalence and future risk of bone disease in urbanised black South African women.

Methods

Experimental design, subjects, inclusion and exclusion criteria

This study formed part of a larger cross-sectional epidemiological field survey, namely the THUSA (Transition and Health during Urbanisation of South Africans) project. This project is also referred to as 'Help' in Setswana. The major aim of the THUSA study was to assess the impact of urbanisation and the consequent demographic transition on the health determinants and status of blacks in the North West province of South Africa. The subjects who participated in the present separate sub-study were selected from among THUSA subjects, except that only girls and women from the age categories 15 - 25 years and 55 - 65 years were included. A cross-sectional comparative study design was used in this sub-study comparing the impact of urbanisation on risk factors for osteoporosis in women of different levels of urbanisation, with stratum 1 being rural and stratum 2 urban.

For several logistical reasons, selection of a total randomised sample of blacks in the North West province was not possible. With the help of the Statistical Consultation Services of the Potchefstroom University for Christian Higher Education, a model was designed to recruit a representative sample. The sample consisted of 'apparently healthy' subjects in different age groups who did not receive therapy that might have an effect on bone loss. All procedures were approved by the Ethics Committee of the Potchefstroom University and informed consent was obtained from the subjects.

Exclusion criteria

The following subjects were not included in the study: *(i)* pregnant and lactating women; *(ii)* subjects known to suffer from any disease (infectious or non-communicable diseases); and *(iii)* subjects using any form of chronic medication (many hypertensive subjects were excluded because of this criterion).

Demographic and medical information, physical activity, diet and anthropometry

Questionnaires were used to gather demographic, medical and dietary information. Researchers in the THUSA project determined the face value of the demographic and medical history questionnaires. Dietary intakes were measured using a validated, culturally sensitive quantitative food frequency questionnaire (FFQ).[11] Validation was done against a 7-day weighed food record and 24-hour urinary nitrogen excretion (using para-aminobenzoic acid (PABA) as marker).[12] The quantitative FFQ was administered during individual interviews by specially trained Setswana-speaking fieldworkers and registered dietitians using food photographs, food models, household utensils and packages to quantify portion sizes. Postgraduate students from the School of Biokinetics took anthropometric measurements. For this study only height and weight were measured, from which BMI was calculated. Weight was measured to the nearest 0.5 kg using a portable electronic balance (Precision Health Scale, A and D Company, Japan). Subjects were barefoot and wore only underwear. Height was measured to the nearest 0.5 mm using a plastic anthropometer (Invicta, IP 1465, UK).

Blood and urine samples

Blood samples were drawn from the vena cephalica using a sterile butterfly infusion set (Johnson and Johnson, 21G, 19 mm) and syringes. Serum was prepared by allowing 50 ml of blood to clot in glass tubes and then centrifuged at 3 000 revolutions per minute (rpm) for 15 minutes. For each subject 30 x 1 ml aliquots were frozen at –20°C for 1 - 4 days and then at –84°C in the laboratory. Spot urine samples were collected from subjects and stored protected from light at –20°C for 1 - 4 days and then at –20°C in the laboratory.

Serum and urinary analyses

Serum PTH was determined using the Intact PTH Parathyroid Hormone 100T kit (Nichols Institute Diagnostics, San-Juan Capistrano, Calif., USA). Serum and urinary calcium were determined using calcium reagent in conjunction with

SYNCHRON CX (systems CX Multi from Beckman, Berea). Bone-specific alkaline phosphatase (BSAP) was determined using Tandem-R Ostase (Hybritech, San Diego, USA). Osteocalcin (OC) was determined using N-tact Osteo SP Osteocalcin IRMA kit (DiaSorin, Stillwater, Minnesota, USA). Cross-linked N-telopeptides from type 1 collagen (NTx) were determined using Osteomark (Ostex, Seattle, Wash., USA.).

Criteria for defining the strata

Stratum I. This stratum included rural people and farm workers. Usually, rural depicts pastoral or agricultural circumstances. In this study, rural blacks were defined as people who were living under the authority of a traditional tribal head (chief, captain or headman) in and around traditional African villages, mostly without running water and electricity. Farm workers worked on commercial farms, mostly owned by white farmers, and lived in brick houses clustered together on the different farms. Running water and electricity were available at some but not all farms.

Stratum II. This stratum included urban middle and upper class subjects. Urban middle class subjects were from established townships or locations found adjacent to all towns and cities in South Africa. The subjects lived in their own brick houses and most had running water and electricity. Urban upper class subjects were recruited from among professional people (nurses, teachers, doctors, government workers, politicians, etc.) and lived either in the established townships or in formerly 'white' residential areas in towns and cities.

Statistical analysis

Data are summarised and presented as means (standard error of the mean). The parameters from the dietary intake, anthropometry, serum analysis and the urinary analysis are all on the interval scale and were analysed using analysis of variance (ANOVA). Bartlett's test was used to confirm homogeneity of variance, and specific differences between the levels of a main effect of interest were established by means of pair-wise comparisons using least significance differences. Testing was done at the 0.05 level of significance.

Results

Table I depicts the serum calcium and magnesium levels of the subjects in both age groups in the two strata. All serum levels were within the normal range. All serum magnesium levels were within normal range and there were no significant differences between age groups in the strata.

Table II shows the information on dietary intakes for the two age groups across the strata. With urbanisation there is an increase in the intake of animal protein, from 29 g to 37 g per day in the 15 - 25-year age group. Compared with the RDA of 45 g total protein, it seems that the subjects' diets are protein-deficient, but the results only take animal protein into consideration. Plant protein constitutes up to 50% of the rural subject's diet, and with urbanisation the trend is towards a higher intake of animal protein. According to blood analyses for albumin (data not shown) the subjects are not protein-

Table I. Serum calcium and magnesium values as measured in the two age groups over the two strata (mean (SEM))

	Age group			
	15 - 25 years		55 - 65 years	
Stratum	Serum calcium (mmol/l)	Serum magnesium (mmol/l)	Serum calcium (mmol/l)	Serum magnesium (mmol/l)
1 (*N* = 20)	2.35 (0.017)	0.88 (0.014)	2.38 (0.035)	0.91 (0.038)
2 (*N* = 20)	2.31 (0.017)	0.87 (0.013)	2.34 (0.021)	0.87 (0.020)

Table II. Dietary intake of subjects according to age group and stratum (mean (SEM))

	Age group			
	15 - 25 years		55 - 65 years	
	Stratum 1 (*N* = 20)	Stratum 2 (*N* = 23)	Stratum 1 (*N* = 11)	Stratum 2 (*N* = 21)
Animal protein (g)	29.9 (2.48)	37.63 (3.56)*	28.35 (2.86)	25.39 (2.22)
Fibre (g)	12.57 (1.34)	15.79 (1.20)*	10.65 (1.21)	14.62 (1.13)*
Calcium (mg)	511.56 (61.69)	387.57 (41.28)*	544.2 (87.87)	392.4 (33.89)*
Magnesium (mg)	266.05 (18.69)	270.30 (18.03)	277.63 (18.56)	278.66 (22.0)
Calcium/protein	17.21 (1.58)	10.69 (0.85)*	17.67 (2.34)	17.50 (2.11)

*$p < 0.05$ between strata in age groups.

deficient and are therefore reasonably well nourished. The typical South African diet contains up to 65 g of total protein, therefore 30% higher than the RDA.[13]

Fibre intake in South Africa is recommended at 25 - 30 g. The subjects all therefore lack fibre in the diet, with values varying between 10 g/day and 15 g/day. In both strata fibre intake increased significantly with urbanisation. All subjects have a sufficient intake of magnesium compared with the RDA of 280 mg/day, except in stratum 1 in the 55 - 65-year age group. All subjects have a low calcium intake, less than 600 mg/day, with the subjects from stratum 2 taking in less than 400 mg/day. The calcium/protein ratio decreases from stratum 1 to 2 in the 15 - 25-year age group, indicating an increased intake of protein with urbanisation with no compensatory increase in intake of protein with urbanisation with no compensatory increase in intake of calcium.

The BMI for the 15 - 25-year age group is significantly lower than for the 55 - 65-year group in both strata, possibly due to age-induced weight gain (Table III). In both strata there is an increase in BMI with urbanisation, although this is not statistically significant.

OC is a marker of bone turnover or remodelling.[14,15] The levels are usually increased during growth, lower until onset of menopause, increased for about 5 years after menopause, and then at a lower stable level until old age. In the 15 - 25-year age group the OC levels for stratum 1 are significantly higher than for stratum 2, indicating a possible effect of urbanisation on bone turnover. In this age group higher levels are indicative of increased bone turnover and growth. It is therefore of concern that OC lowers so significantly towards stratum 2. This could indicate decreased bone growth which may manifest in later age as osteopenia.

BSAP is a marker for osteoblast function.[14,15] Levels through the strata are very similar but are higher in the younger age group, indicative of bone growth.

NTx is a marker for bone degradation.[14,15] As spot samples of urine were used, values were corrected for kidney function by expressing results per mmol creatinine. High levels of NTx are usually detected during growth, with a decrease as bone stabilises, and then an age- and menopause-induced increase. The highest levels of this marker are detected in the 15 - 25-year age group (both strata) and in stratum 2 (55 - 65 years), which will correlate with bone development and high turnover in the young age group and with bone loss in the elderly. The latter observation could also be due to an effect of urbanisation. Table III summarises the values for PTH for both strata and ages. There is a tendency for PTH to increase with age. Stratum 2, age 55 - 65 years, has the highest value. This could be a significant effect of urbanisation in this age group. Alternatively, it confirms the observations of Kleerekoper *et al.*[2] regarding skeletal resistance to PTH in black menopausal women.

Table III also shows the values for urinary calcium excretion. As this was a spot sample and not a 24-hour sample, no definite conclusion can be drawn using these values. Urinary calcium excretion increases with urbanisation in both age groups.

Some correlations between markers of bone turnover were observed across both strata and age groups. Significant correlations between OC and BSAP ($r = 0.298$, $p < 0.02$) and between NTx and BSAP ($r = 0.36$, $p < 0.01$) were observed. BMI was also correlated with OC ($r = -0.27$, $p < 0.03$) indicating that an increase in body mass is associated with a reduction in bone turnover and is therefore bone protective. Fibre was positively correlated with urinary calcium indicating an increased loss of calcium with high fibre intake ($r = 0.34$, $p < 0.006$). Dietary calcium was also strongly correlated with calcium/protein ratio ($r = 0.61$, $p < 0.001$), which is a positive sign in that as dietary protein increases dietary calcium also increases, which is bone protective.

Table IV shows correlations for age 15 - 25 years, strata 1 and 2, showing changes in correlations with urbanisation. In stratum 1 BSAP is correlated with OC ($r = 0.521$, $p < 0.05$) and NTx ($r = 0.670$, $p < 0.05$), suggesting bone growth and increased turnover in young adults. Urinary calcium is correlated with fibre ($r = 0.55$, $p < 0.05$), magnesium ($r = 0.523$, $p < 0.05$) and

Table III. Markers of bone formation./degradation and hormones/factors influencing bone turnover presented in different age groups over strata (mean (SEM))

	Age group			
	15 - 25 years		55 - 65 years	
	Stratum 1 ($N = 17$)	Stratum 2 ($N = 22$)	Stratum 1 ($N = 12$)	Stratum 2 ($N = 18$)
Osteocalcin (ng/ml)	14.07 (1.42)	5.42 (0.83)*	7.89 (1.30)	5.75 (0.71)
BSAP (ug/l)	16.71 (1.41)	14.55 (1.22)	12.83 (1.25)	13.56 (1.04)*
NTx (nM BCE/mM creatinine)	67.35 (6.71)	62.50 (11.36)	51.87 (4.43)	67.02 (10.06)*
PTH (ng/ml)	22.19 (2.29)	17.22 (2.86)	28.65 (3.97)	37.87 (3.66)*
Urinary calcium (mmol/l)	0.64 (0.22)	0.81 (0.24)	0.71 (0.18)	0.82 (0.16)
BMI	22.20 (0.94)	24.20 (0.99)	27.30 (2.09)	30.08 (2.07)

*$p < 0.05$ between strata in age groups.

Table IV. Correlations between parameters of bone turnover and dietary intake for women aged 15 - 25 years in stratum 1 (*N* = 17) and 2 (*N* = 22)

	Stratum 1			Stratum 2		
Parameter	BSAP	NTx	Urinary calcium	BSAP	NTx	Urinary calcium
Osteocalcin (ng/ml)	0.521*	0.510*	0.216	–0.104	0.059	–0.256
NTx (nM BCE/mM creatinine)	0.670*		0.543	0.435		0.285
Protein (g)	0.0325	0.076	–0.014	–0.234	–0.247	0.613*
Fibre (g)	–0.069	0.099	0.550*	–0.422*	0.057	0.575*
Calcium (mg)	0.218	0.345	0.330	–0.148	0.024	0.448*
Magnesium (mg)	–0.226	–0.075	0.523*	–0.358	0.022	0.436*
BMI	–0.361	–0.289	–0.167	0.191	–0.471*	–0.099
Calcium/protein	0.204	0.449	0.474*	0.148	0.411*	0.064

*$p < 0.05$.

BSAP = bone-specific alkaline phosphatase; NTx = N-telopeptides from type 1 collagen; BMI = body mass index.

calcium/protein ratio ($r = 0.474$, $p < 0.05$), showing a strong correlation between diet and urinary calcium excretion. The same age group in stratum 2 loses the correlation between OC, BSAP and NTx, a possible indication of changes in bone parameters with urbanisation. Interestingly, most of the dietary parameters were positively correlated with urinary calcium, indicating that changes in diet accompanying urbanisation could influence calcium retention in the body.

Discussion

During the last two decades osteoporosis has evolved from a relatively rare condition to one with a high incidence in urbanised areas. In the past there was a definite geographical pattern to osteoporosis and fractures. Age-adjusted figures, for example, were 402/100 000 for hip fractures in the USA (1984) versus 31/100 000 in the black population of South Africa.[7] With urbanisation the incidence of hip fractures has increased dramatically in several Asian countries.[16]

There is evidence to suggest that a high protein intake is associated with a negative calcium balance. Phosphate is associated with protein in the diet which increases the synthesis of PTH by depressing serum calcium levels.[17,18] PTH increases calcium reabsorption in the kidneys. An increase in protein intake is shown in Table II where the intake of animal protein increased significantly in 15 - 25-year age group. Increase in protein intake can aggravate calcium deficiency, as high protein intake can compromise calcium absorption and increase calcium excretion. In Table III, urinary calcium increases together with an increase in protein intake. There is evidence of a strong correlation between animal protein intake and incidence of hip fractures ($r = 0.9$). Various trials also support the statement that level of protein intake is correlated with calciuria.[17] The dietary protein-phosphorus relationship is critical to the maintenance of calcium homeostasis on a high-protein diet. This relationship is supported by the correlation between urinary calcium and dietary protein shown in Table IV.

Dietary calcium has a particularly significant effect which may be modified by factors such as physical activity, protein intake and sodium.[17,18] Calcium is a threshold supplement, set at about 500 - 600 mg/day, with the mean dietary intake in the black South African population lower than this. Vegetables may account for some calcium intake while dairy product intake in black South Africans is very low. Calcium intake in our subjects was very low, between 200 mg/day and 500 mg/day. Such low intake may lead to calcium deficiency unless these subjects are hyperabsorptive.[2] With urbanisation, there was a decrease in calcium intake between strata 1 and 2 and together with other changes in the diet such as an increase in animal protein due to urbanisation, the calcium status may be compromised.

Calculation of the calcium/protein ratio could be useful in evaluating urinary calcium excretion.[19] A ratio of 20:1 (mg calcium/g protein) is recommended to ensure optimal calcium balance as well as protein intake. Woo *et al*.[20] found an increase in animal protein intake without a concurrent increase in dietary calcium with urbanisation. This trend is also seen in both groups in this study. Therefore a negative effect on calcium balance and bone turnover can be expected.[21] In stratum 1, subjects aged 15 - 25 years, a high calcium protein ratio is correlated with urinary calcium. In stratum 2 a high calcium/protein ratio is correlated with NTx, indicating an increase in bone loss with change in the ratio (Table IV).

Studies of bone turnover in African-Americans have reported levels lower, the same or higher than those in white women.[1,7,8] Han *et al*.[22] reported lower serum OC in black subjects, similar BSAP levels, low 25-(OH)D and increased 1,25-(OH)2D3 as well as PTH levels. Kleerekoper *et al*.[2] designed a study looking at bone markers as well as bone density across different ethnic groups in the USA in postmenopausal women and his data showed OC and BSAP levels to be similar between black and white subjects. He also detected slightly higher PTH levels in black women and higher 25-(OH) vitamin D levels. Luckey *et al*.[23] reported similar rates of premenopausal bone loss in both white and black groups of patients. However, in early menopause the loss was faster in the white women. More than

5 years post menopause, rates did not differ. Higher bone mass in black patients was ascribed to a higher peak bone mass in early adulthood and slower rates of loss with menopause.

OC levels in stratum 1 subjects aged 15 - 25 years suggest high bone turnover associated with growth, which would mean similar rates of bone formation and resorption. With urbanisation, stratum 2 levels drop, indicating a decrease in turnover. This could mean lower rate of bone accretion in these girls. A single marker cannot be conclusive but it does suggest that the change in lifestyle with urbanisation may suppress bone turnover. This may lead to accretion of a lower peak bone mass in this age group, with subsequent lower bone density at a later stage of life. Kleerekoper *et al*.[2] reported similar or lower levels for BSAP in his group of black patients, while Han *et al*.[22] reported similar levels in his groups. Our results show similar levels for BSAP over strata in each age group.

Measurement of NTx in the urine gives information on collagen degradation. Normal values for a premenopausal woman (age 35 years) is 35 nM BCE/mM creatinine, with a range of 5 - 65. Subjects aged 55 - 65 years in stratum 2 had the highest levels. As OC decreases, NTx increases. This may be an indication of uncoupling of bone formation and resorption possibly resulting in increased loss. In the 15 - 25-year age group, with the significant reduction in OC, a similar significant decrease in NTx levels could be expected which would maintain bone, although more slowly. But as OC decreases NTx excretion remains the same. These young women are therefore into a phase of higher resorption and less formation which may result in bone loss. The peak bone mass to be accrued at this age (late adolescence), could therefore be compromised. In the 55 - 65-year age group, OC decreases with a significant increase in NTx levels, therefore higher resorption can be expected in this age group where bone formation and degradation are uncoupled. A negative correlation is shown between NTx and BMI and a positive correlation with calcium/protein ratio (Table IV). An increase in BMI with urbanisation has been shown in both age groups, although not significantly. BMI could be bone protective; this possibility is confirmed with the measured negative correlation with NTx, therefore slower resorption at higher BMI.

In summary, our results show an effect of decrease in bone formation with urbanisation, a decrease in turnover and an increase in degradation especially in the young age group. A relationship between the bone markers, hormone differences and bone mass was shown in a group of early and late menopausal black South African women, where bone turnover was shown to change with urbanisation.[24] Bone density as measured with ultrasound was shown to decrease towards osteopenia with urbanisation.[24]

Most of the differences between black and white women seem to originate during skeletal growth and development resulting in higher peak bone mass in black girls.[8] The obvious change in diet with urbanisation found in this study may compromise the attainment of a high peak bone mass in young black women. Changes in the diet compromising calcium status increase the possibility of the development of a calcium-deficient black population. With urbanisation a reduction in load-bearing activity also takes place, as hard physical labour becomes much less necessary in everyday life. Eventually the effect of a low calcium intake combined with less exercise and a diet high in animal protein and fibre may manifest as low bone mass. Body weight is protective against bone loss,[6] and an increase in BMI was evident across the strata in all age groups. Therefore, body mass is one factor which could counteract the effect of dietary changes and low calcium intake on the skeleton emerging with urbanisation.

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