Age-Related Inequality between Rates of Formation and Resorption in Various Whole Bones of Rats (43154)

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Abstract. **Variations in rates of bone turnover, consisting of bone formation and bone resorption, were characterized as a function of age and types of bone. Bone formation and bone resorption were quantified and compared in four ages of growing male rats (newborn, 0–2 weeks; weanling, 4–7 weeks; adolescence, 10–14 weeks; and mature, 15–23 weeks) for cephalic bone (calvaria), appendicular bone (femur), axial bones (sixth lumbar vertebrae and sternum), and the pelvis. In all four ages studied, inequality between the rates of bone formation and resorption existed in all bones, and the magnitude of the differences in metabolic imbalance turnover was age and bone type dependent. Formation was consistently higher than resorption in all five bones throughout the entire experimental periods. The ratio of resorption to formation was the lowest at birth (under 0.10:1) for all five bones and then increased. The most rapid bone growth in terms of bone calcium mass occurred at the weanling age (the net gain of calcium per bone per day was the highest) for all bones except the lumbar vertebrae, which occurred at adolescence. The magnitude of the inequality in the rates of bone formation and resorption was greatest in the newborn and diminished with age towards equality. At maturity, the ratio of resorption to formation was under 0.50:1 in the most dense or calcified bones such as the calvarium (0.40:1) and femur (0.49:1), and over 0.50:1 in the relatively thin cortex and well-trabeculated axial bones (0.60:1 in lumbar vertebrae and 0.75:1 in the sternum) and the pelvis (0.91:1). Additionally, large bones (calvaria, long bones, and pelvis) seemed to play an important role in regulating calcium homeostasis starting from the weanling age, since the amount of calcium released from each whole bone per day was high in these bones due to their large mass.** [P.S.E.B.M. 1990, Vol 195]

Normal bone growth is a result of positive balance (net gain) of bone turnover (1), i.e., the excess of bone formation relative to bone resorption, leads to the increase in bone mass during the growing period. Metabolic bone disease and age-related bone loss are due to the imbalances between bone formation and resorption (2–6). Measurements of bone formation or resorption have been made spatially by morphometric methods at the tissue level in the rat (7), dog (8, 9), and human (10, 11). Quantifications of bone resorption have also been done temporally by whole bone isotopic method at the organ level in the rat and rabbit (12, 13). The results of bone turnover (bone formation and resorption) from the previous studies (7–12) reveal the metabolic heterogeneity of the skeletal system between different types of bone and various species of animals. At the organ level, however, bone formation and resorption have not been quantified in the same whole bone for different skeletal sites. It is not clear whether rates of bone formation and resorption are equivalent for all bones, and if the patterns of bone turnover varies with age and bone type. Regional skeletal analysis could be important in terms of understanding why the metabolic consequences of certain diseases such as osteoporosis manifest themselves first in certain skeletal elements (fractures of spine, hip, and wrist in humans [14–16]).

A pharmacokinetic model has been developed in the rat and rabbit (13) based on the kinetic loss of prelabeled [^{3}H]tetracycline from bone (12). To precisely and clearly represent bone resorption and formation on the same bone, to study the pattern of bone turnover, and to compare such patterns among various types of bones and across species, an advanced simultaneous quantification of bone resorption and formation has

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then been developed in mature rats (17). The present study employs the method described above to quantify bone turnover in five bones (calvarium, femur, sixth lumbar vertebrae, sternum, and pelvis). It provides documentation of the age-related inequality between the rates of bone formation and resorption within a given bone, and among various types of bone in growing and mature male rats. The present study represents the rates of bone turnover in both absolute (milligram) and relative (percentage) amount of calcium involved in the formation and resorption process. The absolute values reveal the information about the contribution of calcium to blood from the different types of bone, whereas the relative values provide the pattern of bone turnover and a comparison of such patterns across various types of bone and ages of animals.

Materials and Methods

Animals. All of the animals studied were from Sprague-Dawley strain: 12 newborn (male and female), and 61 other male rats of three different ages (weanling, 4–7 weeks of age; adolescence, 10–14 weeks of age; and mature, 15–23 weeks of age) from Zivic Miller, Zelienople, PA. The rats in each age group were raised from the same original group of similar body weight (±5%). Body weights were checked at the beginning of the study and weekly thereafter. The rats were fed a standard laboratory diet containing 1.0% calcium, 0.65% phosphorus, and 330 IU of vitamin D/100 g (Formulab Chow no. 5008; Purina, Ralston, St. Louis, MO), and given water *ad libitum*. The housing conditions were 72–74°F, 50% humidity.

Material and Equipment. Tetracycline-7-^{3}H ([^{3}H] tetracycline, 0.73 Ci/m*M*) was obtained from New England Nuclear, Boston, MA (18). A liquid scintillation spectrometer (Mark II-Searle Analytic, Des Plaines, IL) (19) was used to determine [^{3}H]tetracycline radioactivity in bone extracts. An automatic titrator (model 4008 Calcette; Precision Systems, Sudbury, MA) was used to measure total calcium content of the bone.

Experimental Rationale. Bone resorption was measured by quantifying the initial calcium mass and the loss of [^{3}H]tetracycline from whole bone at different time intervals. Growing rats were labeled repeatedly with [^{3}H]tetracycline. Bone formation was measured by adding the net increase of calcium mass and the amount of calcium mass resorbed that was reused in bone formation per whole bone at the same time. In this context, bone resorption was independent of bone formation, and bone formation was the summation of newly added calcium and resorbed calcium.

Isotopic Labeling and Sacrifice Schedule. [^{3}H] Tetracycline was repeatedly injected subcutaneously into all rats. For pregnant rats, the total doses of 160 μCi/rat were given in four injections during the last 4 days of gestation. The weanling group received a total dose of 145 μCi/rat in six injections during 10–21 days of age. The adolescent group received a total dose of 231 μCi/rat in 10 injections during 24–56 days of age. The mature group received a total dose of 200 μCi/rat in 16 injections during 28–84 days of age.

There are several principles that underlie the technique of the isotopic labeling. (i) [^{3}H]Tetracycline labels bone mineral during bone formation (20, 21) probably by chelating with calcium (22), and is lost primarily and irreversibly through the biologic process of bone resorption (12, 21, 23) due to rapid excretion by the kidney (12, 13, 23). (ii) Prelabeling of animals during the rapid growing state (12) results in an even distribution of [^{3}H]tetracycline throughout bones (20, 23, 24). (iii) Tritium in [^{3}H]tetracycline does not undergo metabolic transformation *in vivo* nor chemical alteration *in vitro* (18, 25).

In the newborn group (0–2 weeks of age), four rats were sacrificed at birth. The eight remaining rats in the group were placed with a nonlabeled surrogate mother to prevent additional influx of the isotope via lactation from the original radioactive mother, and equally divided prior to sacrifice at 1 and 2 weeks of age. In the weanling group, the rats were sacrificed at 4 weeks of age and at weekly intervals thereafter. Five rats were sacrificed at each time period. In the adolescent group, the rats were sacrificed at 10, 11, 12, and 14 weeks of age. Six rats were sacrificed at each time period. In the mature group, the rats were sacrificed at 15, 17, 19, and 23 weeks of age. Four rats were sacrificed at each time period. All rats were sacrificed by ether overdose. At autopsy, the calvarium, femur, sixth lumbar vertebrae, sternum, and pelvis were recovered intact and cleaned of all soft tissue.

Chemical and Isotopic Measurement. The bones were defatted with $CHCl_3$:CH_3OH (2:1) twice and dried *in vacuo* over NaOH pellets for 1 to 2 days. The bone mineral was extracted repeatedly with 0.5 *N* HCl at 4°C. After centrifuging the bone extract at 2200 rpm for 10 min, the supernatant was measured for total calcium content of the whole bone by an automatic titrator, and analyzed for [^{3}H]tetracycline radioactivity by a liquid scintillation spectrometer.

An aliquot (1 ml) of each bone extraction was taken for measurement of calcium content. All samples were analyzed three times. An aliquot (0.3 ml) of each bone extraction was added to 10 ml of scintillation liquid. A plastic vial with 10 ml of scintillation fluid was the "blank" and its background was 5–6 cpm. The radioactivity of [^{3}H]tetracycline in bone samples was over 10,000 cpm for 10 min of counting and all extractions were counted three times. The data were expressed as total calcium per whole bone (mg/bone) for calcium content, and total radioactivity per whole bone (dpm/bone) for [^{3}H]tetracycline content.

Data Computation and Statistical Analysis. *Bone resorption.* The computation method by Li and Klein (17) was employed. The net change of calcium in whole bones of each experimental group was calculated. The percentage of loss of the radioactive mass of [^{3}H]tetracycline within each group during the experimental period was also calculated, representing the rate of bone resorption (12, 13). The absolute amount of calcium in milligrams resorbed from each bone (bone resorption) was calculated by multiplying the amount of calcium at time zero by the rate of bone resorption.

Bone formation. The total amount of calcium in milligram deposited into each bone (bone formation) was determined by adding the amount of calcium resorbed to the net change in calcium content of each whole bone.

Computation of bone formation and bone resorption. The relative amount (percentage) of calcium deposited into (formation) and resorbed from (resorption) each bone was calculated by dividing each absolute value by the corresponding baseline value (time zero) of the age group. Both absolute (milligram) and relative (percentage) values of calcium involved in formation and resorption were plotted against time to yield linear least-squares regression, respectively. The slope of a given regression constituted the amount of calcium formed or resorbed for each bone per day.

Statistical analysis. Regression was verified by the t test for the regression coefficient. The rates thus calculated were analyzed by analysis of variance within the same age of the five bones, and among four ages of the same bone using biomedical-designed program statistical software (BMDP) from the main frame at the Department of Epidemiology and Biostatistics, School of Medicine, Case Western Reserve University. The metabolic inequality of bone formation and resorption was obtained by subtracting bone resorption from bone formation. The results were expressed in both absolute and relative values.

Results

Turnover rates of the five bones in terms of both absolute (milligram) and relative (percentage) amount of calcium involved in formation and resorption are given in Figures 1 and 2. The turnover rates were different among the groups for the same age of the five bones and for the same bone of the four ages at the $P = 0.05$ level. The inequality of bone formation and resorption is presented in Figure 3.

Absolute Rates. The values for rates of formation and resorption in the five bones were the lowest and the least scattered during the newborn period in terms of absolute amount (Table I). Formation in cephalic bone (calvarium) and appendicular bone (femur) peaked at weanling age, then decreased dramatically between the ages of weanling and adolescence for the

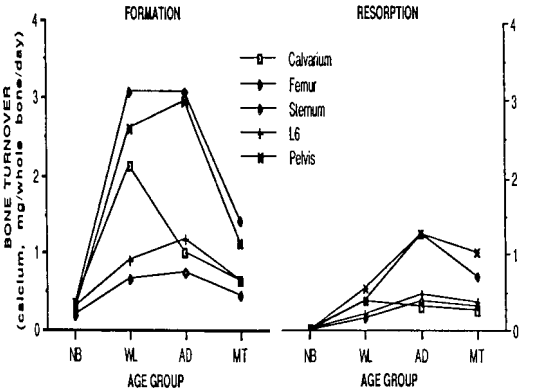

Figure 1. Bone formation (left) and resorption (right) in newborn rats (NB, 0–2 weeks), weanling rat (WL, 4–7 weeks), adolescent rats (AD, 10–14 weeks), and mature rats (MT, 15–23 weeks) for five bones. The values are represented by calcium deposited into (formation) and resorbed from (resorption) the whole bone in absolute (mg/bone/day) amounts.

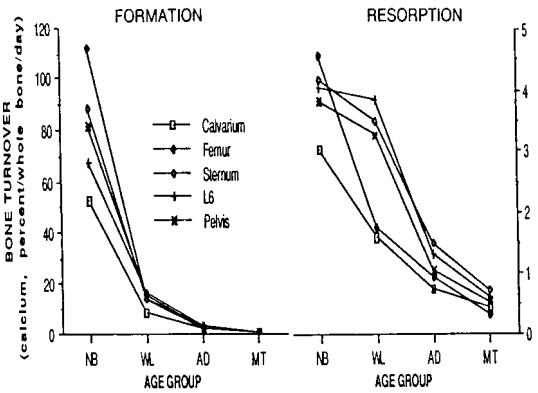

Figure 2. Bone formation (left) and resorption (right) in newborn rats (NB, 0–2 weeks), weanling rat (WL, 4–7 weeks), adolescent rats (AD, 10–14 weeks), and mature rats (MT, 15–23 weeks) for five bones. The values are represented by calcium deposited into (formation) and resorbed from (resorption) the whole bone in relative (percent/bone/day) amounts.

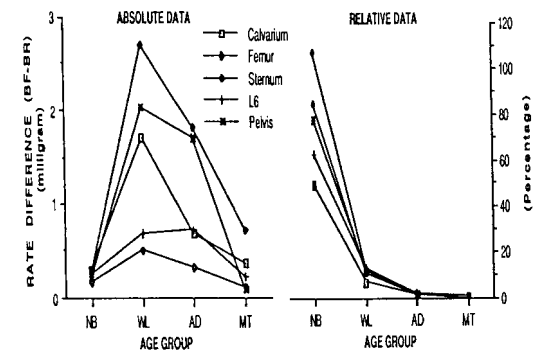

Figure 3. Inequality of bone formation and resorption in newborn rats (NB, 0–2 weeks), weanling rats (WL, 4–7 weeks), adolescent rats (AD, 10–14 weeks), and mature rats (MT, 15–23 weeks) for five bones. The values were obtained by subtracting the bone resorption from formation, and represented in absolute (mg/whole bone/day, left) and relative (percent/whole bone/day, right) amount of calcium.

Table I. Absolute Values (mg) of Calcium Involved in the Formation and Resorption for Each of Five Whole Bones per Day with Age

Bone	Age group							
	NB[a]		WL		AD		MT	
	BF	BR	BF	BR	BF	BR	BF	BR
Calvarium	0.329	0.019[b]	2.116[b]	0.401[c]	0.998[b]	0.317[b]	0.629[c]	0.253[b]
Femur	0.312	0.013[b]	3.090[c]	0.380[b]	3.087[c]	1.264[c]	1.422[c]	0.694[b]
Sternum	0.191	0.009[b]	0.663[c]	0.161[c]	0.728[c]	0.390[b]	0.436[b]	0.326[b]
Lumbar-6	0.275	0.017	0.881[c]	0.199[c]	1.186[c]	0.459[b]	0.616[c]	0.371[c]
Pelvis	0.244	0.011[b]	2.608[c]	0.559[c]	2.955[c]	1.249[b]	1.112[c]	1.009[c]

[a] NB, newborn group: 0–2 weeks; WL, weanling group: 4–7 weeks; AD, adolescent group: 10–14 weeks; MT, mature group: 15–23 weeks; BF, formation; BR, resorption. Mean values when $n = 4$–6.
[b] Statistically different from zero at the $P < 0.05$ level.
[c] Statistically different from zero at the $P < 0.01$ level.

calvarium, and between adolescence and mature for the femur. The peak of formation was approached at adolescent age in axial bones (sixth lumbar vertebrae and sternum) and the pelvis. Resorption in all bones except the calvarium increased with age until the adolescence and decreased thereafter. The highest net gain in calcium mass (mg/bone/day) was at weanling age for all bones (2.710 in femur, 2.049 in pelvis, 1.715 in calvarium, and 0.502 in sternum) except for lumbar vertebrae, which occurred at the adolescence (0.727).

Relative Rates. When the rates of formation and resorption were calculated as a percentage, the maximal values were in the newborn age for both formation and resorption, and decreased from the age of weanling onward (Table II). The marked drop in the rate of formation in all bones occurred between the ages of newborn and weanling. The sharp decrease of resorption rate was between the ages of newborn and weanling in the calvarium and femur, and between the weanling and adolescence in the sternum, lumbar vertebrae, and pelvis.

Differences between Rates of Bone Formation and Resorption. In all four ages studied, formation was consistently higher than resorption in all bones. The ratio of resorption to formation was the lowest at birth (under 0.10:1) and then increased. At maturity, this ratio was under 0.50:1 in calvarium (0.40:1) and femur (0.49:1), and over 0.50:1 in axial bones (in the lumbar vertebrae it was 0.60:1 and the sternum it was 0.75:1) and the pelvis (0.91:1).

The rates of bone formation and resorption were disproportionate in all of the five bones and four ages (Fig. 3). The absolute magnitude of the differences was the lowest at birth for the calvarium and femur, and at maturity for the sternum, vertebrae, and pelvis. This magnitude increased to the highest at the weanling age and decreased from then on. When the data were presented in relative values, the highest magnitude of the inequality was seen at birth for all bones and decreased thereafter. The age-dependent pattern of the absolute and relative magnitude in the inequality resembled the absolute and relative rates of bone formation, respectively.

Discussion

The data developed in this study are significant for their documentation of the patterning of bone turnover in five different bones of the rat skeleton that enjoy, in

Table II. Relative Values (%) of Calcium Involved in the Formation and Resorption for Each of Five Whole Bones per Day with Age

Bone	Age group							
	NB[a]		WL		AD		MT	
	BF	BR	BF	BR	BF	BR	BF	BR
Calvarium	52.40	3.05[b]	8.44[c]	1.60[c]	2.32[b]	0.74[b]	1.11[c]	0.44[c]
Femur	111.90	4.56[b]	14.00[c]	1.75[b]	2.23[c]	0.94[c]	0.66[c]	0.32[b]
Sternum	88.80	4.19[b]	14.40[c]	3.49[c]	2.79[c]	1.49[b]	0.99[b]	0.73[b]
Lumbar-6	67.10	4.06	16.80[c]	3.84[c]	3.37[c]	1.31[b]	1.04[c]	0.62[c]
Pelvis	81.20	3.81[b]	15.30[c]	3.26[c]	2.42[c]	1.03[b]	0.57[c]	0.51[c]

[a] NB, newborn group: 0–2 weeks; WL, weanling group: 4–7 weeks; AD, adolescent group: 10–14 weeks; MT, mature group: 15–23 weeks; BF, formation; BR, resorption. Mean values when $n = 4$–6.
[b] Statistically different from zero at the $P < 0.05$ level.
[c] Statistically different from zero at the $P < 0.01$ level.

part, a different embryonic origin (membrane versus endochondral). They revealed, first, unequal type and age-dependent rates of bone formation and bone resorption, and thus added an unrealized dimension to our heretofore awareness that different skeletal elements exhibit highly individualistic rates of bone turnover (9–11, 26, 27). During the growing period, the rate of bone formation was consistently higher than bone resorption, but the magnitude in the differences was bone type and age dependent. The ratio of bone resorption to bone formation increased gradually with postnatal growth, and may eventually approach one, the equilibrium (metabolic balance) of bone turnover, as seen in the pelvis at maturity (0.91:1).

The rapid reduction of bone resorption rates to low values during growth in the rat may be due to the fact that the rat skeleton is much more highly mineralized than the higher species like dog or human and contains few, if any, mature haversian systems.

The histologic features draw a clear structural distinction between cortical bone and trabecular bone in the skeletal system. Besides the differences in the bony architecture, these two types of bone also vary in the degree of mineralization and pattern of metabolism. In the present study in the rat, the ratio of bone resorption to formation was low in denser bone like calvarium and femur, and high in sternum, lumbar vertebrae, and pelvis from weanling age onward. This ratio was over 0.50:1 in the more trabeculated bones (sternum, lumbar vertebrae, and pelvis) at maturity. The phenomenon that trabecular bone has higher turnover rate than cortical bone has been previously reported in other larger species. In the dog, the degree of mineralization in trabecular bone is much lower than that in cortical bone (28). The ratio of cortical to trabecular calcium as documented by Parks *et al.* (28) is 80:20 in dog. In adult humans, Snyder *et al.* (11) observed that the turnover rate of trabecular bone (10%) is 3–10 times faster than that of cortical bone (2.5%). Additionally, most of the fractures in human osteoporosis occur at sites containing substantial amounts of trabecular bone (16, 29, 30). These facts suggest a strong association between rates of bone turnover and susceptibility of bones to osteoporosis.

Since bone is lost throughout the skeleton with age (16), it should be no surprise that fractures would most often occur at bones having the highest ratio of resorption to formation under the same minimal trauma. Histologically, the surface area that is exposed to the cells being responsible for bone turnover, osteoblast and osteoclast, is larger in trabecular bone than in cortical bone. Therefore, one can speculate that the activity of bone turnover may be proportional to the surface area of bone in direct contact with osteoblast and osteoclast. In other words, bones having a large volume of trabecular structure appear to be more susceptible to osteoporosis (16).

In addition to the type of bony architecture, activities of bone turnover may be affected by several other factors (1, 31–33): blood supply, type of bone marrow, number of marrow cells, mechanical load, muscle distribution, and physical activities. Blood supply is rich in trabecular bone, where the type of surrounding bone marrow is different, with red marrow in vertebrae and yellow marrow in femoral neck and distal radius. Vertebral body, pelvis, and femur belong to weight-bearing bones. Sternum, ribs, thoracic vertebrae, and scapula undergo continuous and frequent cyclic physical activity due to breathing. However, rates of bone turnover vary among these bones (unpublished data). Therefore, pattern of bone turnover may be the summation of multifactorial effects.

The ontogeny of the age-dependent changes in bone turnover was defined optimally when the analyses of bone formation and resorption were presented in terms of their absolute versus relative rates. The *absolute* values clarified the contribution of calcium to blood among various types of bones with age. The *relative* values clarified the patterns of bone turnover among bone types and the comparison of such patterns. Both absolute and relative values need to be considered to avoid misinterpretation of the data. It could mistakenly lead to the interpretation that the growth of bone was the fastest at weanling age from the absolute data (Fig. 3), or that the contribution of calcium from the different types of bones to blood was highly individualistic at birth and then became similar afterwards from the relative data. The correct interpretation will be discussed in more detail below.

Skeleton stores 99% of the body's calcium and is a major regulator for calcium homeostasis. This calcium homeostasis appears to be regulated by the magnitude of bone turnover (1) as well as calcium mass of the bones. From the absolute data, one can speculate that immediately after birth, all bones participated in maintaining the blood calcium level since the activities of bone turnover were high and the calcium masses were similar in all bones. At weanling age, the large bones (skull, pelvis, and long bones) seemed to fulfill the duty of regulating calcium concentration within the body because of the large amount of net changes in calcium mass. Beyond weanling age, calcium homeostasis appears to be controlled mainly by long bones. From the relative data, it appears that the fastest growing bone was the femur and the slowest growing bone was the calvaria at birth. The rates of growth in the five bones seem to approach each other from weanling age onward. The calvaria grew the fastest, whereas the pelvis grew the slowest at maturity.

The rates of bone formation and resorption were unequal, and these inequalities varied according to the

types of bone and ages of animals. With age, the inequalities decreased in all bones. During growth, there was a positive calcium balance in all five bones. The age-related differences in the calcium balance were mirrored by the varying activities and patterns of bone turnover and calcium mass of the bones. Therefore, to avoid misinterpretation of data, accurate measurement of bone turnover must be done by quantifying both bone formation and resorption of the same bone in growing and mature animals.

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