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# Tetracycline Double-Labeling of Iliac Trabecular Bone in 41 Normal Adults

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**Summary.** A histomorphometric evaluation of the iliac crest trabecular bone remodeling was performed after tetracycline double-labeling in 41 normal Danes (12 males and 29 females) aged 19 to 56 years. The fraction of formative (osteoid covered) and resorptive surfaces was unrelated to age but higher in males than in females ($P < 0.02$ and $P < 0.05$, respectively). The appositional rate ($0.65 \pm 0.12$ $\mu$m/day) was unrelated to age and sex, whereas the fractional labeled surfaces were higher ($P < 0.01$) in the males ($0.18 \pm 0.08$ $\mu m^2/\mu m^2$) than in the females ($0.12 \pm 0.05$ $\mu m^2/\mu m^2$), and among the females inversely related to age ($R = -0.38$, $P < 0.05$). The bone formation rate at BMU level ($0.50 \pm 0.20$ $\mu m^3/\mu m^2$/day) was unrelated to sex, but among the females inversely related to age $R = -0.49$, $P < 0.01$). The bone formation rate at tissue level was higher ($P < 0.02$) in the males ($0.13 \pm 0.07$ $\mu m^3/\mu m^2$/day) than in the females ($0.07 \pm 0.03$ $\mu m^3/\mu m^2$/day) and among the females inversely correlated to age ($R = -0.43$, $P < 0.05$). The age- and sex-dependent variations in the dynamic parameters underline the importance of a more elaborated normal material.

**Key words:** Bone biopsy — Tetracycline Labeling — Appositional Rate.

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## Introduction

Conventional histomorphometric analysis of bone biopsies gives information on changes in the static parameters, i.e., the amount of bone; the amount, extent, and width of osteoid seams; and the extent of osteoclastic resorption surfaces. No information, however, is obtained on the pathogenetic alterations in the bone remodeling leading to the observed condition. An increase in the extent of bone formation or resorption surfaces, for instance, may, provided a constant amount of bone is turned over per remodeling cycle, be caused by an increase in the activation frequency of new bone remodeling units [BMU = basic multicellular units (1)] and/or by an increase in the average duration of the formative or resorptive phases, respectively, of the remodeling period. Furthermore, variations in the amount of osteoid may be caused by changes in the extent of bone formation surfaces, or by variations in the appositional rate or the maturation period of osteoid.

Double-labeling with tetracycline is helpful to elucidate the interrelationships mentioned above as not only the extent but also the rate of bone formation can be measured (1). Knowledge of the normal ranges and sex- and age-dependent variations of the different parameters is, however, essential for the interpretation of values obtained in different states and diseases. The aim of the present investigation has, therefore, been to describe the remodeling of iliac crest trabecular bone in 41 normal Danes all double labeled with tetracycline.

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**Table 1.** Measured and calculated values in trabecular bone and significance of sex differences

| | | $S_{fract\ (f)}$ $\mu m^2/\mu m^2$ | $S_{fract\ (r)}$ $\mu m^2/\mu m^2$ | $^uM/t$ $\mu m$/day | $S_{fract\ (lab)}$ $\mu m^2/\mu m^2$ | $^sV_f$ $\mu m^3/\mu m^2$/day | $^sV_{f(BMU)}$ $\mu m^3/\mu m^2$/day |
|---|---|---|---|---|---|---|---|
| Total | Mean | 0.179 | 0.043 | 0.65 | 0.135 | 0.09 | 0.50 |
| N = 41 | SD | 0.068 | 0.016 | 0.12 | 0.063 | 0.05 | 0.20 |
| Males | Mean | 0.219 | 0.052 | 0.68 | 0.183 | 0.13 | 0.58 |
| N = 12 | SD | 0.073 | 0.021 | 0.16 | 0.075 | 0.07 | 0.22 |
| Females | Mean | 0.162 | 0.040 | 0.64 | 0.115 | 0.07 | 0.48 |
| N = 29 | SD | 0.059 | 0.012 | 0.10 | 0.045 | 0.03 | 0.19 |
| *P* | | <0.02 | <0.05 | NS | <0.01 | <0.02 | NS |

$S_{fract\ (f)}$, fractional formation surfaces in trabecular bone; $S_{fract\ (r)}$, fractional resorption surfaces in trabecular bone; $^uM/_t$, appositional rate in trabecular bone; $S_{fract\ (lab)}$, fractional labeled surfaces in trabecular bone; $^sV_f$, bone formation rate, tissue level, surface referent; $^sV_{f(BMU)}$, bone formation rate, BMU level, surface referent

## Material and Methods

The study included 41 volunteers, 12 males aged 23 to 56 years (mean 32) and 29 females aged 19 to 53 years (mean 29), who had given their informed consent to participate. Entry into the investigation was based on normal thyroid, parathyroid, kidney, and liver function. None of the participants received tranquilizers, anticonvulsants, or other chronic medication.

Demethylchlortetracycline 600 mg daily was given in a 2–10–2 day regime. A transcortical iliac crest biopsy (2) was obtained from 2 to 8 days after the last dose of tetracycline was given. The biopsies were embedded undecalcified in methylmetacrylate. Then 7 $\mu$m thick sections were produced and stained with Masson trichrome and Goldner trichrome, and 20 $\mu$m thick sections were mounted unstained.

The following values were determined histomorphometrically on the stained sections (3, 4):

*Fractional formation surfaces in trabecular bone* ($S_{fract(f)}$, $\mu m^2/\mu m^2$) as the extent of osteoid-covered surfaces in decimal fraction of the total trabecular bone surface.

*Fractional resorption surfaces in trabecular bone* ($S_{fract(r)}$, $\mu m^2/\mu m^2$) as the extent of Howship's lacunae in decimal fraction of the total trabecular bone surface.

The following values were determined using fluorescent microscopy on the unstained sections:

*Appositional rate in trabecular bone* ($^uM/t$, $\mu$m/day) as the mean of 4 extreme measurements, almost equally spaced, between the middle of the lines in all double-labeled zones ($^uM$) divided by the interval of days (t) between the given doses of tetracycline. The value is uncorrected (u) for oblique cutting (3).

*Fractional labeled surfaces in trabecular bone* ($S_{fract(lab)}$, $\mu m^2/\mu m^2$) as the extent of single- or double-labeled surfaces in decimal fraction of the total trabecular bone surface.

The following values of trabecular bone remodeling were calculated:

*Bone formation rate, tissue level, surface referent* [$^sV_f$, $\mu m^3/\mu m^2$/day (3)] as:

$$^sV_f = S_{fract(lab)} \times {}^uM/t$$

which gives the amount of mineralized bone being made per unit surface area of trabecular bone per day uncorrected for obliquity of the plane of section.

*Bone formation rate, BMU level, surface referent* [$^sV_{f(BMU)}$, $\mu m^3/\mu m^2$/day (3)] as:

$$^sV_{f(BMU)} = \frac{S_{fract(lab)} \times {}^uM/t}{S_{fract(f)}}$$

which gives the average amount of mineralized bone being made per day per unit osteoid-covered surface uncorrected for obliquity of the plane of section.

In the present paper the perimeters measured in a two-dimensional section are transformed into surface areas in a three-dimensional structure in accordance with the terminology of Frost (3). This transformation is stereologically correct provided that the measured structure is isotropic, and the sectional plane is randomly chosen (5).

### *Statistical Analysis*

The statistical significance of differences in group means was determined by the Wilcoxon test for two samples. The interdependence of bone histomorphometric data and age was assessed by means of Spearman's coefficient of rank correlation suit-

**Table 2.** Age dependency of measured and calculated values in trabecular bone

| | | $S_{fract\ (f)}$ $\mu m^2/um^2$ | $S_{fract\ (r)}$ $\mu m^2/\mu m^2$ | $^{u}M/t$ $\mu m/day$ | $S_{fract\ (lab)}$ $\mu m^2/um^2$ | $^{s}V_f$ $\mu m^3/\mu m^2/day$ | $^{s}V_{f(BMU)}$ $\mu m^3/\mu m^2/day$ |
|---|---|---|---|---|---|---|---|
| Total | R | 0.05 | 0.07 | −0.18 | −0.11 | −0.19 | −0.37 |
| N = 41 | p | NS | NS | NS | NS | NS | <0.02 |
| Males | R | 0.04 | −0.33 | −0.33 | 0.13 | −0.15 | −0.37 |
| N = 12 | p | NS | NS | NS | NS | NS | NS |
| Females | R | −0.11 | 0.07 | −0.14 | −0.38 | −0.43 | −0.49 |
| N = 29 | p | NS | NS | NS | <0.05 | <0.05 | <0.01 |

Abbreviations as in Table 1

able for any continuous distribution. In the figures a linear regression line is constructed and its inclination compared to zero in order to suggest a cutoff point, for comparing values above and below the age interval of the population studied.

## Results

Table 1 gives the mean values (± SD) of the measured and calculated parameters in trabecular bone and the significance of differences between the sexes.

Table 2 shows the interdependence of bone histomorphometric data and age in each sex and in the total population (Spearman's rank correlation). Graphic representation of some of the data is given in Figure 1.

Among the static parameters a significant higher fraction of formative surfaces ($P < 0.02$) and resorptive surfaces ($P < 0.05$) was found in the males compared with the females. No age dependency was found in either the male or female group.

Among the dynamic parameters no sex- or age-dependency was found in the appositional rate, whereas both the fractional labeled surfaces and the calculated value of bone formation rate at tissue level were found significantly higher in the males than in the females ($P < 0.01$ and $P < 0.02$, respectively). Furthermore, a decrease with increasing age was found among the females in the fraction of labeled surfaces ($P < 0.05$) and in the bone formation rate both at tissue level ($P < 0.05$) and at BMU level ($P < 0.01$). No significant age-related changes were found in the rather small group of males.

## Discussion

In the present study, the dynamics of trabecular bone formation in the iliac crest were investigated in 41 normal adults after double-labeling with tetracycline.

The findings in the static parameters of bone resorption and formation surfaces confirm our previous results (4) and corroborate those of Meunier, Edouard, and Courpron (6, 8). Like Meunier and Edouard (6) we found a higher fraction of formative surfaces in males than in females, but unlike Meunier et al. (7) we found in the present investigation a similar difference in the fraction of resorptive surfaces. The static parameters suggest either a higher

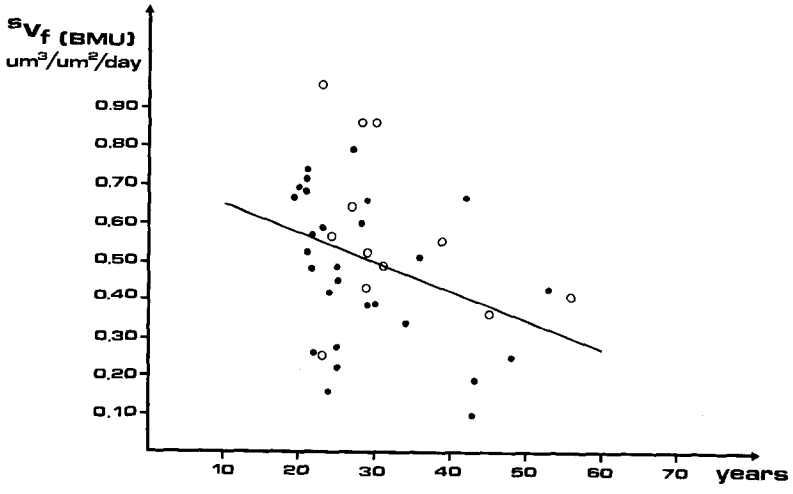


**Fig. 1.** Correlation between bone formation rate at BMU level $^{s}V_{f(BMU)}$ in $\mu m^3/\mu m^2/day$ and age in years in the two sexes ($y = -0.01x + 0.72$). ○ males; • females

activation frequency of new remodeling cycles in trabecular bone in males than in females or a prolonged average life span of both the resorptive and formative phase of the remodeling cycle (1). A third possibility is a greater average extent of the single BMU in males than in females.

Among the dynamic parameters, the appositional rate, which reflects the osteoblastic activity at the active cellular level, proved to be sex- and age-independent with a mean value and a standard deviation very close to that found in a smaller group of individuals by Meunier (9). In that investigation the mean age was considerably higher than in the present, which further confirms that the appositional rate in trabecular bone is age-independent. This is in contrast to previous findings in cortical bone (1) showing a decrease in the appositional rate with increasing age. The bone formation rate at BMU level, which reflects the average activity of active as well as inactive osteoblasts, was in a similar way sex-independent because of parallel variations in the fractional labeled surfaces and the fractional formation surfaces. The bone formation rate at tissue level, however, was found to be higher in the males than in the females, because of the higher fraction of labeled surfaces in the male group.

These results indicate, provided a constant amount and mean thickness of bone is being turned over per remodeling cycle in the two sexes, that the activation frequency of new remodeling cycles is higher in males than in females and that the life span of the formative phase of the remodeling cycle is equal in the two sexes. It should be underlined, however, that the amount and mean thickness of trabecular bone turned over per remodeling cycle have not been determined in the present study. The assumption that these moieties of bone are sex-independent is based on measurements in cortical bone (1).

In the female group an age-related decrease was found in the fraction of labeled surfaces and in the bone formation rate at both BMU level and tissue level. Lack of any significant changes with age in these parameters in the male group may be explained by the smaller number of individuals in this group. However, the observed decrease in bone formation rate at tissue level with age among the females may contribute to the more rapid loss of bone in females than in males with age (4, 8).

The demonstrated age- and sex-dependent variations in the dynamic parameters emphasize the importance of a more elaborate normal material including younger and older age groups.

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