0013-7227/03/$15.00/0
*Printed in U.S.A.*

The Journal of Clinical Endocrinology & Metabolism 88(3):1043–1047

doi: 10.1210/jc.2002-021367

# Racial Differences in Bone Turnover and Calcium Metabolism in Adolescent Females

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**Blacks develop a higher peak bone mass than whites which is associated with a reduced risk for bone fracture. The physiological basis for the difference in bone mass was investigated by metabolic balance and calcium kinetic studies in adolescent black and white girls. The hypothesis that the greater peak bone mass in blacks compared with whites is due to suppressed bone resorption was tested. Subjects were housed in a supervised environment for 3 wk during which time they consumed a controlled diet and collected all excreta. Subjects were given stable calcium isotopes orally and intravenously after 1 wk adaptation. Blacks have greater calcium retention (mean ± SD, 11.5 ± 6.1 *vs.* 7.3 ± 4.1 mmol/d, $P < 0.05$) consistent with greater bone formation rates (49.4 ± 13.5 *vs.* 36.5 ± 13.6 mmol/d, $P < 0.05$) relative to bone resorption rates (37.4 ± 13.2 *vs.* 29.4 ± 10.9 mmol/d, $P = 0.07$), increased calcium absorption efficiency (54 ± 19 *vs.* 38 ± 18%, $P < 0.05$) and decreased urinary calcium (1.15 ± 0.95 *vs.* 2.50 ± 1.35 mmol/d, $P < 0.001$), compared with whites. The racial differences in calcium retention in adolescence can account for the racial differences in bone mass of adults. (*J Clin Endocrinol Metab* 88: 1043–1047, 2003)**

AMERICAN BLACKS HAVE higher bone mass than whites (1, 2). In children, although areal bone mineral density was higher in black than white girls at several sites (3, 4), vertebral volumetric bone density did not differ in prepubertal black and white girls (5). A seeming paradox exists when trying to understand the mechanism responsible for racial differences in peak bone mass. Blacks have been reported to have lower levels of biochemical markers of bone formation and bone resorption, compared with whites, in many studies in both children (6, 7) and adults (1, 8–11) but not all (12). This has led to the hypothesis that peak bone mass is greater and bone loss is lower in blacks than whites because of suppressed bone turnover in blacks, especially bone resorption. Some histomorphometric studies in adults support the lower biochemical marker data, whereas others do not. In one study (13), static measures of bone turnover (osteoid volume, osteoid surface, osteoid thickness, and eroded surface) were greater in blacks than whites (13); whereas, in other studies, investigators found either no racial differences in either static or dynamic (activation frequency) measures of bone turnover in premenopausal women or lower bone turnover in black than white adults (14–16). Furthermore, blacks seem to be vitamin-D-insufficient, as assessed by low serum 25-hydroxyvitamin D levels and high serum 1,25-dihydroxyvitamin D and PTH levels (1, 8, 9). In this state, a high rate of bone turnover would be expected.

Kinetic studies concurrent with metabolic studies are ideal for measuring rates of bone formation and resorption; however, this information is incomplete from the literature. Short-term studies, with and without the use of tracers, have reported reduced urinary calcium levels but no difference in calcium absorption in black, compared with white, adults (8, 10) and children (7). Abrams *et al.* (17) reported similar findings in premenarcheal girls but, in contrast, found significantly greater rates of calcium absorption and reduced rates of urinary calcium in postmenarcheal black, compared with white, girls of age 4.9–16.7 yr. However, subjects were not matched for age, postmenarcheal age, or calcium load, which may influence calcium metabolism. Although stable isotopic tracers were used in the Abrams study, to determine that bone formation rates were significantly higher in blacks than whites, none of the previous kinetic studies determined bone resorption rates. This requires calcium kinetic analysis under steady state conditions, *i.e.* a controlled diet.

The differences in peak bone mass are largely determined at the time of adolescent bone growth. To test the hypothesis that blacks have higher peak bone mass than whites because of lower bone turnover rates, we conducted a 14-d metabolic balance study (after adaptation to the diet) and measured calcium kinetics and biochemical markers of bone turnover in black and white adolescent girls matched for weight and sexual maturity during puberty, a stage of accelerated growth.

## Subjects and Methods

### *Subjects and design*

Healthy black adolescent girls were recruited, to compare with white girls previously studied for calcium metabolism under the same experimental design in the same summer months (18). Exclusion criteria included dietary calcium intakes of less than 16 mmol/d to exclude milk avoiders, less than 10th or greater than 90th percentile weight for height for age (19), medical conditions or medications affecting calcium me-

Abbreviations: DpD, Deoxypyridinoline; NTx, N-teleopeptides of type I collagen; SAAM program, Simulation, Analysis and Modeling program.

tabolism, fewer than three black grandparents, pregnancy, eating disorder, or tobacco use. Health was determined by questionnaire and physical examination. Prestudy nutrient intakes were determined by 3-d diet records, which were analyzed by Nutritionist IV Diet Analysis (First Databook Division, CA 1995). Menarcheal age was determined by questionnaire and by telephone follow-up in those girls who had not reached menarche. Height was determined by a wall stadiometer, and weight was determined by a calibrated electronic scale. Total body bone mineral density and content were determined by dual-energy x-ray absorptiometry on a Lunar Corp. (Madison, WI) IQ using adult software. Precision of total body bone mineral content was 2%.

Of the 21 black girls who started the study, 13 volunteered and completed the kinetic study, and 18 subjects completed the balance study. Three girls left the study because of homesickness. Because of the expense of the stable isotopes, only 13 subjects were given the oral dose, and 12 subjects were given both an oral and iv dose. Data were excluded from subjects if there was evidence of noncompliance. Balance was calculated in 14 subjects, calcium absorption in 13 subjects, and complete kinetics were determined in 10 subjects. All subjects were studied under protocols signed by themselves and their guardians and approved by Purdue University and Indiana University School of Medicine Institutional Review Boards.

The study simulated a summer camp environment, in a sorority house that was converted into a metabolic facility at Purdue University. Polyethylene glycol (PEG E3350; Dow Corning Corp., Midland, MI) was used to determine completeness of fecal collections, as previously described (18). A consistent calcium:PEG fecal ratio, after 1 wk, supported the assumption that equilibrium had been established. Twenty-four-hour urine and feces were collected in acid-washed containers. Urinary creatinine, over 14 d, was used to normalize the daily urine calcium to 24-h periods, in the event that timing of collections was imprecise.

The first 7 d of the study served as a period of equilibration, and the last 14 d served as the experimental period. The diet consisted of a 4-d cycle menu that was controlled for calcium [28.2 ± 1.9 mmol/d (1129 ± 74 mg/d)], protein (69.9 ± 28.8 g/d), fat (87.9 ± 9.1 g/d), and magnesium (7.46 ± 1.9 mmol/d). All food and beverages were prepared with deionized water and weighed to the nearest 0.1 g. Subjects were asked to consume all food, including deionized water rinsing of high calcium foods. Discretionary salt, sodium chloride, was not allowed. A composite of each day's meals and snacks was frozen in acid-washed containers for later analysis. Subjects were weighed each morning, on rising, wearing light clothing and no shoes. Body weights were maintained (with beverages) within 2 kg from baseline. Subjects were asked to consume up to two cans of 7-Up and flavored deionized water (regular or diet) to control weight fluctuations. These beverages contained negligible minerals. Calcium retention was determined as dietary intake minus urine and fecal excreta.

A double stable calcium isotope kinetic study was performed during the last 2 wk of the experimental period. Subjects were admitted to the General Clinical Research Center for administration of the stable isotope and subsequent collections from a catheter for the first 6 h post administration. After an overnight fast, venous catheters were placed in both arms, on waking at 0600 h, and fasting blood and urine were collected. An oral dose of 0.9 mmol $^{44}Ca$ was administered, accompanied by a breakfast containing approximately 6.25 mmol calcium. One hour after the oral dose, an iv dose of 0.95 mmol $^{42}Ca$ was administered iv. Blood was taken at 10, 20, 30, 60, 90, 120, 150, and 180 min; 4, 5, 6, 11, and 23 h; and 3, 5, 7, 9, 11, and 13 d after administration of the iv dose.

Calcium kinetic data from serum, urine, and feces were analyzed using the WinSAAM (Windows version of Simulation, Analysis and Modeling) program and a three-compartmental model, as previously described for the white girls (20). Assumptions in fitting the data were that kinetics were identical for oral and iv tracers and that subjects were in steady state during the 14-d kinetic study after administration of the tracer.

### *Analysis*

Twenty-four-hour urines were measured for creatinine, on unacidified aliquots, using a chemical analyzer (Cobas Mira, Roche Diagnostic Systems, Inc., Branchburg, NJ). The remaining urine sample was acidified with 1% (by vol) concentrated HCl. Fecal samples were diluted with deionized water and concentrated HCl and were homogenized with a stomacher (Tekmar Co., Cincinnati, OH). Aliquots were frozen at −10 C for future analysis. A turbidimetric assay was used for measurement of PEG in fecal homogenate (21, 22).

For mineral analysis, aliquots of homogenized food and fecal samples were analyzed in triplicate. Aliquots were lyophilized (Dura-Dry Freeze Dryer, Model PAC-TC-V4; FTS Systems, Inc., Stone Ridge, NY) and ashed in a muffle furnace at 600 C for at least 48 h, and ashed samples or acidified urine samples were diluted with 0.5 mol HCl/liter containing 0.5% lanthanum as lanthanum chloride. Total calcium was measured in urine, feces, and food by atomic absorption spectroscopy (5100 PC; Perkin-Elmer, Inc., Shelton, CT). Calcium from wheat flour (National Bureau of Standards Wheat Flour 1567) averaged 266 ± 3.7 ppm (coefficient of variation, 2.25%), compared with the certified value of 278 ± 36 ppm. Calcium stable isotope ratios were determined by fast atom-bombardment mass spectrometry (23). Dietary composites were also analyzed for protein by Kjeldahl analysis, and fat by Soxhlet extraction (24).

At the end of the metabolic balance period, after an 8-h overnight fast, blood was drawn, and the second morning urine void was collected. Blood was left to clot for 30 min, serum was removed and stored at −70 C, and urine was stored at −40 C. Serum was analyzed for PTH 1–84 by a two-site immunoassay (Nichols Institute Diagnostics, San Clemente, CA). The interassay CV was 9% at 25 mg/liter. Also, 25-hydroxyvitamin D and 1,25 dihydroxyvitamin D were analyzed by protein binding assays after purification by HPLC. Total serum alkaline phosphatase was determined enzymatically by standard techniques, using paranitrophenol as substrate. Serum pyridinoline was analyzed by enzyme immunoassay, using kits from Quidel Corp. (San Diego, CA). The interassay variation was 8.7%. Serum tartrate resistant acid phosphatase was measured enzymatically with paranitrophenolphosphate as substrate. Serum was incubated for 1 h at 36 C, before assay, to destroy inhibitors. Urine cross-linked N-teleopeptides of type I collagen (NTx) were measured by an enzyme-linked immunoabsorbant assay (Osteomark; Ostex International, Inc., Seattle, WA). Urinary free deoxypyridinoline (fDpD) was measured by ELISA kits from Quidel Corp. The interassay variation was 8% at 98 nM. Values for biochemistries were measured during the year of the study, except for serum pyridinoline, which was measured in both groups simultaneously in 2002. All assays were identical for both studies.

### *Statistical analysis*

Student's *t* tests, assuming unequal variances, were performed to compare group means between blacks and the white girls previously studied (18). For kinetic parameters, population values were determined using the multiple-studies feature of the SAAM program (25).

## Results

Subject characteristics are given in Table 1. Black girls were younger than white girls, to achieve similar postmenarcheal age. They were also similar in height, weight, and body mass index. The black girls had a higher total body bone mineral content and density than whites. An evaluation of prestudy nutrient intakes showed that macronutrient composition was similar between groups, but black girls were consuming significantly less calcium than white girls.

Black girls had higher serum 1, 25-dihydroxyvitamin D and lower serum calcium levels than white girls. Differences in biochemical markers of bone turnover because of race did not achieve significance.

Net calcium retention by balance was significantly ($P < 0.05$) higher for blacks than for white girls, as shown in Table 2. Although designed to use the same dietary intake of calcium in both studies, by analysis, calcium intake for the black balance study averaged approximately 5 mmol/d less. However, after adjusting urinary and fecal outcomes in whites to an intake of 28.2 mmol/d (the study intake of blacks), using the regression model designed by Jackman *et al.* (26), signif-

**TABLE 1.** Physical and biochemical characteristics of black and white adolescent girls (mean ± SD)

| | Blacks (n = 14) | Whites (n = 14) |
|---|---|---|
| Chronological age (yr) | 12.8 ± 1.2 | 13.7 ± 0.9[a] |
| Postmenarcheal age (months)[b] | 9.9 ± 20.8 | 8.6 ± 15 |
| Height (cm) | 160 ± 7 | 159 ± 5 |
| Weight (kg) | 57 ± 11 | 54 ± 10 |
| Body mass index (kg/m$^2$) | 22.1 ± 3.9 | 21.4 ± 3.7 |
| Total body bone mineral density (g/cm$^2$) | 1.13 ± 0.09 | 1.05 ± 0.06[c] |
| Total body bone mineral content (g) | 2418 ± 459 | 2100 ± 327[a] |
| Total body bone calcium (g) | 918 ± 174 | 798 ± 125[a] |
| Prestudy intakes | | |
| Energy (KJ) | 8111 ± 1602 | 8270 ± 1628 |
| Calcium (mmol) | 18.2 ± 6.6 | 25.3 ± 18.4[a] |
| Protein (g) | 71 ± 21 | 70 ± 15 |
| Fat (g) | 75 ± 22 | 78 ± 21 |
| Phosphorus (mg) | 1158 ± 400 | 1204 ± 380 |
| Fiber (g) | 9 ± 3 | 8 ± 5 |
| Serum PTH (pg/ml) | 32.6 ± 17.5 | 29.9 ± 7.4 |
| Serum 1,25-dihydroxyvitamin D (pM) | 119.8 ± 36.4 | 93.6 ± 18.5[a] |
| Serum 25-hydroxyvitamin D (nM) | 69.1 ± 23.5 | 66.2 ± 20.4 |
| Serum calcium (mM) | 2.48 ± 0.08 | 2.65 ± 0.18[c] |
| Serum total alkaline phosphatase (IU/liter) | 257 ± 110 | 204 ± 77 |
| Serum pyridinoline (nM) | 2.5 ± 0.6 | 2.8 ± 0.5 |
| Serum tartrate-resistant acid phosphatase (IU/liter) | 19.7 ± 4.9 | 17.8 ± 6.7 |
| Urinary free Dpd:Cr (mmol/mmol) | 13.5 ± 4.7 | 14.0 ± 4.8 |
| Urinary NTx:Cr (nM BCE/mM Cr) | 279 ± 172 | 344 ± 139 |

Cr, Creatinine; BCE, bone collagen equivalents. Means are significantly different, [a], $P < 0.05$; [c], $P < 0.01$.
[b] Five girls in each group were premenarcheal at the time of the study but were queried about onset of menarche later.

**TABLE 2.** Calcium metabolism and bone turnover in black and white girls (mean ± SD)[a]

| | Blacks | Whites |
|---|---|---|
| Balance study | | |
| Dietary Ca (mmol/d) | 28.2 ± 1.9 | 33.2 ± 2.5 |
| Urinary Ca (mmol/d) | 0.93 ± 0.76 | 2.65 ± 1.1[b] |
| Fecal Ca (mmol/d) | 16.5 ± 5.3 | 22.5 ± 2.9[b] |
| Net Ca retention (mmol/d) | 11.5 ± 6.1 | 7.3 ± 4.1[b] |
| Kinetic study | | |
| Absorption (%) | 54 ± 19 | 38 ± 18[b] |
| Rate of absorption (mmol/d) | 15.9 ± 4.7 | 12.4 ± 5.8 |
| Urinary excretion (mmol/d) | 1.15 ± 0.95 | 2.50 ± 1.35[c] |
| Endogenous excretion (mmol/d) | 2.73 ± 1.25 | 2.80 ± 0.88 |
| Fecal excretion (mmol/d) | 17.0 ± 4.33 | 23.0 ± 6.33[c] |
| Bone deposition (mmol/d) | 49.4 ± 13.5 | 36.5 ± 13.6[b] |
| Bone resorption (mmol/d) | 37.4 ± 13.2 | 29.4 ± 10.9 |
| Bone balance (mmol/d) | 12.1 ± 4.5 | 7.03 ± 6.9[b] |

Means are significantly different at [b] $P < 0.05$; [c] $P < 0.001$.
[a] n = 14 for both groups, except n = 13 for absorption and n = 10 for other kinetic measures in black girls.

icant differences remained in total balance and in urinary and fecal excretion of calcium.

Calcium balance and kinetic data are shown in Table 2. Calcium absorption efficiency was significantly greater for black than white girls. The total amount of absorbed calcium tended to be higher in blacks ($P = 0.06$). Urinary calcium excretion in black girls was 46% of that of white girls. Fecal calcium loss in black girls was 26% lower than that of white girls, reflecting the 70% higher absorption efficiency in black girls. Endogenous fecal excretion was not different between the races. Blacks retained approximately 70% of absorbed calcium, whereas whites retained approximately 60% of absorbed calcium. Bone resorption rates tended ($P = 0.07$) to be greater in black girls, but bone formation rates were significantly higher, by 35%, in black than white girls. Net bone turnover or retention (bone formation rates minus bone resorption rates) was significantly higher in blacks than in whites.

Racial differences in calcium retention can explain racial differences in adult bone mineral content. To do this, however, we need to take into account the relationship between calcium retention and postmenarcheal age. Using data from white women with postmenarcheal ages from –2 to 19 yr, the following nonlinear regression model was estimated (18): Daily calcium retention = $\beta_0 \times e$ ($\beta_1 \times$ postmenarcheal age). Here, $\beta_0$ is the calcium retention corresponding to menarche, $e$ is the base for natural logarithms, and $\beta_1$ describes the rate at which calcium retention decreases with postmenarcheal age.

To estimate the parameters of this model for black women, we first estimated retention corresponding to the prestudy calcium intakes in Table 1, using the regression model given by Jackman *et al.* (26). Analysis indicated that the parameter $\beta_0$ depended on race, but $\beta_1$ did not. These curves are plotted in Fig. 1. An estimate of the cumulative difference in retained calcium from menarche to adulthood is given by the area between the two curves. Converted to bone mass, the difference is approximately 12%.

## Discussion

Racial differences were found in several measures that control whole-body calcium metabolism during the period of rapid growth. Calcium absorption efficiency was increased. This is consistent with observations by some in children, but not by others, depending on maturational age (6, 17); and the differences are similar to those observed in adults (8). Urinary calcium losses in blacks, compared with white girls, were half as much, a finding consistently reported by others in children (9, 27, 28) and by some studies in adults (6, 29)

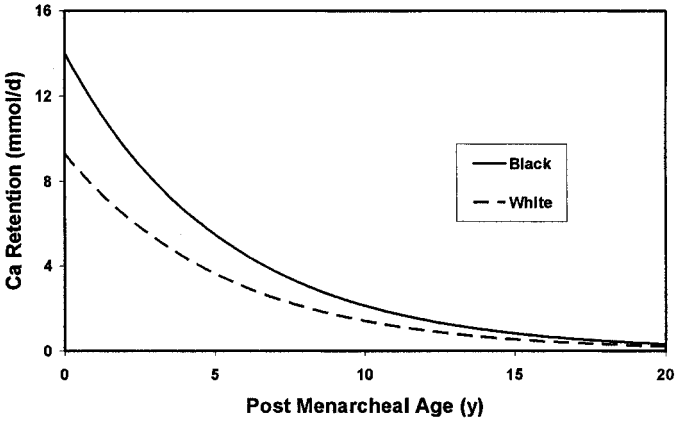


FIG. 1. Model fit for calcium retention, as a function of postmenarcheal age, in black and white females. *Solid line*, Black females; *dashed line*, white females. The cumulative racial difference in bone mass, based on calcium accretion from onset of menarche to 20 yr post menarche, is predicted to be 12%.

but not by others (8). Metabolic calcium balance studies have not been previously reported in blacks; so, the observation that fecal calcium excretion is lower in blacks, compared with whites, but endogenous fecal calcium losses are similar, is original. Collectively, these differences led to 57% greater calcium retention in black than in white girls during this period of peak calcium accretion. This efficient use of calcium occurred even though 11 of the 17 black girls who gave consent tested positive as lactose maldigesters, by hydrogen breath analysis, as reported earlier (30). The high level of calcium intake in this study was achieved through dairy products, primarily milk, and was well tolerated even in the girls who were lactose maldigesters.

Blacks have substantially higher bone mineral density than whites, and we observed this difference even in our small sample of adolescent girls. Racial differences in bone mineral density ranged from 12–18% at various sites, as determined by bone densitometry, in 362 postmenopausal women; and racial differences in vertebral quantitative computed tomography, a volumetric measure of bone density, was even higher at 40% (1). In a broader representation of the American population, age-adjusted femoral neck bone mineral content and density of blacks (n = 2129) was 10% and 13% higher, respectively, than whites (n = 3251), in adult women, 20 yr and older, participating in the NHANES III, 1988–94 survey (2). Although genetic differences in body size and muscle mass index partially account for racial differences in bone, adjusting for body mass index removes only about one-fifth of the difference in bone mass (31). In our study, girls were not different in the measured anthropometric measures, but we did not measure sitting height or leg length. Groups were also not different in sexual maturity, another potential confounder of racial differences in bone or calcium accretion. We have previously reported the rapid decline in calcium retention with postmenarcheal age in white females (18), which parallels the rapid decline in bone accrual rate after the peak, which occurs (on average) at age 11.8 in white girls (32). We applied the method used previously for white females (18) to describe the relationship of calcium retention and postmenarcheal age to both races, and we projected the bone mass differences that would be observed at 20 yr postmenarcheal age (Fig. 1). Racial differences in adult bone mass predicted in this manner would be approximately 12%. This is consistent with observed racial differences in bone mass (1, 30). It is apparent, from this figure, that much of adult differences in bone mass develops during adolescence.

Rates of bone turnover were 35% greater in black than white girls. This observation extends the estimation of bone formation rates based on short-term collections by Abrams *et al.* (17). Their estimates for postmenarcheal girls (35.8 mmol/d for white girls and 49 mmol/d for black girls) were very similar to values in Table 2, despite a wide range of calcium intakes. In the metabolic balance study combined with kinetic analysis reported here, bone resorption rates could also be determined. They were also higher in blacks, but the difference, *i.e.* bone balance, was significantly more positive in blacks. Black girls had higher serum 1,25-dihydroxyvitamin D than did white girls, consistent with lower serum calcium, increased calcium absorption, and increased bone resorption rates. This is different from the apparent skeletal resistance to the resorptive actions of PTH in black women of age 25–40 yr (29). Furthermore, it removes the seeming paradox, at least in adolescents, between insufficient vitamin D status, elevated PTH, and suppressed bone remodeling rates based on biochemical markers of bone turnover cited in the literature (1, 6–11). In later life, suppression of bone resorption is associated with reduced bone loss; but in adolescence, it can mean greater bone gains, if bone formation is unchanged or increased, as in this study.

Biochemical markers of bone turnover are used as indirect measures for bone formation (total serum alkaline phosphatase) and resorption (pyridinoline, free DpD, tartrate resistant acid phosphatase, NTx) (33). Although Henry and Eastell (12) determined there were no ethnic differences in biochemical markers of bone turnover in 20- to 37-yr-old white and African-Caribbean men and women, others have reported lower levels of the markers in blacks than whites in both pre- and postmenopausal women (1, 9–11) and children (6, 7), suggesting that bone resorption was suppressed in blacks. In this study, biochemical markers of bone turnover did not support suppressed bone turnover in black (compared with white) adolescents. Whether this can be attributed to the small sample size, coupled with high variability of biochemical markers of bone turnover and their indirect nature, is uncertain. Nevertheless, the more direct measures of bone turnover, by calcium kinetic analysis, demonstrated higher bone turnover, especially higher bone formation rates, in black than white adolescents.

A limitation of this study is the necessarily small sample size, because of the expense of the kinetics studies, which limits generalizability to the larger population. Also a limitation is the separation in time of studies in whites and black girls. In a recent study, we have confirmed that black adolescent girls retained 6 mmol/d more calcium than white girls studied simultaneously on calcium intakes of 20 mmol/d (34).

Racial differences in calcium metabolism showed that black adolescents absorb and retain calcium more efficiently than whites. Bone turnover is greater in blacks, favoring net

bone accretion. These differences may account for bone mass differences observed in adulthood. Future research will undoubtedly elucidate the genetic factors that program these differences.

## Acknowledgments

Received August 28, 2002. Accepted November 25, 2002.

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This work was supported by U.S. Public Health Service Grants R01-AR-40553 and M07-RR-00750.

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