Evidence for Dramatically Increased Bone Turnover in Spontaneously Hypertensive Rats

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Using the ^{3}H-tetracycline model, whole-body skeletal bone resorption was compared among male and female spontaneously hypertensive (SHR) rats and normotensive Wistar Kyoto (WKy) and Sprague-Dawley (SD) rats. Immature animals undergoing rapid skeletal growth and bone sculpting showed a tendency for decreased indices of skeletal resorption in females compared with males. By 24 weeks of age, the indices of the rate of resorption and extent of metabolically reactive bone in male rats were decreased a mean of 68% and 74%, respectively, compared with values obtained at 8 weeks. By comparison, values for 24-week-old females decreased only 26% and 56%, respectively, evidence for a significantly elevated level of resorptive activity in mature females compared with males in each of the 3 rat strains. Within-sex comparisons of 24-week-old animals indicated that bone resorptive activity was similar between normotensive male and normotensive female groups. By comparison, the resorptive activity was significantly increased in both male and female hypertensive rats compared with normotensive controls. This condition was exaggerated in female hypertensive rats, which showed an approximate 81% and 44% increase in the indices of rate of resorption and extent of metabolically reactive bone compared with normotensive WKy controls. The results indicate a marked sexual dichotomy in the decline of skeletal bone resorptive activity following maturation and slowing of skeletal growth. They further indicate a significant elevation of whole skeleton bone turnover in male SHR rats and dramatically increased bone turnover in female SHR rats.

THERE IS SUBSTANTIAL indirect evidence suggesting a possible link between hypertension and osteoporotic disease. High levels of parathyroid hormone (PTH) are found in osteoporotic[1] and hypertensive[2] patients. Patients with osteoporosis often remain in a hyperparathyroid state,[3,4] and it is reported that a large portion of individuals with osteoporosis have blood pressure that is higher than normal.[5,6] Patients with essential hypertension, as well as animal models of hypertension (spontaneously hypertensive [SHR] rats), show decreased plasma ionic calcium, increased PTH, and increased urinary excretion of calcium.[2,7-9] Blood pressure in both humans and SHR rats is reduced by parathyroidectomy, whereas increased dietary calcium has been shown both to delay the onset and to reduce the severity of hypertension.[10-12] Hence, it may be reasonable to speculate that bone metabolism is altered in hypertension where PTH is elevated and calcium metabolism is abnormal.

The experimental evaluation of bone metabolism in hypertension is limited. Prior studies indicated that bone calcium content[13,14] was not different between SHR and normotensive animals, and it has been shown more recently that the development of skeletal mass is normal or enhanced in the hypertensive rat.[15] However, other studies found evidence of decreased cortical bone mass and an increased number of osteoclasts in the SHR rat.[16,17] More recently, evidence for decreased trabecular bone volume and increased bone turnover has been reported for the SHR rat,[18,19] suggesting moderate bone loss in older SHR animals.

In light of the conflicting evidence for potential abnormalities in the bone metabolism of hypertensive animals, the present study was undertaken to compare bone resorption among SHR rats and 2 normotensive rat strains. The ^{3}H-tetracycline model was used in these studies to provide a measure of whole-body bone resorptive activity, as compared with prior studies using selected individual bone measurements. The results indicate a marked increase in skeletal bone resorption in SHR rats that is apparent in both young growing animals and mature rats. The results are consistent with a recent study[20] showing that female SHR rats exhibit a tendency for increased age-related cancellous and cortical bone loss with evidence of early and increased sensitivity to ovariectomy-induced bone loss.

MATERIALS AND METHODS

The animals were Sprague-Dawley rats (SD/HlaBR; Hilltop Lab Animals, Scottsdale, PA), Wistar Kyoto rats (WKy/NCrlBR; Charles River, Wilmington, MA), and Aoki-Okamoto SHR rats (SHR/NCrlBR; Charles River). They were maintained in colony rooms at 23° ± 2°C on a 12-hour light/dark photoperiod and were allowed free access to Purina Rat Chow (Ralston Purina, St Louis, MO) and tap water. To verify the hypertensive and normotensive condition of the groups, the systolic blood pressure of rats from each strain was measured with the animal conscious using a programed electrosphygmomanometer with a pneumatic pulse transducer and tail cuff.

^{3}H-Tetracycline Method for Measurement of Whole Skeleton Bone Resorption

Whole skeleton bone resorption was measured as previously described.[21] Thirteen days prior to the start of urine collection, a total of 12 male and 12 female rats of each of the SHR, WKy, and SD strains at either 6 weeks or 22 weeks of age underwent a series of subcutaneous injections of ^{3}H-tetracycline (NET-141 Tetracycline, [7-^{3}HCN]; New England Nuclear, Boston, MA) dissolved in 0.05 mol/L HCl containing ascorbic acid as a reducing agent. At the end of the isotope-labeling period, 8-week-old rats had received a total of 25 µCi per rat in 5 injections and 24-week-old rats received a total of 35 µCi per rat of ^{3}H-tetracycline in 5 injections on days 1, 4, 7, 10, and 13. Following the final injection, each rat was transferred to a standard metabolic chamber and its food consumption and body weight were monitored daily. The first 24-hour urine sample was discarded, and the urine was collected thereafter at 24-hour intervals for a period of 35 days. Urine volume was

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recorded daily, and a 0.5-mL aliquot from each sample was combined with 10 mL Scintiverse E (Fisher Scientific, Pittsburgh, PA) to determine ^{3}H-tetracycline content by liquid scintillation spectrometry. The urinary ^{3}H-tetracycline loss curve data for each rat were normalized by dividing by the dry skeletal mass previously determined for each rat strain.[15] The normalization factors (grams) were as follows: 8-week male/female: SD, 10.1/9.7; WKy, 6.9/8.0; and SHR, 5.9/5.6; and 24-week male/female: SD, 18.8/12.8; WKy, 18.8/12.7; and SHR, 14.8/10.4. Individual curves were then analyzed by Table Curve 2D Automated Curve Fitting Software (Jandel Scientific, San Rafael, CA). Based on the equation obtained, the curves were further analyzed to obtain the following parameters for the slow compartment of label loss that is thought to represent cell-mediated bone resorption: (1) y-intercept, the rate of label loss from the actively resorbing bone compartment immediately following termination of label loading, providing a measure of the rate of resorption uncomplicated by previous depletion of label from resorbing bone; and (2) area under the curve or pool size, a measure of the extent of metabolically reactive bone resorbed during depletion of the available pool of ^{3}H-tetracycline label.

Statistics

The regression parameters were analyzed for significant differences by 3-factor ANOVA (Super ANOVA; Abacus Concepts, Berkeley, CA). Blood pressure data were analyzed by 2-way ANOVA followed by "contrasts" (Abacus Concepts) in making specific, preplanned comparisons. Differences were considered significant at a P level less than .05. Data are presented as the mean ± SEM throughout.

RESULTS

Figure 1 shows the pattern for blood pressure in SHR rats and normotensive WKy and SD rats in the interval from 8 to 24 weeks of age. Both male and female SHR rats showed an elevation of blood pressure to hypertensive levels by 8 weeks of age. Thereafter, blood pressure continued to increase, with severe hypertension realized in 24-week-old animals. Blood pressure in WKy, the parent strain of SHR rats,[22] and SD rats also increased with maturity but was well below the level in SHR rats. Each of the rat strains investigated showed a dichotomy of blood pressure between the sexes, with the values significantly lower in females versus males after 12 weeks of age.

Skeletal Resorption Kinetic Parameters

Significant differences were observed among rapidly growing 8-week-old animals of the 3 rat strains for both the rate of resorption (Table 1) and the pool size of label loss (Table 2). WKy resorption values were increased compared with SD values at 8 weeks. By comparison, the rate of resorption and the pool size in both male and female SHR rats were significantly increased compared with the parent-strain WKy values at each measurement. Although the rate of resorption and the pool size tended to be reduced in females compared with males, there was no consistent evidence of between-sex differences in resorption parameters in rapidly growing 8-week-old animals.

As expected, there was a marked decrease in the male values for the rate of resorption (60% to 70%) and the pool size (75% to 80%) between 8 and 24 weeks of age. Female values also decreased for the rate of resorption (15% to 35%) and pool size (45% to 60%), but to a lesser extent versus the males. In 24-week-old animals, there was a clear sexual dichotomy, with an elevated female rate of resorption and pool size recorded for each of the 3 rat strains. Within-sex comparisons indicated no differences in the rate of resorption (Table 1) or pool size (Table 2) between normotensive 24-week-old WKy and SD rats. By comparison, male SHR rats showed a significantly increased rate of resorption compared with parent-strain WKy but a similar pool size relative to WKy rats at 24 weeks. The 24-week-old female SHR rats showed significant increases in both resorption and ^{3}H-tetracycline label pool size compared with the normotensive WKy controls.

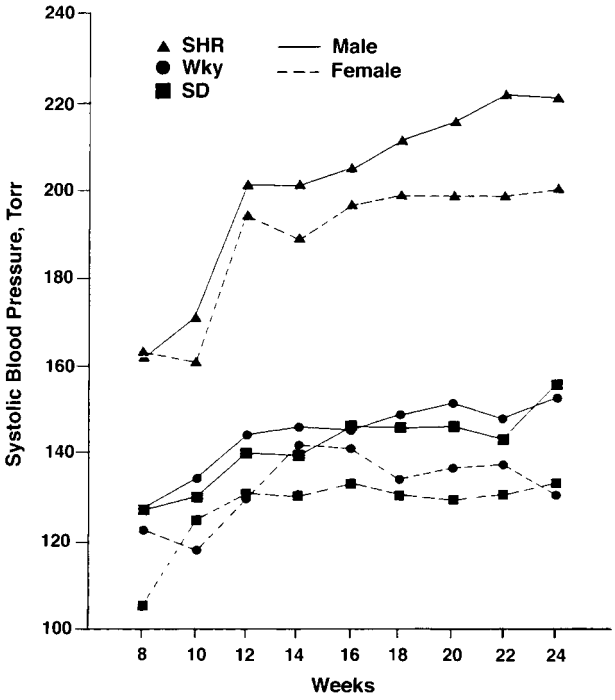

Fig 1. Time course for systolic blood pressure in male and female SHR, WKy, and SD rats between 8 and 24 weeks of age. Values represent the mean of 6 animals.

DISCUSSION

The relationship between hypertension and increased bone turnover is not certain. The SHR rat is a widely studied model of essential hypertension that exhibits properties of abnormal calcium metabolism similar to those found in human hyperten-

Table 1. Rate of ^{3}H-Tetracycline Label Loss (y-intercept) in 8- and 24-Week-Old Male and Female SHR, WKy, and SD Rats

Group	Male	Female	Female/Male
8 week			
SD	14.7 ± 1.1	14.9 ± 1.8	1.01
WKy	27.0 ± 1.1*	18.9 ± 0.8‡	0.70
SHR	34.3 ± 1.1†	32.7 ± 1.4†	0.95
24 week			
SD	5.8 ± 0.6	12.9 ± 0.6‡	2.22
WKy	8.2 ± 0.6	12.5 ± 0.8‡	1.56
SHR	9.6 ± 0.4†	22.6 ± 2.4†‡	2.35

NOTE. Data are presented as dpm/g dry skeleton mass × 10^{-3}; values are the mean ± SEM of 6 animals.

*Significant *v* SD.

†Significant *v* WKy.

‡Significant between sexes of the same strain.

Table 2. ^{3}H-Tetracycline Label Pool Size of 8- and 24-Week-Old Male and Female SHR, WKy, and SD Rats

Group	Male	Female	Female/Male
8 week			
SD	532.0 ± 22.2	494.4 ± 32.8	0.92
WKy	832.3 ± 19.5*	647.1 ± 22.5*‡	0.78
SHR	958.2 ± 17.4†	901.2 ± 44.0†	0.94
24 week			
SD	138.8 ± 10.8	260.1 ± 5.7‡	1.87
WKy	178.0 ± 11.0	248.0 ± 4.5‡	1.39
SHR	205.2 ± 10.3	357.0 ± 20.2†‡	1.74

NOTE. Data are presented as dpm/g DSM × 10^{-3}; values are the mean ± SEM of 6 animals.

*Significant *v* SD.

†Significant *v* WKy.

‡Significant between sexes of the same strain.

sives. Urinary calcium loss is increased in SHR rats, with hypercalcuria reported by 17 weeks of age in males with an associated decrease in serum ionized calcium and an elevation in PTH levels.[23] Although it is still an area of controversy, there is an extensive literature suggesting that gastrointestinal handling of calcium is significantly altered in SHR (reviewed in Hatton et al[24]). Taken together, the literature clearly indicates a significant disturbance of various factors regulating calcium metabolism in hypertension that could affect bone mineral metabolism. Hence, one explanation may be that the increase in bone turnover reflects one aspect of a potentially interrelated constellation of abnormalities of calcium metabolism in the SHR strain that may or may not be casually related to a blood pressure elevation. Lalande et al[25] have shown that the sulfonamide diuretic indapamide, which has been used successfully in the treatment of hypertension, can prevent sodium-induced bone resorption and bone loss independently of changes in serum PTH in SHR rats fed 8% sodium diets. This observation suggests that a common mechanism links hypertension and increased bone turnover in SHR rats. However, their results further suggest that factors other than altered calcium metabolism may contribute to bone metabolism in hypertensives. In studies of bones from 11 strains of mice, Beamer et al[26] have demonstrated significant genetically determined differences in the total and cortical bone density, mineral content, and density of specific bones. They concluded that there is a major genetic influence on bone density among different strains of mice, and that different parameters of bone development such as length and density are under regulation by different genes. Recent study[27] has further indicated highly significant between-strain differences in femoral bone formation and mineral apposition rates in mice maintained under identical dietary and housing conditions. Taken together, the results open the possibility that unresolved genetic factors contribute to differences in bone turnover between hypertensive and normotensive rat models.

Various studies to evaluate individual bone density,[28,29] ash weight,[16] and histomorphometric characteristics[19,30] are generally consistent with increased bone catabolism and reduced bone mineralization in mature SHR rats. Citing the potential limitations in extrapolating individual bone data to the intact skeleton, a recent study examined the whole skeletal dry weight and calcium content of SHR rats in comparison to Wky rats.[15] Skeletal development expressed as the dry skeletal mass as a percent of body weight was significantly increased in the females of each strain at both 8 weeks (~25%) and 24 weeks (~35%) of age. Within-sex comparisons further indicated that skeletal development of SHR rats was significantly increased compared with WKy controls in 24-week-old males and in females at both 8 weeks and 24 weeks of age. Moreover, they showed that the calcium content per gram of bone was identical between the 2 rat strains. Hence, whole-body assessment indicated robust development of skeletal mass and normal calcium content in the skeleton of females compared with males and in hypertensive versus normotensive rats. Together with the present findings of elevated whole skeletal bone resorption, these results indicate that despite normal or enhanced skeleton mass, whole-body bone turnover is markedly increased in hypertensive rats, possibly leading to altered histomorphology[19,30] and individual-bone structural quality. Consistent with this view, Liang et al[20] reported histomorphometric evidence that the ratio of cancellous bone to cortical bone was increased in SHR rats, whereas they showed an age-related loss in these bone types not observed in WKy rats. Furthermore, earlier and greater cancellous bone loss was noted in SHR rats following ovariectomy. They concluded that SHR rats sustain a high rate of bone turnover with the potential for increased susceptibility to factors leading to osteopenia.

The results indicate strong between-sex differences in mature animals, with females exhibiting both an increased rate of resorption (Table 1) and increased pool size (Table 2) compared with males. The reason for increased resorptive activity in females is uncertain. The turnover of bone in rats at 24 weeks of age would be expected to involve significant remodeling and modeling activities associated with repair and bone sculpting.[31] In addition, skeletal bone turnover represents a major mechanism in extracellular fluid calcium homeostasis. Because it seems unlikely that remodeling or modeling would be elevated in unbred females compared with males, a likely explanation is that the increased resorptive activity is in some fashion linked to calcium homeostasis in the female. This, in turn, would appear consistent with the exacerbation of the phenomenon in SHR rats which exhibit evidence of disturbed calcium homeostasis.

In summary, a comparison among SHR, WKy, and SD rat strains, as expected, indicated that whole-body skeletal bone resorption was dramatically reduced between rapidly growing 8-week-old and mature 24-week-old animals. Furthermore, in mature animals, whole skeletal resorptive activity was significantly elevated in females of each strain compared with males. An increase in resorptive activity in SHR rats compared with normotensive animals was detected as early as 8 weeks of age. Increased resorption was clearly present in hypertensives at 24 weeks of age and was particularly severe in female SHR rats. The cause of increased bone turnover in females or hypertensives is not certain, but may be linked to the demands of whole-body calcium homeostasis in these animals. In light of previous study,[15] the results suggest that the stress placed on the skeleton by calcium homeostasis could be a major factor to increase skeletal mass in the mature animal in circumstances where adequate dietary calcium is available. On the other hand, the high basal level of bone turnover in female and SHR animals would be expected to increase their susceptibility to bone loss in circumstances of calcium deficiency or osteoporotic disease. The clinical implications of the present study are uncertain and will require additional research.

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