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Static and Dynamic Bone Histomorphometry in Children With Osteogenesis Imperfecta

F. RAUCH,[1] R. TRAVERS,[1] A. M. PARFITT,[2] and F. H. GLORIEUX[1]

[1]*Genetics Unit, Shriners Hospital, and the Departments of Surgery and Pediatrics, McGill University, Montréal, Canada*
[2]*Division of Endocrinology and Metabolism, University of Arkansas for Medical Sciences, Little Rock, AR, USA*

Osteogenesis imperfecta (OI) is a genetic disorder characterized by increased bone fragility and low bone mass. Four clinical types are commonly distinguished. Schematically, type I is the mildest phenotype, type II is usually lethal, type III is the most severe form compatible with postnatal survival, and type IV is moderately severe. Although mutations affecting collagen type I are responsible for the disease in most patients, the mechanisms by which the genetic defects cause abnormal bone development have not been well characterized. Therefore, we evaluated quantitative static and dynamic histomorphometric parameters in tetracycline-labeled iliac bone biopsies from 70 children, aged 1.5 to 13.5 years, with OI types I (n = 32), III (n = 11), and IV (n = 27). Results were compared with those of 27 age-matched controls without metabolic bone disease. Biopsy core width, cortical width, and cancellous bone volume were clearly decreased in all OI types. Decreased cancellous bone volume was due to a 41%–57% reduction in trabecular number and a 15%–27% lower trabecular thickness. Regression analyses revealed that trabecular number did not vary with age in either controls or OI patients, indicating that no trabecular loss occurred. The annual increase in trabecular thickness was 5.8 μm in controls and 3.6 μm in type I OI, whereas no trabecular thickening was evident in type III and IV OI. Wall thickness, which reflects the amount of bone formed during a remodeling cycle, was decreased by 14% in a subgroup of 17 type I OI patients, but was not determined in the other OI types. The remodeling balance was less positive in type I OI than in controls, and probably close to zero in types III and IV. Surface-based parameters of bone remodeling were increased in all OI types, indicating increased recruitment of remodeling units. No defect in matrix mineralization was found. In conclusion, there was evidence of defects in all three mechanisms, which normally lead to an increase in bone mass during childhood; that is, modeling of external bone size and shape, production of secondary trabeculae by endochondral ossification, and thickening of secondary trabeculae by remodeling. Thus, OI might be regarded as a disease in which a single genetic defect in the osteoblast interferes with multiple mechanisms that normally ensure adaptation of the skeleton to the increasing mechanical needs during growth. (Bone 26:581–589; 2000)

Key Words: Bone fragility; Children; Histomorphometry; Modeling; Osteogenesis imperfecta (OI); Remodeling.

Introduction

Osteogenesis imperfecta (OI) is a heritable disorder that is characterized by increased bone fragility and low bone mass.[30] Often, bone shape is also abnormal with metaphyseal flaring and thin diaphyses. Four clinical types are commonly distinguished.[32] Type I OI comprises patients with a mild presentation and a low normal or slightly reduced height, whereas type II is usually lethal in the perinatal period. Type III OI is the most severe form in children surviving the neonatal period. These patients have a well-defined phenotype, including extremely short stature, progressive bone deformity and growth plate fractures. Patients who do not fit into one of the aforementioned categories are usually classified as having type IV OI.

In most OI patients, the disease is caused by mutations in either the procollagen type I α1 or the procollagen type I α2 gene.[30] The pathophysiological effects of these mutations on the skeleton are not completely understood. The obvious direct effect is a perturbed osteoblast function, the cell type that expresses the mutated gene product in bone.[30] In addition, there are indirect effects, as abnormalities in collagen production and secondary changes in the organic and inorganic bone matrix components[4,9,18,31,35,36] alter the environment for all cell types in bone. However, these indirect consequences of matrix abnormalities are not well characterized.

To elucidate how these direct and indirect effects of OI mutations affect bone development, more histomorphometric data are necessary. Quantitative histomorphometry is the only available method to study bone cell function within the in vivo structural context. As of yet, there is a surprising scarcity of information on the bone tissue characteristics of OI. Early qualitative studies on bone histology in OI evaluated samples obtained at sites of deformity or fracture during surgical procedures, and thus results were obscured by injury or repair reactions.[29] The first quantitative studies used bone specimens from the rib and were limited to the analysis of a few parameters.[1,37]

Few data have been obtained using the current standard bone histomorphometric procedure; that is, quantitative analysis of tetracycline-labeled iliac bone samples. Apart from several case reports, results from three small series of patients have been

Address for correspondence and reprints: Dr. Francis H. Glorieux, Genetics Unit, Shriners Hospital for Children, 1529 Cedar Avenue, Montréal, Qc H3G 1A6, Canada. E-mail: glorieux@shriners.mcgill.ca

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Table 1. Structural parameters

	Controls	Type I	Type III	Type IV	*p* (all)	*p* (OI)
n (M/F)	27 (17/10)	32 (20/12)	11 (6/5)	27 (14/13)		
Age (yr)	7.6 ± 3.9	7.6 ± 3.8	7.9 ± 2.9	7.5 ± 3.6	0.99	0.95
C.Wi (mm)	5.9 [4.8–7.4]	4.3 [2.7–5.5]	2.9 [2.4–4.3]	2.8 [2.3–3.9]	<0.0001	0.07
Ct.Wi (μm)	870 ± 340	520 ± 200	313 ± 200	370 ± 170	<0.0001	0.003
BV/TV (%)	21.2 ± 4.8	11.0 ± 5.2	6.4 ± 2.9	7.2 ± 4.1	<0.0001	0.03
Tb.Th (μm)	124 ± 28	105 ± 25	91 ± 20	98 ± 26	<0.0001	0.28
Tb.N (/mm)	1.72 ± 0.24	1.03 ± 0.39	0.72 ± 0.28	0.74 ± 0.32	<0.0001	0.006
BS/BV (mm^2/mm^3)	17.9 ± 3.8	19.8 ± 4.7	27.7 ± 9.6	21.9 ± 4.4	<0.0001	0.0007

Data expressed as mean ± SD or median [interquartile range]; *p* values obtained by analysis of variance (ANOVA) or Kruskal–Wallis test, as appropriate; *p* (all), significance of variation between all four groups of subjects; *p* (OI), significance of variation between OI groups.
KEY: BS/BV, bone surface per bone volume; BV/TV, cancellous bone volume; C.Wi, core width; Ct.Wi, cortical width; Tb.N, trabecular number; Tb.Th, trabecular thickness.

published.[2,20,34] One of these[20] examined adults, the other two included nine and four children, respectively. These studies provided valuable preliminary information, but were hampered by small sample numbers and the lack of adequate control groups.

Here we present quantitative static and dynamic histomorphometric data of tetracycline-labeled iliac bone specimens from 70 children, between 1.5 and 13.5 years of age, with types I, III, and IV OI. Results are compared with those of 27 age-matched controls without metabolic bone disease. The aims of this study are to assess the abnormalities of bone structure in OI and to analyze their age-dependency during bone development. Furthermore, we attempt to explain the observed structural abnormalities on the basis of indices reflecting bone cell function.

Subjects and Methods

Subjects

The patient population comprised 70 children with OI, aged 1.5 to 13.5 years (**Table 1**). The patients were clinically classified according to the criteria established by Sillence.[32] All patients had a history of frequent fractures and low bone mass, as assessed by dual-energy X-ray absorptiometry. Family history was positive for increased bone fragility in many cases, but this was not a prerequisite for the diagnosis of OI. Type I was diagnosed in patients who had a mild course, were of normal or slightly reduced stature, and had little or no bone deformity. Type III patients had high fracture rates, severe short stature, marked bone deformities, and a typically triangular face. The type IV group comprised patients with a moderate-to-severe course, variable short stature, and moderate-to-severe bone deformities. Only children who could unambiguously be assigned a specific type of OI were included in the present analysis. We did not include children in the type IV OI group, who presented features of the newly identified type V OI,[14] or who had a recessive form of OI.[15] None of the participants of the present study had received pharmacological treatment other than vitamin and calcium supplementation in the 6 months preceding the biopsy.

The control population consisted of 27 age-matched children (age 1.5–13.7 years; Table 1), in whom iliac bone biopsies were obtained during various orthopedic procedures for conditions such as lower limb deformities, scoliosis, clubfeet, and other problems, which require corrective surgery. These individuals were free of metabolic bone disease and were biopsied with the aim to establish reference data, as described elsewhere.[16] Some of the samples from OI patients and the control group have been investigated previously in backscattered electron microscopy studies.[4,18] Informed consent was obtained in each instance from the subject and/or a legal guardian, as appropriate. The study protocol was approved by the ethics committee of the Shriners Hospital.

Bone Biopsy and Histomorphometry

Transiliac bone samples were collected on days 4 or 5 after dual labeling with demeclocycline (15–20 mg/kg per day taken orally during two 2-day periods separated by a 10-day-free interval). Biopsy preparation and histomorphometric analyses with the exception of wall thickness measurements were performed with the standard procedures used at the Shriners Hospital, as described previously.[16]

Wall thickness was determined on Goldner-stained sections. The distance from osteoid-covered bone surfaces to the abrupt change in collagen fiber orientation, as seen under polarized light, was measured at 50 μm intervals. Wall thickness was calculated from these measurements using the reconstruction method described by Steiniche et al.[33] Lamellae are thinner and less smooth in OI patients than in healthy children (see *Results*). Therefore, it proved very difficult to reliably identify abrupt changes in fiber orientation in many patients with type I disease and almost all OI type III and IV patients. For this reason, wall thickness measurements were limited to controls and a subgroup of 17 patients with type I OI.

Measurements were carried out using a digitizing table with OSTEOMEASURE software (Osteometrics, Inc., Atlanta, GA). Nomenclature and abbreviations follow the recommendations of the American Society for Bone and Mineral Research.[24]

Statistical Analysis

Differences among OI types and controls were tested by analysis of variance (ANOVA) if data followed a gaussian distribution, or by Kruskal–Wallis test for nonnormally distributed data. Comparisons between two groups were done by *t*-test. All tests were two-tailed and, throughout the study, $p < 0.05$ was considered significant. Associations are given as Pearson correlation coefficients. To determine age-dependent confidence intervals (CI) for structural parameters, results of the control group were subjected to regression analyses testing a variety of curve fits (linear, logarithmic, square, cubic, exponential, S model). The type of regression yielding the best fit was used to calculate the 95% CI for the data. Thus, linear regression was used for cancellous bone volume (BV/TV), trabecular thickness (Tb.Th), and trabecular number (Tb.N), whereas an S-model regression ($y = e^{(b0 + b1/x)}$) was used for core width (C.Wi) and cortical

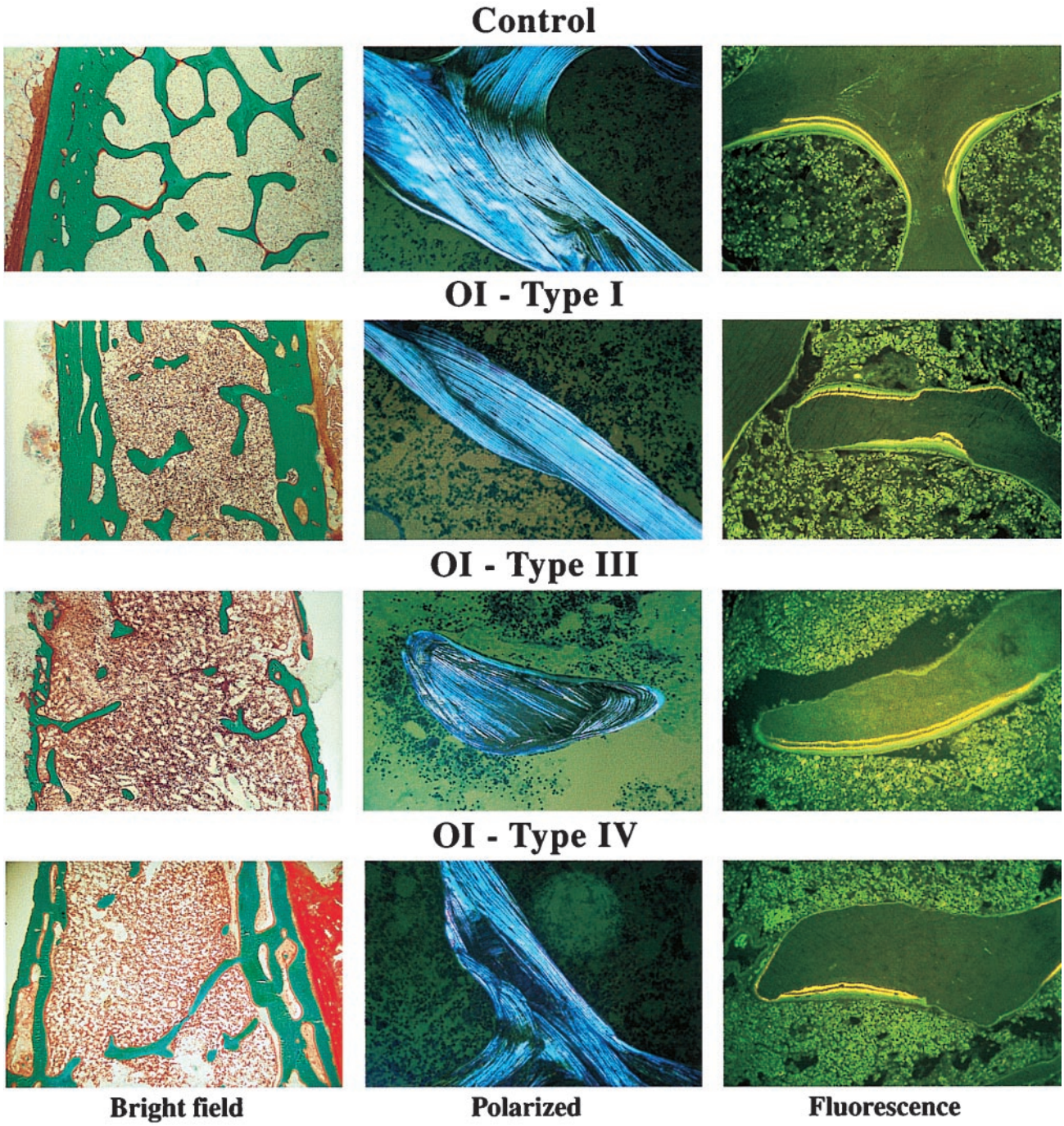

Figure 1. Typical sections of biopsies from a control subject (boy, 9 years) and OI patients (type I: girl, 5 years; type III: boy, 9 years; type IV: boy, 13 years). Original magnifications: left column, ×32, middle column, ×200; right column, ×200.

width (Ct.Wi). These calculations were performed using SPSS software, version 6.0 for WINDOWS (SPSS Inc., Chicago, IL).

Results

Figure 1 shows typical iliac bone sections from a control subject and from patients with OI types I, III, and IV. The decrease in biopsy size and in the amounts of cortical and cancellous bone are readily apparent in the OI samples. As seen under polarized light, a lamellar pattern is visible in the OI types presented here, but lamellae appear thinner and less smooth than in controls. Also, an increased number of osteocytes was evident in most samples, but this was not quantified in the present study. Inspection of the tetracycline labels under fluorescent light does reveals no obvious disturbances of the mineralization process. Quantitative histomorphometric parameters were similar in both genders. Therefore, results of girls and boys were analyzed together.

Structural Parameters

As expected, all indices of bone structure differed significantly between OI groups and controls (Table 1). The width of the

biopsy core (C.Wi) as well as indicators of cortical and cancellous bone mass (Ct.Wi, BV/TV) were clearly decreased. Low cancellous bone volume in the OI groups was due to two factors. First, trabecular thickness was decreased by 15%–27%. Second, there were 41%–57% fewer trabeculae per millimeter of cross section. This was associated with a clearly increased bone surface/volume ratio (BS/BV).

The age variations of the structural parameters are shown in **Figure 2**. Both core width (Figure 2A) and cortical width (Figure 2B) increased with age in healthy children. Core width did not change with age in any OI type ($p > 0.10$ each), but cortical width increased slightly in types I and IV OI. In the cancellous compartment, bone volume increased in controls, but did not change significantly with age in any OI group (Figure 2C). Trabecular thickness was within the 95% confidence interval of controls in all of the 19 OI patients <5 years of age (Figure 2D). However, trabecular thickness did not increase with age in types III and IV and increased less in type I than in controls. Consequently, trabecular thickness was <95% confidence interval of controls in 9 of the 17 OI patients (52%) >10 years of age. The second determinant of cancellous bone volume, trabecular number, exhibited no age dependency in either OI or control groups (Figure 2E).

Comparisons among the three groups of OI patients revealed that the reduction in bone mass parameters generally reflected clinical severity, although there was considerable overlap (Table 1). Both cortical width and cancellous bone volume were greater in type I OI than in the other types. The differences in cancellous bone volume between OI types were mostly due to differences in trabecular number rather than thickness.

Bone Formation Parameters

Histomorphometric parameters reflecting cancellous bone formation are given in **Table 2**. Osteoid thickness tended to be somewhat lower in OI patients. However, the difference between groups was not significant by ANOVA and was of borderline significance, when OI patients were compared with controls as a single group ($p = 0.09$, *t*-test).

All bone surface-based formation indices (OS/BS, Ob.S/BS, MS/BS, BFR/BS) were markedly increased in the three OI groups (Table 2 and **Table 3**). Most noticeable was the 2.2–3.7-fold increase in osteoblast surface. Even though a greater extent of osteoid surface was covered by osteoblasts (Ob.S/OS) in OI patients, mineral apposition rate (MAR) was decreased. A similar decrease was found for adjusted apposition rate (Aj.AR), but due to the higher variability of this parameter the difference vs. controls reached significance only when all OI patients were treated as one group ($p = 0.02$, *t*-test). As for indicators of osteoid mineralization, mineralizing surface per osteoid surface (MS/OS), and mineralization lag time (Mlt), no significant differences between OI patients and controls were found.

To further evaluate osteoblast function, bone formation rate was calculated relative to various referents (Table 3). Bone surface- and volume-based bone formation rates were increased in OI patients. However, related to osteoblast surface, bone formation rate was only about half of the control result. Due to the decrease in bone volume, tissue-based bone formation rates were 30%–40% lower than in controls.

Wall thickness (W.Th), a measure that reflects the amount of bone formed during a remodeling cycle, and parameters derived from it were determined in controls and in a subgroup of patients with type I OI (**Table 4**). As the mean age in these patients was somewhat higher than in the whole group of type I patients, we selected an age-matched group of 17 individuals from the control population to compare wall thickness and derived indices. Wall thickness was 14% lower in the type I OI group than in controls, whereas activation frequency (Ac.f) was 59% higher. In contrast, formation period (FP) was similar in type I OI and controls.

Bone Resorption Parameters

Erosion surface (ES/BS) was increased in types III and IV, but not in type I OI (**Table 5**). Both osteoclast surface (Oc.S/BS) and osteoclast number (N.Oc/B.Pm) were higher in OI groups than in controls.

Discussion

In the present study we analyzed quantitative histological features in the three classical OI types that are compatible with postnatal survival. One of the most obvious abnormalities in OI is decreased bone mass, which has been reported in a number of previous histological and radiological studies.[1,2,7,10,20,28,34,37] Our data provide evidence that there are defects in all three mechanisms, which normally lead to an increase in bone mass during childhood. These are: (1) modeling of external bone size and shape; (2) production of secondary trabeculae by endochondral ossification; and (3) thickening of secondary trabeculae by remodeling. As our analyses focused mostly on secondary trabeculae, the defects in trabecular thickening could be evaluated in detail, whereas evidence for impaired modeling and endochondral ossification was mostly indirect (see later).

Bone Modeling

The external size of the biopsy core did not increase with age in OI patients, and cortical width was generally markedly below normal. The differences between the OI groups and controls appeared to be larger than might be expected from the overall smaller body frame in OI. Similar observations have been made in radiogrammetric studies of the second metacarpal[13,27] and by quantitative computed tomography at the femoral diaphysis.[19] As external bone size and cortical width during growth are determined by modeling processes,[12] these observations suggest a modeling defect in OI. This is an important aspect of the disease, as deficient bone modeling will result in a smaller cross section and thinner cortices of long bones, and thus reduced bone strength. To characterize the modeling defect in more detail, more information is required about bone cell activity on periosteal and endocortical bone surfaces. In addition, as modeling processes are thought to be determined by the muscle forces that act on bone,[12] studies on muscle development in OI might help to understand the bone modeling defect.

Production of Trabeculae

Apart from diminished external size and cortical width, OI is also characterized by decreased cancellous bone mass, which is largely due to decreased trabecular number. Low trabecular number can either result from increased loss or decreased production of trabeculae. Our results do not provide any evidence that children with OI lose secondary trabeculae, as trabecular number remained constant with age. By exclusion, this suggests that fewer secondary trabeculae are produced.

In the ilium—and, by analogy, in the vertebral bodies—secondary trabeculae arise either through "cancellization" of endocortical bone when the cortices drift apart from each other, or through the better known process of endochondral ossification.[3] The external size of the biopsies did not change with age in the OI groups and therefore, cortical cancellization probably did not play a major role in the OI groups. Thus, low trabecular

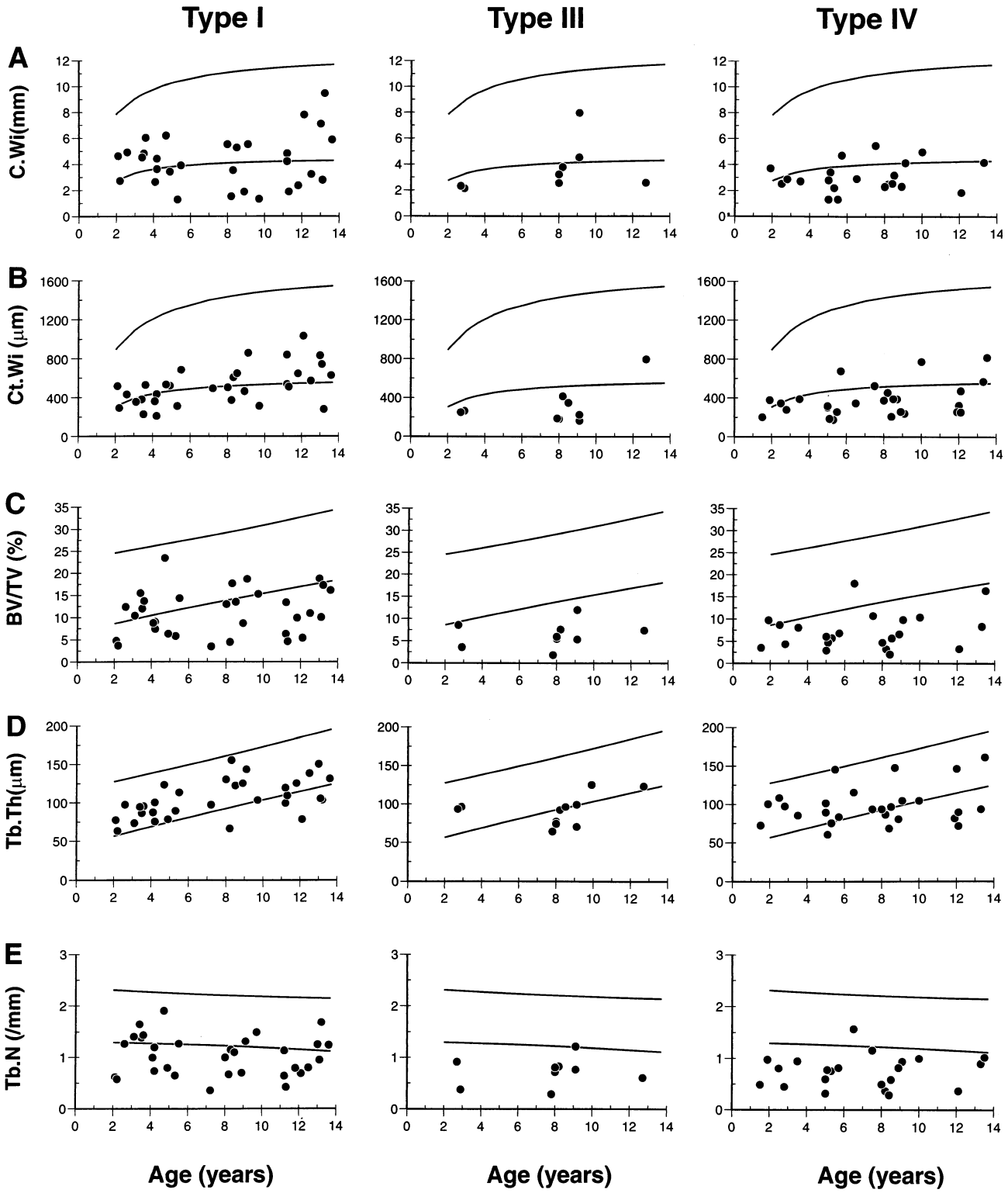

Figure 2. Age variation of structural histomorphometric parameters in OI. Lines indicate the 95% confidence interval of control data. (A) Biopsy core width (C.Wi). Correlations with age: $p = 0.01$ in controls; $p =$ not significant in any OI type. (B) Cortical width (Ct.Wi). Correlations with age: $p = 0.002$ in controls, $p < 0.001$ in type I OI; $p =$ not significant in type III; $p = 0.02$ in type IV. (C) Cancellous bone volume (BV/TV). Correlations with age: $p < 0.0001$ in controls; $p =$ not significant in any OI type. (D) Trabecular thickness (Tb.Th). Correlations with age: $p < 0.0001$ in controls (regression equation: $y = 80.5 + 5.76 * x$); $p = 0.0005$ in type I OI (regression equation: $y = 77.3 + 3.58 * x$), $p =$ not significant in type III and IV OI. (E) Trabecular number (Tb.N). Correlations with age not significant in any group.

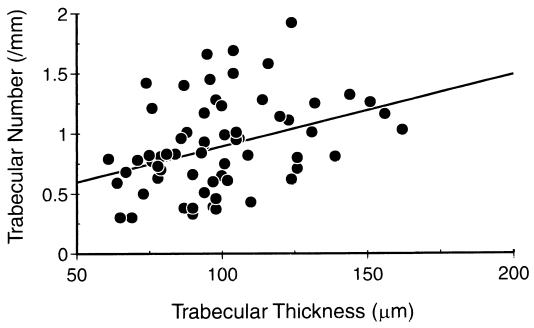

Figure 3. Relationship between trabecular thickness and trabecular number in specimens from all OI patients ($r = 0.36$; $p = 0.002$).

number is most likely the consequence of decreased production during endochondral ossification.

Normally, formation of trabeculae starts at the growth plate-metaphyseal bone interface, where osteoblasts deposit bone matrix on septae made of calcified cartilage.[3] About 80% of these primary trabeculae are quickly removed during the ensuing remodeling to secondary spongiosa.[8] If the function of osteoblasts in the metaphysis is decreased as it is in secondary cancellous bone (see later), thinner primary trabeculae will be formed. Thinner trabeculae are more likely to be perforated by osteoclasts and to be removed completely. Thus, the final outcome of decreased osteoblast activity on mineralized cartilage and primary trabeculae would be the formation of fewer secondary trabeculae.

This model makes several assumptions for which our data do not provide direct evidence. However, it does explain not only why trabecular number is decreased in OI, but also why trabecular thickness in the youngest OI patients is close to normal: The elimination of thin trabeculae during trabecular morphogenesis would tend to "normalize" the average thickness of the remaining trabeculae, yet at the cost of decreased trabecular number. If this scenario is correct, a positive association should be detectable between trabecular thickness and number. This was indeed the case, as shown in **Figure 3**.

Trabecular Thickening

During childhood, cancellous bone volume does not increase in OI patients like it does in healthy children. As shown in the present study, this is mainly due to absent or inadequate thickening of secondary trabeculae. Changes in trabecular thickness are probably the result of remodeling processes. To understand the events leading to decreased or absent trabecular thickening, it may therefore be helpful to remember that bone remodeling has two independently regulated aspects:[22] first, the number of new remodeling teams of osteoclasts and osteoblasts recruited per unit time (the best histomorphometric estimate of this quantity is activation frequency); and, second, the average amount of bone turned over in individual remodeling cycles (the best estimate of the average volume of bone matrix produced in an individual remodeling cycle is mean wall thickness).[25] The amount of bone resorbed is very difficult to measure in an individual bone section. However, a method has been developed to calculate erosion depth in groups of subjects, which will be used in the subsequent calculations.[23] The difference between the amount of bone resorbed by osteoclasts and the amount of bone formed by a team of osteoblasts in the same remodeling cycle is called the remodeling balance.[22]

In the control population, the annual increase in trabecular thickness averaged 5.8 μm at between 2 and 13 years of age, as can be derived from the slope of the regression line in Figure 2D. In type I OI, the yearly increase in trabecular thickness was only 3.6 μm, whereas no significant trabecular thickening was detected in types III and IV. This indicates that a positive remodeling balance was present in control subjects and in type I OI, but not in types III and IV. Because a trabecular cross section has two surfaces, the annual increase on each surface was 2.9 μm in controls and 1.8 μm in type I OI.

Wall thickness was determined in a subset of patients with OI type I and in controls, which allows the calculation of activation frequencies and a more detailed analysis of remodeling events in these two groups. As indicated by activation frequency, a given location of the cancellous bone surface underwent 1.04 remodeling cycles per year in controls and 1.65 remodeling cycles per year in type I OI patients. Thus, there was a positive balance per remodeling cycle of 2.9 μm/1.04 = 2.8 μm in controls and 1.8 μm/1.65 = 1.1 μm in type I OI. As mean wall thickness was 44.2 μm in controls and 38.1 μm in the type I OI group, the mean erosion depth was calculated to be (44.2 μm − 2.8 μm) = 41.4 μm in controls and (38.1 μm − 1.1 μm) = 37.0 μm in type I OI (**Figure 4**). These considerations show that the performance of both osteoblast and osteoclast teams was decreased in type I OI. However, the amount of bone produced by the osteoblast team during a remodeling cycle was 14% lower than in controls, whereas the amount of bone resorbed by osteoclasts was de-

Table 2. Bone formation parameters

	Controls	Type I	Type III	Type IV	*p* (all)	*p* (OI)
O.Th (μm)	6.2 ± 1.5	5.5 ± 1.7	5.8 ± 2.0	5.6 ± 1.3	0.38	0.86
OV/BV (%)	3.1 ± 1.3	5.2 ± 2.6	7.6 ± 2.8	6.2 ± 2.4	<0.0001	0.01
OS/BS (%)	29 ± 10	48 ± 14	56 ± 7	54 ± 15	<0.0001	0.17
MS/BS (%)	13.7 ± 4.7	23.1 ± 9.7	23.7 ± 6.2	26.4 ± 10.0	<0.0001	0.43
Ob.S/BS (%)	8.0 ± 4.2	19.4 ± 9.5	30.0 ± 14.0	25.2 ± 10.9	<0.0001	0.02
MS/OS (%)	45 ± 17	48 ± 16	43 ± 14	50 ± 13	0.58	0.45
Ob.S/OS (%)	27 ± 13	39 ± 14	53 ± 21	46 ± 15	<0.0001	0.05
MAR (μm/d)	0.98 ± 0.13	0.73 ± 0.18	0.85 ± 0.23	0.74 ± 0.15	<0.0001	0.19
Aj.AR (μm/d)	0.44 ± 0.15	0.35 ± 0.14	0.38 ± 0.18	0.37 ± 0.12	0.14	0.84
Mlt (d)	14.8 [11.9–16.7]	16.5 [12.5–19.8]	12.9 [11.5–31.6]	14.8 [12.0–21.1]	0.61	0.74

Data expressed as mean ± SD or median [interquartile range]; *p* values obtained by ANOVA or Kruskal–Wallis test, as appropriate; *p* (all), significance of variation between all four groups of subjects; *p* (OI), significance of variation between OI groups.
KEY: Aj.AR, adjusted apposition rate; MAR, mineral apposition rate; Mlt, mineralization lag time; MS/BS, mineralizing surface per bone surface; MS/OS, mineralizing surface per osteoid surface; Ob.S/BS, osteoblast surface per osteoid surface; OS/BS, osteoid surface per bone surface; O.Th, osteoid thickness; OV/BV, osteoid volume per bone volume.

Table 3. Bone formation rate using different referents

	Controls	Type I	Type III	Type IV	*p* (all)	*p* (OI)
BFR/BS ($\mu m^3/\mu m^2/y$)	49 ± 17	77 ± 34	69 ± 37	72 ± 33	0.03	0.39
BFR/BV (%/y)	82 ± 36	116 ± 62	184 ± 69	139 ± 50	<0.0001	0.01
BFR/Ob.S ($\mu m^3/\mu m^2/y$)	534 [407–888]	263 [206–417]	263 [138–430]	303 [183–494]	<0.0001	0.76
BFR/TV (%/y)	15.6 [12.4–23.7]	11.0 [5.3–18.2]	10.3 [5.3–17.5]	9.6 [5.1–12.3]	0.006	0.55

Data expressed as mean ± SD or median [interquartile range]; *p* values obtained by ANOVA or Kruskal–Wallis test, as appropriate; *p* (all), significance of variation between all four groups of subjects; *p* (OI), significance of variation between OI groups.
KEY: BFR/BS, bone formation rate per bone surface; BFR/BV, BFR per bone volume; BFR/Ob.S, BFR per osteoblast surface; BFR/TV, BFR per tissue volume.

creased by only 11%. This small imbalance in favor of "osteoclast activity" was sufficient to prevent normal trabecular thickening in type I OI.

Lower wall thickness means that the end result of osteoblast team function during a remodeling cycle was diminished. Our data also shed some light on the processes responsible for this insufficient end result. Mineral apposition rate and adjusted apposition rate were low, indicating that osteoblast teams produce a smaller amount of matrix per unit time. Because the active formation time at a remodeling site (formation period) was normal in OI, the sluggish pace of matrix production leads to a decreased total amount of work done. This slowness of matrix production occurs despite an increased number of osteoblast team members, as indicated by the increased Ob.S/OS ratio. Therefore, the amount of work achieved by an individual cell must be clearly decreased. This was also suggested by the analysis of bone formation rate related to osteoblast surface (BFR/Ob.S), which, in OI patients, was only about half of the control value. This was not unexpected, as lower production of organic bone matrix per osteoblast was also found in cell culture experiments.[21] Interestingly, BFR/Ob.S was similar between types I, III, and IV OI, indicating that the differences in disease severity were not due to differences in the amounts of bone matrix produced by osteoblasts.

Although the amount of bone turned over in individual remodeling cycles is decreased in OI, the number of remodeling cycles that take place on a given bone surface per unit time is increased. This was directly shown only in type I OI, where activation frequency was about 60% above the control result. However, activation frequency without doubt was also increased in types III and IV OI, as those parameters that depend mainly on activation frequency (OS/BS, Ob.S/BS, MS/BS, BFR/BS, Oc.S/BS, ES/BS)[26] were invariably increased. These observations confirm previous studies of pediatric OI patients.[1,2,7,28,37] The relationships between formation and resorption surfaces were similar to controls (data not shown), indicating that the temporal and spatial coupling of remodeling events was intact in OI.

At first sight, these observations appear to be at variance with studies of biochemical markers of bone metabolism. For example, low serum levels of the bone formation parameter, collagen type I C-terminal propeptide,[5] and high urinary levels of the resorption marker, collagen type I N-telopeptide, relative to creatinine[6] might be interpreted to indicate decreased bone formation rates and a severe imbalance between formation and resorption. However, it should be noted that the total production of these molecules depends not only on the activity of bone

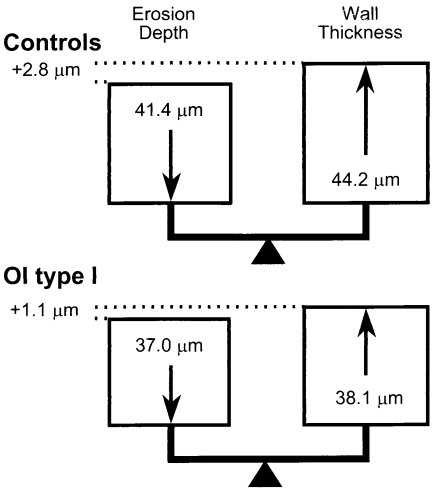

Figure 4. The remodeling balance in controls and in patients with type I OI.

Table 4. Wall thickness and derived parameters in controls and patients with type I OI

	Controls	Type I	*p*
n (M/F)	17 (9/8)	17 (12/5)	
Age (yr)	9.0 ± 3.6	9.1 ± 3.8	0.97
W.Th (μm)	44.2 ± 5.7	38.1 ± 4.0	0.0001
Ac.f (cycles/yr)	1.04 ± 0.38	1.65 ± 0.76	0.008
FP (d)	106 [80–147]	98 [88–144]	0.85

Data expressed as mean ± SD or median [interquartile range]; *p* values obtained by *t*-test or *U*-test, as appropriate.
KEY: Ac.f, activation frequency; FP, formation period; W.Th, wall thickness.

Table 5. Bone resorption parameters

	Controls	Type I	Type III	Type IV	*p* (all)	*p* (OI)
ES/BS (%)	16.3 [11.6–18.1]	15.6 [13.7–21.8]	25.6 [21.9–30.8]	19.7 [15.0–26.4]	0.003	0.01
Oc.S/BS (%)	1.14 [0.67–1.79]	1.37 [1.05–1.70]	2.15 [1.10–2.80]	1.68 [1.07–2.61]	0.03	0.22
N.Oc/B.Pm (/mm)	0.35 ± 0.18	0.47 ± 0.29	0.69 ± 0.36	0.51 ± 0.29	0.008	0.12

Data expressed as mean ± SD or median [interquartile range]; *p* values obtained by ANOVA or Kruskal–Wallis test, as appropriate; *p* (all), significance of variation between all four groups of subjects; *p* (OI), significance of variation between OI groups.
KEY: ES/BS, eroded surface per bone surface; N.Oc/B.Pm, number of osteoclasts per bone perimeter; Oc.S/BS, osteoclast surface per bone surface.

turnover, but also on bone mass. Results of urinary bone markers in addition are influenced by muscle mass, as their concentrations in urine are normalized to creatinine to correct for variations in water diuresis.[17] These interfering factors may at least partly explain the differences in results obtained with biochemical and histomorphometric parameters of bone turnover.

The cause of increased recruitment of remodeling teams is not clear, but increased microdamage in the bone matrix due to impaired mechanical resistance is a likely explanation. If so, increased remodeling in OI may be largely ineffective in improving the quality of the bone tissue, as the newly deposited matrix harbors the same structural defect as the "old" matrix. Other conceivable etiologies for increased remodeling in OI include the repeated immobilization of many severely affected OI patients, as well as decreased mechanosensing or impaired osteocyte viability secondary to impaired fluid flow in canaliculi surrounded by brittle mineralized matrix. These topics need to be addressed in future studies.

Matrix mineralization was not detectably affected in OI. None of our patients had a grossly increased mineralization lag time (>100 days) and none had an osteoid thickness of >12 μm. Of course, this does not exclude more subtle abnormalities of mineralization, such as decreased mineral crystal size or disorganized crystal arrangement.[35,36]

Synthesis

This study provides evidence that there are defects in all three mechanisms that normally lead to an increase in bone mass during childhood (i.e., bone modeling, production of trabeculae by endochondral ossification, and remodeling). Although our data confirm that, on the single cell level, bone formation is quantitatively decreased in OI, it is likely that, in addition, "qualitative" defects play a role in the pathogenesis of the disease.[4,9,18,31,35,36] For example, type III OI is clinically far more severe than type I, but bone formation rate per osteoblast surface was very similar in the two types. Both quantitative and qualitative defects may contribute to alteration of the mechanical properties of the bone tissue, clinically recognizable as brittleness. The resulting macroscopic changes may be interpreted as a decreased responsiveness of the bone to mechanical loads.[11] Thus, OI can be regarded as a disease in which a single genetic defect in the osteoblast interferes with multiple mechanisms that normally ensure adaptation of the skeleton to the increasing mechanical needs during growth.

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