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Intracortical remodeling during human bone development—A histomorphometric study

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Abstract

Although intracortical bone remodeling is a key aspect of bone physiology, very little is known about this process during human bone development. In this study, we examined transiliac bone samples from 56 individuals between 1.5 and 22.9 years of age (31 female; tetracycline labeling present in 42 subjects) who did not have evidence of metabolic bone disease. Parameters of osteonal structure (osteon diameter, wall thickness, diameter of osteonal canals) and dynamic measures of intracortical remodeling were determined separately for the external and internal cortex. We found that measures of osteonal structure were independent of age. However, the percentage of osteons showing metabolic activity was lower in the older study subjects, corresponding to a slowdown in the turnover of cortical bone. Most dynamic parameters of bone metabolism were higher in the internal cortex than in the external cortex. Cortical porosity was negatively associated with age on the external, but not on the internal cortex. The bone forming activity that refills the remodeling cavities seemed to favor the side of the osteonal canal that faced towards the periosteum. In summary, intracortical remodeling activity varies markedly during bone development, and is slightly asymmetric between the two cortices of an iliac bone specimen. Remodeling during development is thus an age-dependent process that varies with location even within the same bone.

Keywords: Bone metabolism; Bone histomorphometry; Cortex; Ilium; Remodeling

Introduction

Bone tissue is continuously renewed through remodeling, which consists of the sequential removal and replacement of small packets of bone [1]. In accordance with the fundamental importance of this process for bone health and disease, remodeling is one of the most intensely studied aspects of bone biology.

The basic features of the remodeling process were first described by Frost's group, who examined cortical bone in rib samples [2]. Later, when the ilium became the standard site for obtaining bone biopsy samples in humans, the focus of clinical histomorphometric analysis shifted from cortical to trabecular bone. However, cortical bone makes up about 80% of the bone mass in the human body, and therefore intracortical bone remodeling is without doubt an important aspect of bone physiology and pathophysiology throughout life. For example, high cortical porosity due to elevated intracortical remodeling rates has been implicated in the high incidence of fractures both in children and in postmenopausal women [3,4].

Iliac bone histomorphometry in healthy adults has shown that cortical porosity increases with age in both sexes [5]. This is due to a larger size of osteonal canals, which in turn is explained by a negative remodeling balance. Much less information is available about intracortical remodeling during human bone development. More than 40 years ago, Epker et al. noted that intracortical remodeling activity was considerably higher in the second than in the fourth decade of life, but detailed analyses are lacking [6].

We have previously reported on histomorphometric results in the cancellous bone of children, adolescents and young adults who had no signs of a generalized bone disorder [7,8].

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More recently, we have evaluated bone cell activity on periosteal, endocortical and intracortical bone surfaces in the same group of individuals [9]. In the present study, we extend these latter analyses to provide a more detailed assessment of the intracortical remodeling process during human bone development.

Subjects and methods

Subjects

The study population comprised 56 Caucasian subjects (31 female, 25 male; age 1.5 to 22.9 years), in whom bone biopsies were obtained during surgery for various orthopedic conditions. All subjects were ambulatory, had normal renal function as assessed by measurement of serum creatinine and had no evidence of any metabolic bone disease. Orthopedic conditions included lower limb deformities ($n=14$), scoliosis ($n=24$), clubfeet ($n=4$) and other problems that required corrective surgery (exostoses, cubitus valgus, equinovarus of the foot) ($n=14$). None was immobilized prior to surgery or received medications known to affect bone metabolism.

As described earlier, the original study population had included 58 individuals [7]. However, biopsy specimens from two patients had to be excluded for the present analysis, because neither cortex was sufficiently preserved for histomorphometric analysis. The study cohort was selected so that the ages were reasonably evenly distributed. It was not a goal of this study to assess gender-specific differences. Therefore, there was an uneven gender-distribution among age groups. Informed consent was obtained in each instance from the subject and/or a legal guardian. The study protocol was approved by the Ethics Committee of the Shriners Hospital for Children.

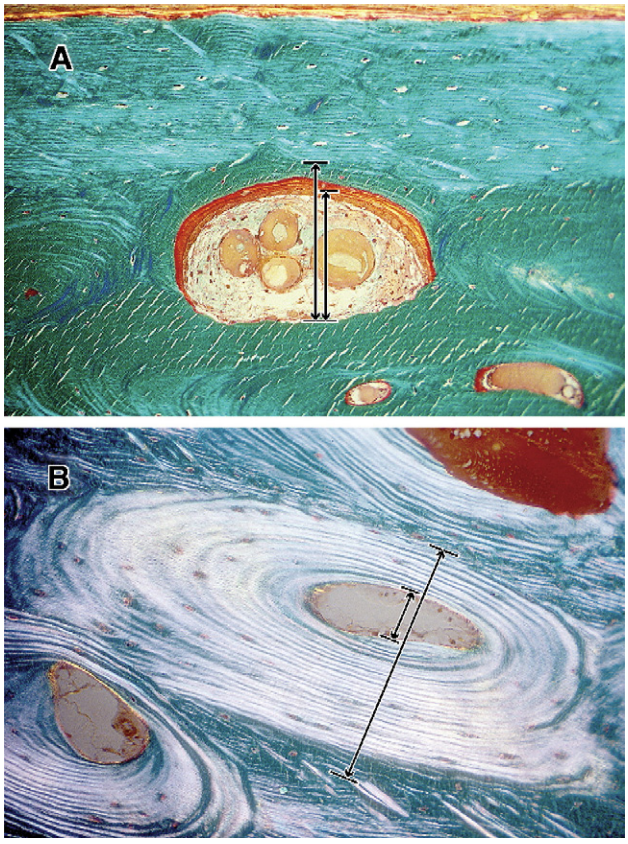

Fig. 1. Examples of active (A) and quiescent (B) osteons. In each case, the longer arrow shows the osteon diameter, the shorter arrow indicates the canal diameter. Goldner staining. Original magnification 200. (A) The osteonal canal is lined by eroded surface (lower part) and by osteoid (upper part). The periosteal surface of the cortex is at the upper border of the picture (not shown). The osteoid is thus located on the part of the osteonal canal facing the periosteum. (B) The osteonal canal is in an eccentric position. Here the periosteal surface of the cortex is at the lower border of the picture (not shown).

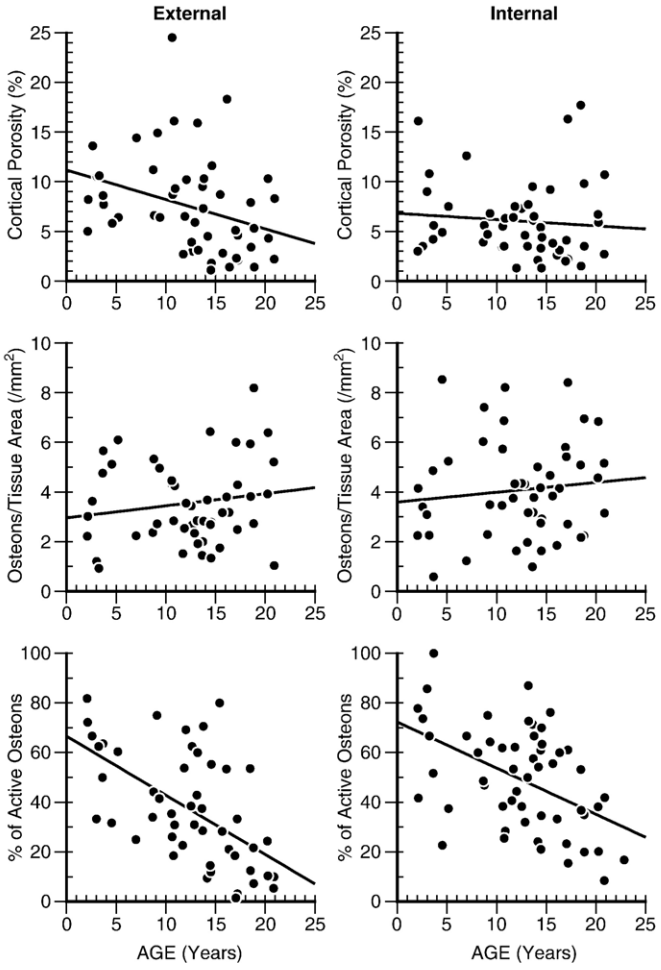

Fig. 2. Age-dependency of structural intracortical parameters in the external and internal cortex of transiliac bone samples.

Bone biopsy

Full-thickness transiliac bone biopsies were obtained with a Bordier trephine (5 or 7 mm core diameter) under general anesthesia, from a site located 2 cm behind the anterior superior iliac spine. In 42 subjects, biopsies were collected on the 4th or 5th day after dual labeling with demeclocycline (Declomycin®; 15–20 mg/kg/day taken orally during two 2-day periods separated by a 10-day free interval). No side effects of the biopsy procedure were noted other than transient local discomfort.

Biopsy specimens were fixed in 10% phosphate-buffered formalin (pH 7.1) and kept at room temperature for 48–72 h. They were then dehydrated in increasing concentrations of ethanol, cleared with xylene and embedded in methylmethacrylate. After proper polymerization, the blocks were trimmed with an Isomet diamond saw (Buchler, IL, USA) to remove the excess of plastic. Undecalcified 6 μm thick sections were cut with a Polycut E microtome (Reichert-Jung, Heidelberg, Germany), placed on chromium-alum gelatine coated slides and left to dry at 50°C for 18 h. For each specimen, 2–5 series of consecutive sections were cut at least 150 μm apart. The sections were deplastified with ethylene glycol monoethyl acetate to allow for optimal staining. In each series, three consecutive sections were selected. Two were

Table 1
Linear regression analyses on the relationship between histomorphometric results and age

	External					Internal				
	N	Intercept	Slope	*R*	*P*	*N*	Intercept	Slope	*R*	*P*
Structural parameters										
Cortical porosity (%)	50	11.2	−0.30	−0.32	0.01	55	6.8	−0.07	0.10	0.24
Osteons per tissue area (/mm^2)	50	2.96	0.049	0.16	0.14	55	4.09	0.036	0.09	0.27
Percentage of quiescent osteons (%)	50	15	3.3	0.70	<0.001	55	19	2.3	0.54	<0.001
Percentage of active osteons (%)	50	85	−3.3	−0.70	<0.001	55	81	−2.3	−0.54	<0.001
Quiescent osteon diameter (μm)	50	166	0.54	0.09	0.27	53	144	0.83	0.15	0.15
Active osteon diameter (μm)	50	193	−0.46	−0.06	0.34	55	161	0.64	0.10	0.24
Quiescent canal diameter (μm)	50	39	0.058	0.02	0.44	53	26	0.32	0.16	0.13
Active canal diameter (μm)	50	129	−1.63	−0.20	0.08	55	100	−0.91	−0.18	0.09
Average canal diameter (μm)	50	87	−1.7	−0.42	0.001	55	77	−1.4	−0.33	0.008
Wall thickness (μm)	50	64	0.24	0.10	0.24	53	59	0.25	0.10	0.23
Dynamic parameters										
Bone formation rate per bone surface (μm^3/μm^2/year)	37	163	−6.8	−0.73	<0.001	41	147	−3.1	−0.30	0.03
Bone formation rate per bone volume (%)	37	54	−2.5	−0.73	<0.001	41	43	−1.1	−0.33	0.02
Mineralizing surface per bone surface (%)	37	35.8	−1.3	−0.62	<0.001	41	29.9	−0.18	−0.09	0.28
Mineral apposition rate (μm/day)	37	1.45	−0.032	−0.52	<0.001	41	1.36	−0.026	−0.51	<0.001
Activation frequency (/year)	37	2.9	−0.13	−0.75	<0.001	41	2.5	−0.055	−0.31	0.03
Mineralization lag time (day)	37	8.2	0.26	0.25	0.07	41	7.3	0.27	0.37	0.008
Formation period (day)	37	39	5.0	0.40	0.007	41	71	1.8	0.23	0.07

stained with either toluidine blue (pH 3.7) or Goldner Trichrome, and the third mounted unstained for fluorescence microscopy.

Histomorphometry

Cortical analyses were performed separately for external and internal cortices. The in situ orientation of the sample had been marked at the time of biopsy by cutting off muscle tissue from the internal cortex after expelling the sample from the biopsy needle. It was therefore possible to identify the external cortex in the sectioned sample by the presence of abundant muscle cells attached to the periosteal surface. In some samples, only one of the cortices was sufficiently well preserved to be suitable for analysis, explaining the difference in the number of external and internal cortices that were analyzed. The following standard primary measures were obtained: Cortical tissue area, cortical bone area, bone perimeter, double label perimeter, interlabel distance (measured at multiple locations spaced by 50 μm) and single label perimeter.

In addition to these routine measures, osteonal structure was assessed. Osteon diameter and osteonal canal diameter were measured as the shortest diameter of the entire osteon and of the canal cross-section, respectively, as described by Brockstedt et al. [5] (Fig. 1). Osteon diameter and osteonal canal diameter were measured separately for quiescent secondary osteons and 'active' secondary osteons. Osteons were classified as 'quiescent' when their canal was covered by lining cells and osteoid was absent. Osteons were regarded as 'active', when osteoid or erosions were present on the canal surface. Wall thickness was calculated for quiescent secondary osteons as the difference between osteon diameter and osteonal canal diameter divided by two. Measures of osteonal structure were limited to intact osteons circumscribed by a cement line, as osteonal diameter cannot be determined in osteonal fragments.

Active osteons were further classified according to the aspect of their canals. When the canal surface was eroded in its entire circumference, the osteon was categorized as resorptive. When the canals were completely covered by osteoid, the osteon was classified as formative. When both erosions and osteoid were present, the osteon was classified as 'mixed' (Fig. 1). The orientation of bone formation within mixed osteons was also assessed, according to whether the osteoid was predominantly located on the half of the osteonal canal facing the periosteum or the half facing the endocortical surface (Fig. 1).

All analyses were carried out at a magnification of 200. A digitizing table with the Osteomeasure® software (Osteometrics Inc., Decatur, GA, USA) was used for all measurements. Three-dimensional parameters were derived from the primary measures using standard formulae [7]. Nomenclature and abbreviation follows the recommendations of the American Society for Bone and Mineral Research [10].

Statistical analyses

Comparisons between results on the external and internal cortex were evaluated for significance using paired *t*-tests. The association between histomorphometric results and age was assessed by linear regression analysis.

Table 2
Comparison between histomorphometric results on external and internal cortices

	N	External	Internal	*P*
Structural parameters				
Cortical porosity (%)	49	7.6±5.0	6.0±3.8	0.08
Osteons per tissue area (/mm^2)	49	4.2±1.8	4.2±2.1	0.91
Percentage of quiescent osteons (%)	49	56±25	48±23	0.007
Percentage of active osteons (%)	49	44±25	52±23	0.007
Quiescent osteon diameter (μm)	47	174±32	158±28	0.003
Active osteon diameter (μm)	49	189±40	170±35	0.02
Quiescent canal diameter (μm)	47	41±15	30±11	<0.001
Active canal diameter (μm)	49	110±44	89±27	0.003
Average canal diameter (μm)	49	66±22	61±23	0.13
Wall thickness (μm)	47	67±13	64±12	0.17
Dynamic parameters				
Bone formation rate per bone surface (μm^3/μm^2/year)	37	77±48	103±56	0.006
Bone formation rate per bone volume (%)	36	22±18	28±16	0.07
Mineralizing surface per bone surface (%)	37	19±11	26±11	0.001
Mineral apposition rate (μm/day)	37	1.04±0.32	1.00±0.31	0.59
Activation frequency (/year)	36	1.3±0.9	1.7±0.9	0.01
Mineralization lag time (day)	36	11.5±5.6	10.8±3.8	0.53
Formation period (day)	36	103±66	98±41	0.71

Values are mean±SD. *P* values were calculated by paired *t*-test.

R values represent Pearson's correlation coefficient. All tests were two-tailed and throughout the study $P<0.05$ was considered significant. These calculations were performed using the SPSS software, version 11.5 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Osteonal structure

No age-dependency was found on either cortex for osteonal density (number of intact osteons per tissue area), osteon diameter, wall thickness and the diameter of active and quiescent osteonal canals (Fig. 2; Table 1). However, the percentage of active osteons decreased with age. As osteonal canals exhibiting metabolic activity are larger than quiescent ones, the age-dependent decrease in the proportion of active osteons translated into a shrinking average canal diameter (Table 1). These changes were more pronounced on the external than on the internal cortex. Consequently, cortical porosity significantly decreased with age on the external, but not on the internal cortex.

Average cortical porosity in the entire group was similar between the external and internal cortex (Table 2). Nevertheless, osteonal structure clearly differed between the two cortices, as the proportion of active osteons was higher in the internal cortex and both active and quiescent osteonal canals had a smaller diameter on the internal than on the external cortex.

Active osteons were analyzed in more detail. On average, 13% (SD 12%) of active osteons had only eroded surfaces on their canals, whereas in 43% (SD 19%) the canals were entirely covered by osteoid. The remaining 44% (SD 20%) of active osteons were classified as 'mixed', as their canal surfaces were partly eroded and partly covered by osteoid. This distribution was similar between the internal and external cortices.

Mixed osteons were further subclassified according to whether the osteoid was located predominantly on the half of the osteonal canal facing towards to the periosteum or the half closer to the trabecular compartment (Fig. 1). It was found that in an average of 58% (SD 25%) of osteons, the osteoid was located predominantly in the periosteal half of the canal, whereas the osteoid faced towards the trabecular compartment in only 9% (SD 13%) of osteons. In the remaining mixed osteons, there was no clear preponderance of osteoid on either half of the osteonal canal. This asymmetry was similar between the two cortices and did not vary with age. Fig. 3 summarizes these findings in a schematic manner.

Dynamic measures of intracortical remodeling

Bone formation rate per bone surface was negatively associated with age in both the internal and the external cortex (Table 1). On the external cortex, this decrease was the combined result of decreases in both the mineralizing surface and the mineral apposition rate, whereas on the internal cortex

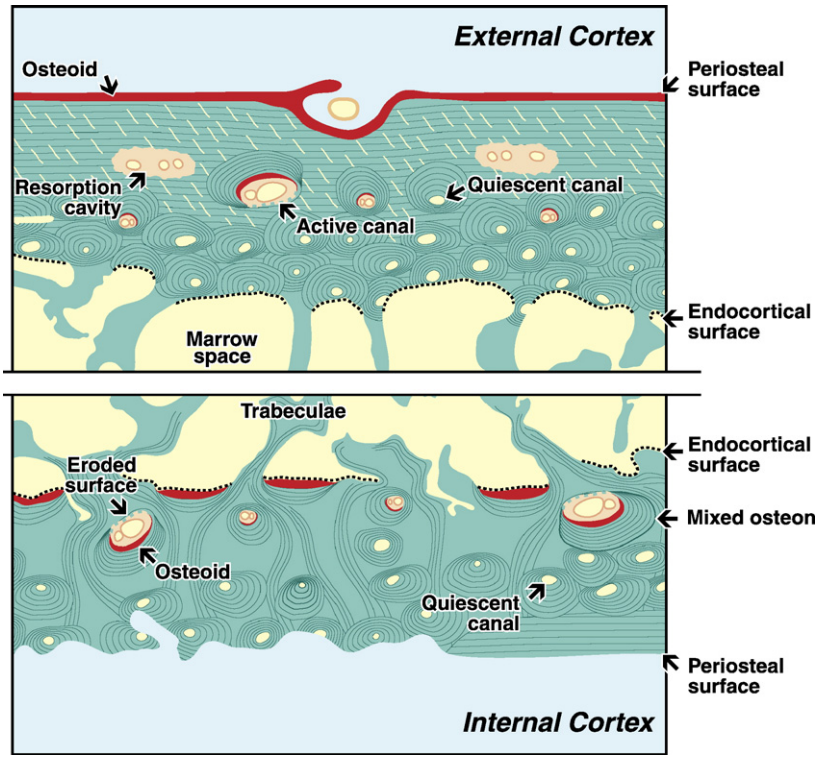

Fig. 3. Schematic drawings of the external and internal iliac cortex. Osteons are indicated as target-shaped structures. Mixed osteons have canals that are partly lined by erosions and partly by osteoid. In such mixed osteons, the osteoid is predominantly located on the half of the canal that is facing towards the periosteal surface. In mature (quiescent) osteons, the canal is usually in an eccentric position, located in the half of the osteon that faces towards the endocortical surface.

only mineral apposition rate declined. The overall effect of these changes was that the yearly turnover of cortical bone, as estimated from bone formation rate per bone volume decreased significantly with age on both cortices. Activation frequency decreased significantly with age on both cortices (Table 1), whereas mineralization lag time increased significantly on the internal cortex only (Table 1). Formation period increased only on the external cortex.

Comparing the two cortices, average bone formation rate per bone surface, mineralizing surface and activation frequency were higher in the internal cortex (Table 2). In addition, there was a trend towards higher values for bone formation rate per bone volume in the internal cortex. No significant difference between cortices was found for mineral apposition rate, mineralization lag time and formation period.

To put these results into perspective, the developmental changes in cortical bone formation activity were compared to those in cancellous bone. As reported before, the average bone formation rate per bone surface in the entire study cohort was higher in cortical bone (77 $\mu m^3/\mu m^2$/year [SD 49] in the external cortex, 103 $\mu m^3/\mu m^2$/year [SD 56] in the internal cortex) than in cancellous bone (39 $\mu m^3/\mu m^2$/year [SD 17]) [9]. However, regression analyses show that between 2 and 20 years of age, bone formation rate decreased more rapidly in the external cortex (by 82%) than in cancellous bone (by 70%) (Table 1; Fig. 4). Consequently, the regression equations shown in Table 1 and Fig. 4 predict that the bone formation rate per bone surface of an average 20-year-old subject is similar in the external cortex and in cancellous bone (27 $\mu m^3/\mu m^3$/y in both cases). In the internal cortex, bone formation rate decreases much less between 2 and 20 years of age (by 40%) than in the external cortex and in cancellous bone. Regression analysis therefore predicts that at 20 years of age bone formation rate in the internal cortex is still about three times higher (85 $\mu m^3/\mu m^3$/year) than in the other two remodeling compartments.

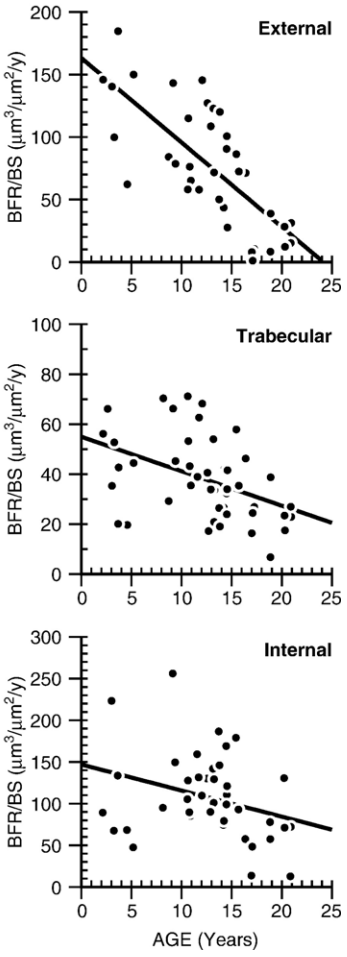

Fig. 4. Age-dependency of bone formation rate per bone surface (BFR/BS) in the two cortices and the cancellous compartment of transiliac bone samples. The regression equations for the cortices are shown in Table 1. For cancellous bone, the regression equation was: BFR/BS = 55–1.4 × age.

Discussion

In the present study, we found that intracortical remodeling activity varies markedly during bone development, but that the structural features of osteons (osteon diameter, wall thickness and the diameter of active and quiescent osteonal canals) remain constant. The density of intact osteons also remained stable throughout the age range of the present study, which is in accordance with studies of the 6th rib [11].

Even though the diameter of active and quiescent osteonal canals remained unchanged during bone development, the average size of osteonal canals decreased. The explanation for this apparent paradox is that the proportion of metabolically active osteons decreased with age. Active canals are always larger than quiescent canals, because the intracortical remodeling activity consists either of making canals bigger (at resorptive sites) or bringing larger canals back to the size of quiescent canals (at formative sites). A smaller proportion of active canals therefore translates into a smaller average canal size and thus into lower cortical porosity.

The higher proportion of active canals in the younger study participants was a direct consequence of the higher activation frequency in these individuals, indicating that, in any given time period, more remodeling cycles occur in younger children. Nevertheless, all aspects of osteonal structure (osteon diameter, osteonal canal diameter, wall thickness) were independent of age. It therefore appears that the difference between the amount of bone resorbed and formed during a remodeling cycle (the so-called remodeling balance) remains constant during cortical bone development.

Intracortical remodeling differed somewhat between the external and the internal cortex. Osteonal dimensions were larger in the external cortex, whereas the average surface-based bone formation rate was higher in the internal cortex. Interestingly, a similar difference in bone formation rate between the two cortices has been reported in adult women suffering from osteoporosis [12]. It is thus possible that the asymmetry between the two cortices is not limited to the period of bone growth and development, but rather is a general feature of iliac bone. The age-dependent changes in remodeling activity

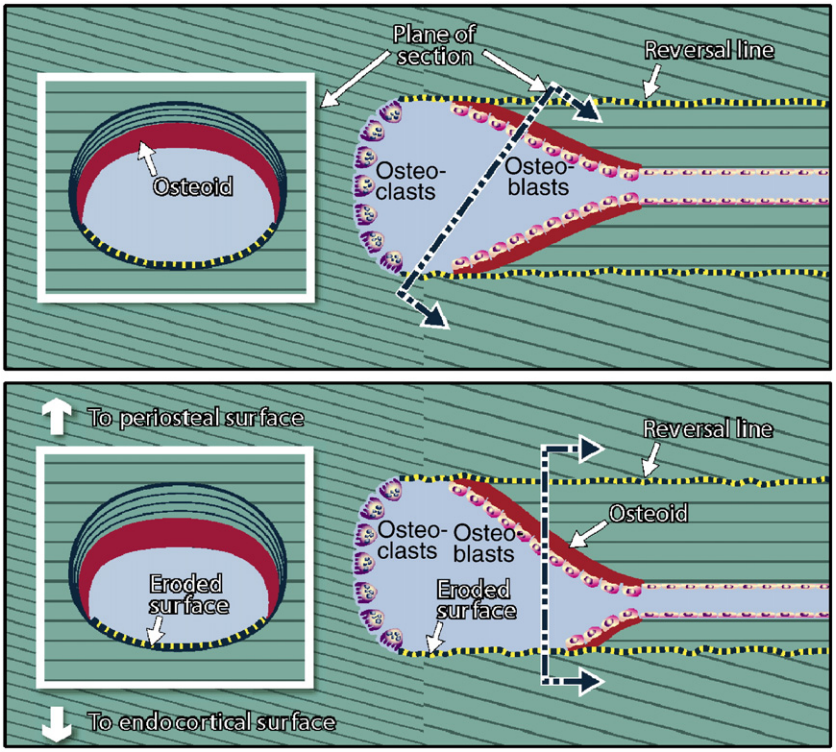

Fig. 5. Scenarios to explain the occurrence of osteonal canals that are partly lined by erosions and partly by osteoid. Upper panel: A symmetric osteon is sectioned obliquely. Lower panel: An asymmetric osteon is sectioned perpendicularly to the main axis.

were different in cortical and trabecular bone, but overall bone formation rate per bone surface was higher in cortical than in cancellous bone. This again is similar to observations that have been made in healthy adults [13]. The site-specificity of remodeling points to the importance of local factors in the control of bone remodeling.

The highly site-specific nature of the intracortical remodeling process is also highlighted by the observation that the metabolic activity within a single osteon was clearly asymmetric. In almost half of the metabolically active osteons, the canals were lined by both eroded surface and osteoid. This might be explained as the result of an oblique section through an osteon (Fig. 5, upper panel). However, this hypothesis would not explain our second observation, namely that osteoid was far more frequently found on the half of the osteonal canal facing the periosteum.

More than four decades ago, Frost's group had observed osteonal canals containing both eroded and osteoid surfaces and had suggested that these asymmetric osteons were moving through the cortex ('waltzing osteons') [14]. Yet, we very rarely observed the presence of osteoclasts in such mixed osteons (data not shown). The presence of eroded surface in such canals does not necessarily mean that bone resorption was actively continuing. The same eroded aspect would be expected, if bone formation started earlier on the side of the osteonal canal that faces the periosteum (Fig. 5 lower panel). This scenario would also explain why quiescent osteonal canals are usually located in an eccentric position within the osteon (Figs. 1B and 3). The mechanism leading to such an asymmetric bone formation activity within an osteonal canal is obscure at present.

This study has several limitations. The samples were obtained during surgical procedures for localized conditions, and therefore study participants were not strictly healthy. However, none of the study subjects had signs of a generalized bone disorder. It is therefore plausible to assume that the results largely reflect the effects of bone development rather than these patients' conditions. Other limitations are that the sexes are unequally distributed in the various age ranges and that pubertal status was not recorded at the time of biopsy sample collection. The present material therefore does not allow addressing the sex-specific effects of puberty on intracortical remodeling.

In summary, the present study shows that intracortical remodeling activity varies markedly during bone development, but that the structural features of osteonal bone remain constant during that time. Bone cells behave differently in the external and internal cortex and in the cancellous compartment of an iliac bone sample. The bone forming activity that refills the remodeling cavities seems to favor the side of the osteonal canal that faces towards the periosteum. All these observations suggest that there must be tight local control of bone cell activity during intracortical remodeling in young subjects.

Acknowledgments

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