Age related histomorphometric changes in bone in normal British men and women

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Abstract

***Aims*—To define the iliac crest histomorphometry of static variables in 234 individuals aged 16–100 years (91 men, 143 women) and of dynamic variables in 84 individuals aged 19–94 years (33 men, 51 women) from the North West of England.**

***Methods*—Iliac crest biopsy specimens were sectioned, undecalcified, and examined using image analysis.**

***Results*—The decrease in the quantity of cortical and trabecular bone and the connectivity of trabecular bone was more pronounced in women than men. This was associated with a reduction in bone formation and increased bone resorption which was greater in women at both the tissue and cellular level. Some of these histomorphometric differences first became evident at the natural menopause, and therefore provide clues as to the cause of the high prevalence of osteoporosis in postmenopausal women.**

***Conclusions*—These results show an age and sex dependent variation both in static and dynamic parameters, which differ, in some respects, from other studies and confirm the need for large regional studies to provide a database of normal morphometric results for a specific population.**

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Histomorphometric analysis of bone biopsy specimens has an important role in the diagnosis and treatment of metabolic bone diseases. Analysis consists of an accurate measurement of dynamic and static parameters related to bone metabolism. Previous reviews of histomorphometric data have mainly been derived from post mortem studies, usually in cases of sudden death. Though providing much useful information, only static variables were assessed and underlying endocrine or metabolic bone disease could not be excluded with certainty.

Over the past 20 years iliac crest biopsy specimens of healthy subjects of different ages have been studied in France, Denmark, America, Norway and South Africa.[1–5] Though many of the results from these studies are comparable, they show that geographical and interracial variations mean that regional laboratories must have appropriate control data for the population under study. So far, only the results of one British study[6] are available: this study was based in the South of England on data from a total of 41 people of both sexes. But demographic and geographic differences in the United Kingdom, and variations between individuals mean that the smaller the group the less sound the data.

Methods

Iliac crest biopsy specimens containing adequate amounts of cortical and trabecular bone from 84 normal subjects and 150 necropsies were studied. All were caucasian. The necropsy cases were selected from individuals who had died suddenly and who had not been admitted for more than 24 hours before they died. None had a history of previous gastrointestinal disease or gastrointestinal surgery and no necropsy evidence of liver, renal, or metabolic bone disease. They comprised 91 men and 143 women. Their ages ranged from 16 to 100 years. The causes of death were predominantly myocardial infarction, intracranial haemorrhage, trauma and massive pulmonary embolism (table 1).

The 84 subjects (33 men, 51 women) in whom dynamic parameters were assessed were selected from patients admitted to Manchester Royal Infirmary, Manchester, and Hope Hospital, Salford, for different surgical procedures. These included hip and knee replacements for osteoarthritis, and hernia repair. Their ages ranged from 19 to 94 years. None of the subjects had any history of metabolic bone disease or other systemic disease. Liver, thyroid, and renal function, checked before surgery, were normal. None was taking corticosteroids or other medication known to alter calcium metabolism or skeletal structure. Written consent was obtained from all subjects before surgery. Bone biopsy was performed under general anaesthesia at the same time as the operation was carried out. The study was approved by the hospital ethical committees.

While awaiting admission for bone biopsy, the 84 live subjects received oral demethylchlor-tetracycline (Ledermycin, Lederle) (10-15 mg/kg body weight) in two divided doses over 24 hours. The same dose schedule was

Table 1 Causes of death in post mortem subjects

Causes of death	*N* =
Myocardial infarction	47
Intracranial haemorrhage	39
Trauma	26
Pulmonary embolism	18
Miscellaneous	20
Total	150

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Table 2 Histomorphometric parameters with methods of measurement

Parameters	*Units*	*Methods*
Activation frequency ~	Days	1/ (EP + FP + QP)
Adjusted appositional rate ~	μm/day	(MS/OS) × MAR
Bone formation rate ~	% year	(MAR × MS × BS/BV)100
Bone surface	μm	LM*
Bone volume	%	AM» (BV/TV) × 100
Cortical thickness	μm	2PM^ × π/4
Cortical volume	mm³	AM⋙ (CV/TV) × 100
Erosion depth ⌞	μm	2PM^
Active erosion period ~	Day	(a.ES/OS) × FP
Erosion rate ~	mm/year	E.De/a.EP × 1/1000 × 365
Eroded surface ▶ (absolute)	μm	LM*
Active eroded surface ◀	μm	LM*
Eroded surface (relative)	%	(ES/BS) × 100
Formation period ~	Day	W.Th/Aj.AR
Label interval	Day	Time between labels
Double label surface	μm	LM*
Inter label distance	μm	LM*
Single label surface	μm	sLs + dLs
Total label surface	μm	2PM^
Mineralisation apposition rate (cortical)	μm/day	iLd/LP
Mineralisation apposition rate (trabecular)	μm/day	iLd/LP
Mineralisation lag time ~	Day	O.Th/Aj.AR
Mineralising surface (corrected)	μm	dLs + ½sLs
Mineralising surface (double)	%	dLs/tLs
Mineralising surface total (absolute)	μm	tLs
Mineralising surface absolute (bone referent)	%	MS/BS (dLs + ½sLs)
Mineralising surface (osteoid referent)	%	MS/OS (sLs + dLs)
Number of osteoclasts (cortical)	/mm²	10^{-2}(NOc.c/TV.c)
Number of osteoclasts (trabecular)	/mm²	10^{-2}(NOc.t/TV.t)
Number of osteoclasts (subcortical)	/mm	10^{-2}NOc.s/LM*
Osteoblast surface	%	Ob.S/BS
Osteoblast surface	%	Oc.S/BS
Osteoid surface (absolute)	μm	LM*
Osteoid surface (relative)	%	(OS/BS) × 100
Osteoid thickness	μm	OWi × π/4
Osteoid width	μm	2PM^
Osteoid volume	%	OV/BV
Quiescent period ~	Days	(QS + OS)FP
Quiescent surface ~	μm	BS-(ES + OS)
Trabecular number	/mm²	numbers/area
Trabecular separation	μm	2PM^
Trabecular thickness	μm	2PM^
Tissue volume	mm²	AM⋙
Wall thickness	μm	2PM^ × π/4
Wall thickness (cortical)	μm	2PM^ × π/4
Wall width	μm	2PM^

~ calculated values; *length, » area and ^2 point measurement; ⌞ mean depth of lacunae that contained preosteoblasts or unmineralised osteoid); ▶ scalloped surface depth 1 lamella or more; ◀ ES interfaced with osteoclasts or small mononuclear cells.

MAR = mineral apposition rate
dLs = double labelled surface
sLs = single labelled surface
Tb.N = trabecular number
Tb.Th = trabecular thickness
W.Th = wall thickness
TBV = trabecular bone volume
OS/BS = osteoid surface
MARt = mineral apposition rate in trabecular bone
BFR/BS = bone formation rate at bone surface
BFR/BV = bone formation rate of bone volume
Aj.AR = adjusted apposition rate
Mlt = mineralisation lag time
FP = formation period
Ac.f = activation frequency
ES/BS = eroded surfaces
OcS/BS = osteoclast surface
EP = erosion period
Ct.Th = cortical thickness
CV = cortical volume
W. Thc = cortical wall thickness
MAR c = mineral apposition rate in cortical bone
E.De = erosion depth
a. Ep = active erosion period
ER = erosion rate
ES = eroded surface (absolute)
a. ES = active eroded surface
ES/BS = eroded surface (relative)
LP = label interval
iLs = inter label distance
tLs = total label surface
MS.c = mineralising surface (corrected)
MSd = mineralising surface (double)
MSt = mineralising surface total (absolute)
MS/BS = mineralising surface absolute (bone referent)
MS/OS = mineralising surface (osteoid referent)
NOc.c = number of osteoclasts (cortical)
NOc.t = number of osteoclasts (trabecular)
Noc.s = number of osteoclasts (subcortical)
Ob.S = osteoblast surface
Oc.S = osteoclast surface
OS = osteoid surface (absolute)
OS/BS = osteoid surface (relative)
O.Th = osteoid thickness
O.Wi = osteoid width
OV = osteoid volume
QP = quiescent period
QS = quiescent surface
TN = trabecular number
TS = trabecular separation
TV = tissue volume

repeated after an interval of 10 days. Urine specimens from each individual were examined for fluorescence in ultraviolet light on the day before each dose to eliminate previous use of tetracycline or the presence of another fluorochrome, and a day later to confirm compliance and absorption. Biopsy was carried out four to seven days after the last dose. This double tetracycline lebelling was necessary[7] for the measurement of mineral apposition rate and other dynamic variables in trabecular and cortical bone, which were performed on the biopsy specimens.

A full thickness, transiliac bone biopsy specimen was taken in normal subjects and in necropsy cases using either an 8 mm Bordier or Lalor trephine biopsy needle. The biopsy specimen was taken from a standardised location 2 cm below and behind the anterior superior iliac spine.[8] Biopsy material was fixed in ethanol for two to three days. Dehydration was continued in ethanol. Tissue was placed in three changes of LR white resin monomer (London Resin Co). The tissue polymerisation was carried out overnight at 60° C in an anoxic environment. Twenty seven 5 μm step serial sections were cut through a block with a Tungsten tipped knife on an LKB powered microtome. Groups of three sections were stained with toluidine blue (pH 4·2), modified Giemsa, or Von Kossa techniques. For fluorescence microscopy, 20 μm unstained sections were cut at the different levels throughout the block.

Measurements were made by semiautomated image analysis on the whole of each of the nine toluidine blue stained sections using a VIDS II image analyser with user defined parameters of length, area, and two point discrimination measurement. The exception was the mineral apposition rate (MAR), which was measured using an eyepiece graticule in a fluorescence microscope to perform two point analysis at four regularly spaced positions along each front delineated by double fluorescence label. The length of mineralising surface was divided into two components—those having two labels and those having only one label. The latter represents regions of the bone surface that either finished or started mineralising in the interval between the two labels being given. To define more accurately certain parameters of bone formation, a corrected value of length of mineralising surface was constructed, taking account of single labelling, using the standard method of defining the true active mineralising surface as

Table 3 Dynamic trabecular and cortical parameters (mean (SD))

		Age						
Numbers of patients		*16–30 M 3/F 4*	*31–40 M 5/F 5*	*41–50 M 6/F 8*	*51–60 M 6/F 10*	*61–70 M 5/F 10*	*71–80 M 5/F 8*	*> 81 M 3/F 6*
MARt	M	0·64(0·12)	0·63(0·34)	0·62(0·19)	0·53(0·19)	0·59(0·20)	0·58(0·12)	0·56(0·19)
μm/day	F	0·63(0·14)	0·63(0·18)	0·61(0·11)	0·57(0·23)	0·56(0·21)	0·49(0·13)	0·52(0·17)
MARc	M	0·74(0·27)	0·76(0·21)	0·74(0·31)	0·73(0·21)	0·71(0·32)	0·68(0·41)	0·67(0·13)
μm/day	F	0·73(0·19)	0·71(0·18)	0·75(0·28)	0·71(0·23)	0·68(0·12)	0·62(0·21)	0·60(0·13)
BFR/BV % year	M	25·4(8·9)	25·3(14·9)	24·8(17·6)	23·7(9·9)	20·2(16·5)	22·5(12·4)	21·1(7·9)
(dLs + ½sLs)	F	25·3(7·6)	26·1(17·1)	25·2(16·5)	22·5(10·5)	18·3(11·9)	17·9(5·2)	16·9(9·0)
BFR/BS	M	1·6(0·4)	1·5(1·0)	1·4(0·6)	1·8(0·6)	1·2(0·6)	1·4(0·3)	1·4(0·5)
$\mu m^3/\mu m^2/yr$ (dLs + ½sLs)	F	1·6(0·4)	1·6(0·7)	1·8(1·0)	1·3(0·9)	1·5(1·2)	1·1(0·5)	0·9(0·4)
BFR/TV	M	3·7(1·5)	4·0(2·8)	3·6(2·2)	2·9(2·7)	3·3(2·0)	3·1(2·4)	2·8(1·6)
% yr(dLs + ½sLs)	F	3·8(0·9)	3·8(1·5)	3·9(2·3)	2·1(2·6)	2·7(2·5)	2·3(1·9)	2·0(1·8)
MS/BS %	M	7·8(1·9)	7·5(3·6)	7·4(3·8)	7·5(2·1)	7·6(3·7)	6·9(1·8)	7·1(2·9)
dLs + ½sLs	F	7·2(0·8)	7·4(2·2)	7·4(3·7)	7·7(2·5)	7·2(4·7)	6·8(4·1)	6·1(3·1)
MSt/OS	M	79·5(4·8)	78·4(12·9)	77·5(13·4)	79·4(11·1)	75·8(15·3)	80·1(9·2)	76·2(5·1)
sLs + dLs	F	78·0(5·9)	81·0(10·9)	79·9(14·5)	80·1(15·1)	78·3(12·6)	77·5(13·3)	77·0(13·1)
MSd	M	62·4(2·6)	62·5(7·5)	59·3(8·4)	57·5(7·7)	55·4(9·3)	54·3(6·4)	52·5(4·8)
(dLs + tLs)	F	61·1(2·3)	59·7(7·6)	58·0(10·1)	53·4(11·1)	43·3(7·4)	45·6(9·6)	46·6(6·8)
Aj.Ar μm/dayx10^{-2}	M	41·3(12·4)	38·0(31·1)	32·4(26·5)	29·6(5·5)	27·5(20·6)	22·5(13·4)	24·2(9·8)
(dLs + ½sLs)	F	34·7(15·9)	36·2(24·1)	30·0(21·5)	27·5(19·9)	26·6(8·4)	22·4(20·3)	22·1(17·4)
FP (day)	M	158·7(107·5)	184·6(122·3)	175·4(86·4)	160·1(91·8)	179·5(87·2)	185·2(83·6)	185·3(134)
W.Th/Aj.AR	F	161·7(88·8)	158·9(101·6)	190·3(59·6)	146·2(126)	168·8(113)	173·9(81·7)	191·4(120)
Ac.f (day)	M	6·9(4·0)	6·2(3·3)	7·4(4·8)	7·8(4·2)	7·9(2·9)	8·3(5·1	8·6(5·2)
1(EP + FP + QP)	F	5·8(3·4)	6·2(3·0)	7·5(3·6)	6·4(2·1)	8·1(4·9)	8·3(5·1)	7·9(4·6)
Mlt (day)	M	17·6(7·6)	18·4(6·3)	17·3(6·8)	18·9(5·8)	17·8(7·3)	19·4(8·0)	18·0(4·6)
O.Th/Aj.AR	F	19·2(5·7)	16·3(9·1)	17·5(7·3)	21·2(6·8)	20·4(8·1)	19·5(6·4)	18·6(7·3)
E.De	M	52·1(28·4)	57·9(30·6)	49·7(23·4)	48·1(15·8)	49·3(12·6)	48·1(18·8)	47·4(26·9)
(μm)	F	61·0(31·1)	56·2(20·1)	59·3(30·2)	54·3(14·6)	50·2(17·7)	48·6(22·7)	47·3(20·4)
ER	M	0·44(0·19)	0·39(0·23)	0·52(0·16)	0·37(0·21)	0·42(0·18)	0·36(0·21)	0·35(0·17)
(mm/year)	F	0·54(0·38)	0·38(0·21)	0·42(0·19)	0·48(0·20)	0·40(0·24)	0·33(0·15)	0·36(0·16)
EP (day)	M	134·6(57·4)	121·7(81·3)	140·5(90·8)	118·9(51·6)	130·6(62·4)	129·4(80·1)	101·9(46·3)
(aES/OS)xFP	F	119·7(65·8)	131·2(61·1)	122·2(59·6)	117·3(53·4)	11·6(51·8)	108·9(41·6)	100·1(70·0)

the double labelled plus half the single labelled surfaces (dLs + ½sLs) (table 2). The parameters were measured using standard techniques[9–12] and defined according to the terminology proposed by Parfitt and colleagues.[13]

The static and dynamic parameters obtained by measurement or calculation are shown in tables 3, 4 and 5. A differential stain was used to identify the osteoid surface, defined as an unmineralised surface of at least 3 μm in thickness. Osteoid width (O.Wi) was then measured at four equidistant sites on each osteoid seam.

All measurements were based on the mean of 20 separate fields using the standard constant for section obliquity which is 1·2. Most measurements were converted to three-dimensional variables in accordance with the recommendations of Parfitt *et al.*[9] Where volume is expressed as a proportion of another volume, for example, bone volume equals the volume of bone expressed as a proportion of the volume of the tissue. Conversion of measured two-dimensional to derived three-dimensional numerics required no change in the ratio, as defined by the theorem of

Table 4 Static trabecular histomorphometric parameters

		Age								
Numbers of patients		*16–20 M 4,*1/ F 4,*1*	*21–30 M 9,*2/ F 10,*3*	*31–40 M 5,*5/ F 9,*5*	*41–50 M 8,*6/ F 14,*8*	*51–60 M 5,*6/ F 14,*10*	*61–70 M 10,*5/ F 20,*10*	*71–80 M 5,*5/ F 9,*8*	*81–90 M 8,*2/ F 6,*4*	*91–100 M 4,*1/ F4,*2*
BV/TV	M	23·1(4·5)	23·9(5·0)	22·0(3·9)	21·9(5·3)	20·6(5·2)	19·2(5·0)	17·7(4·7)	16·1(4·6)	15·2(3·4)
%	F	24·1(5·1)	23·8(4·7)	22·6(4·8)	19·9(6·2)	17·5(5·8)	16·0(4·2)	14·6(5·8)	13·2(3·8)	11·9(3·1)
W.Th	M	51·7(5·6)	49·8(5·8)	49·1(7·3)	45·6(4·9)	42·3(5·3)	44·5(3·7)	37·7(3·7)	34·8(2·8)	35·1(5·9)
(μm)	F	52·6(7·2)	53·2(4·7)	52·9(5·9)	48·9(4·3)	39·7(3·7)	34·3(3·8)	32·8(6·9)	31·3(3·6)	30·6(4·7)
OS/BS %	M	18·2(5·7)	16·1(5·3)	14·0(4·6)	16·5(5·4)	17·1(6·1)	12·4(4·2)	11·3(3·3)	10·8(2·7)	10·1(3·0)
	F	16·1(4·8)	16·2(4·7)	15·3(3·8)	14·4(4·1)	12·6(3·1)	13·1(4·1)	11·1(3·6)	11·3(2·8)	9·2(2·1)
OS/BV %	M	4·3(2·1)	3·6(1·9)	3·5(1·9)	3·1(1·2)	3·0(1·6)	2·4(1·1)	2·7(1·0)	2·3(0·7)	2·4(1·2)
	F	3·0(1·4)	3·0(1·0)	3·1(1·0)	2·9(1·0)	2·4(1·1)	1·5(0·6)	1·7(0·3)	2·2(1·0)	2·2(0·4)
O.Th	M	9·9(2·5)	8·6(3·2)	9·7(4·6)	9·4(3·9)	8·7(2·0)	8·6(2·5	8·5(2·2)	8·5(1·9)	8·2(4·4)
(μm)	F	9·0(4·0)	8·7(3·0)	8·7(3·0)	8·4(4·1)	8·2(3·6)	8·2(3·7)	8·0(4·5)	7·6(3·7)	7·5(4·2)
Obs/BS	M	5·6(1·6)	5·4(2·0)	6·0(1·1)	5·2(2·1)	4·6(1·0)	4·7(1·1)	4·2(1·1)	4·5(1·3)	4·2(2·1)
%	F	6·2(2·2)	6·0(2·2)	5·0(1·6)	5·4(2·0)	6·0(1·8)	4·3(1·8)	4·2(1·0)	3·9(0·8)	3·9(2·1)
ES/BS %	M	3·7(1·6)	3·7(1·2)	4·5(1·9)	4·1(1·8)	3·6(1·0)	3·5(1·5)	3·8(1·5)	3·9(1·3)	3·5(1·1)
	F	3·2(0·8)	4·3(1·6)	4·1(1·2)	4·6(1·8)	4·6(1·6)	4·4(2·0)	4·8(2·0)	5·2(2·1)	4·8(2·8)
OcS/BS%	M	0·6(0·3)	0·6(0·3)	0·6(0·4)	0·6(0·2)	0·7(0·3)	0·7(0·3)	0·7(0·5)	0·7(0·4)	0·7(0·3)
	F	0·5(0·2)	0·6(0·3)	0·5(0·2)	0·6(0·5)	0·7(0·4)	0·8(0·5)	0·8(0·6)	0·8(0·5)	0·8(0·4)
NOc/TV	M	5·9(4·0–21)†	4·8(0·1–18)†	4·5(0·2–19)†	4·9(0·3–20)†	5·3(0·3–22)†	5·9(0·4–22)†	5·5(0·2–24)†	5·6(0·3–22)†	5·7(0·4–22)†
/mm^2	F	5·3(0·7–20)†	4·5(0·1–19)†	4·3(0·2–19)†	5·2(0·7–21)†	6·1(0·8–22)†	6·0(0·8–23)†	6·5(0·1–26)†	6·9(1·3–35)†	6·8(1·2–31)†
Tb Th	M	140(23)	141(27)	138(24)	136(25)	137(27)	138(28)	136(31)	135(30)	135(35)
(μm)	F	140(23)	142(22)	146(19)	140(23)	140(40)	139(38)	136(35)	139(35)	136(34)
Tb N	M	1·7(0·4)	1·7(0·4)	1·7(0·4)	1·7(0·4)	1·6(0·4)	1·5(0·4)	1·5(0·4)	1·4(0·4)	1·4(0·3)
(/mm)	F	1·8(0·5)	1·8(0·4)	1·7(0·4)	1·7(0·4)	1·5(0·4)	1·4(0·4)	1·3(0·3)	1·3(0·2)	1·2(0·2)
Tb Sp	M	470(76)	454(53)	494(82)	513(102)	515(113)	566(126)	601(109)	666(100)	676(123)
(μm)	F	481(63)	468(91)	505(99)	529(143)	557(135)	602(171)	652(126)	709(115)	713(159)

*Biopsies; †range

Table 5 Static cortical bone parameters

No of cases		Ages 16–20 M 5/F 5	21–30 M 11/F 13	31–40 M 10/F 16	41–50 M 14/F 22	51–60 M 11/F 24	61–70 M 15/F 30	71–80 M 10/F 17	81–90 M 10/F 10	90–100 M 5/F 6
NOc.s (/mm)	M	5·3(1·2)	2·5(1·3)	1·3(0·4)	1·1(0·5)	1·7(0·4)	1·6(0·6)	1·5(0·7)	2·0(0·8)	1·6(0·4)
(NOc.s/LM*)	F	6·1(1·4)	2·0(1·1)	1·6(0·3)	1·7(0·6)	1·9(1·0)	2·1(1·0)	2·2(0·9)	3·5(1·2)	3·1(1·6)
C.Th (μm)	M	1245(376)	1276(434)	1136(298)	1159(297)	1303(348)	1191(110)	1078(344)	1117(398)	1096(497)
(2PM^ × π/4)	F	1264(234)	1303(348)	1151(276)	1224(397)	1151(210)	1057(401)	1003(466)	894(555)	893(504)
CV (mm³)	M	97·6(2·8)	97·6(3·1)	95·8(3·6)	97·3(2·7)	94·4(1·0)	93·6(0·9)	93·4(4·8)	93·8(3·6)	93·1(5·9)
(CV/TV)100	F	95·4(4·3)	97·1(3·2)	96·5(4·6)	94·4(5·8)	91·8(2·9)	89·3(7·4)	86·5(6·5)	85·0(8·4)	83·3(8·9)
NOc.c (/mm²)	M	2·0(0·4)	1·8(0·6)	2·4(0·7)	2·5(1·3)	1·6(0·9)	2·1(1·0)	1·8(0·7)	2·7(0·9)	2·2(1·8)
NOc.c/TVcx10⁻²	F	2·5(0·2)	1·6(0·9)	2·2(0·9)	2·7(1·4)	3·2(1·8)	3·0(0·7)	3·2(0·9)	4·2(1·8)	3·8(0·6)
W.Th.c (μm)	M	53·5(7·0)	54·8(6·3)	56·1(8·5)	54·0(9·9)	50·3(3·2)	52·7(10·8)	57·6(6·5)	47·5(9·7)	46·5(9·4)
W.Wi × π/4	F	56·2(4·7)	55·5(9·6)	55·7(6·3)	53·5(10·1)	50·6(3·0)	43·5(6·6)	42·2(10·4)	47·9(11·1)	41·1(5·8)

*length measurement; 2PM^ two point measurement.

DeLesse,[14] but where spherical geometry was involved, the stereological constant $\pi/4$ was used to convert two-dimensional measurements to three-dimensional values.

Results

Trabecular bone volume (TBV) was marginally greater in early life in women than men and decreased with age in both sexes. Between the fifth and sixth decades of life, this decrease was more rapid in women than men. When compared with the mean bone volume of early adulthood, the decrease reached significance in women aged 61 to 70 years (56% reduction; $p = 0.05$) and in men aged 81 to 90 years (34% reduction; $p = 0.05$).

When the structural parameters of trabeculae were assessed, young women in this study showed slightly increased numbers of trabeculae compared with young men, but the trabecular number (Tb. N) decreased with age in men and women. This is consistent with the observation of Meunier *et al.*[15] The decrease was more pronounced in this study in women after the sixth decade which contrasts with some studies[5,16] but agrees with a previous British study.[17] Trabecular thickness (Tb.Th) decreased with age, but more appreciably in women than men. This is at variance with all other studies which have described either no age related decline in trabecular thickness,[15,16] or a decline with age in men only,[17–19] or an increase with age.[20] Trabecular separation increased with age: women showed a relatively increased separation of trabeculae compared with men, implying that as women age not only do they lose trabecular thickness but also trabecular "connectedness". This is compatible with the obser-vations of others.[21–23]

When parameters of osteoblast activity were studied, a decline was seen in wall thickness (W.Th) which was similar to the changes in TBV; in men there was a reduction of 32·1% and in women, a reduction of 42·9% with age. In women, most of the reduction in wall thickness occurred after the menopause, an observation in keeping with those of previous studies.[24–27] Osteoid surface (OS/BS) decreased with age in both sexes. When compared with the mean osteoid surface in subjects up to 50 years old, the decrease in osteoid surface reached significance at 61 to 70 years in both men ($p = 0.02$) and women ($p = 0.02$). The overall decline in osteoid surface was 39·1% in men and 42·8% in women and was associated with a similar fall in osteoid volume. The greatest rate of fall in osteoid surface and volume occurred in women aged 40 to 60 years. Osteoid thickness fell with aging to a lesser degree in men (17·2%) and women (16·7%).

There was a moderate but similar decrease in mineral apposition rate in trabecular bone (MARt) with age in men and women up to age 50 years, but there was a relatively more pronounced decline in women older than 50. This preferential decline was similar to the observation made by Recker *et al*[28] in postmenopausal women. The total mineralising surface varied little with respect to osteoid surface but there was a relative decrease in the number of double labelled surfaces (dLs) in men and women, indicating that in the 10 days between labels, fewer bone forming units were active. The fall in bone formation rate (bone surface and bone volume) (BFR/BS and BFR/BV) was greater in women than in men and the greatest fall in women occurred in those aged 40 to 60 years of age. The adjusted appositional rate (Aj.AR) decreased in both sexes in a similar manner with time.

The mineralisation lag time (Mlt) did not change appreciably with aging in either sex. The formation period (FP) in men showed only minor changes up to the age of 50 years, but subsequently increased progressively. In women there was an irregular increase in formation period with age, a change not so obvious in men. This differs from the observation of a reduction in formation period with aging in normal and osteoporotic women.[25] Activation frequency (Ac.f) did not change significantly, but there was an upward trend in both sexes with age.

Osteoclast parameters showed that eroded surfaces (ES/BS) remained unchanged with age in men but increased in women. Likewise, osteoclast surface (OcS/BS) varied little with age in men, but increased in women about the time of the natural menopause and remained increased subsequently. Both men and women showed a decline in the depth of eroded cavities with aging. Women, when compared with men, however, showed relatively deep eroded cavities until the age of 60. The erosion period (EP) reduced with age, and women, overall, showed a relatively

shorter erosion period when compared with men in the same age group. Erosion rate decreased slightly with age in both sexes.

Cortical thickness (Ct.Th) decreased with age, with a greater reduction seen in women. There was also a decrease in cortical volume (CV) and cortical wall thickness (W.Thc) with age, equivalent to that seen with the trabecular parameters. The decrease was relatively greater in women than men. The mineral apposition rate in cortical bone (MARc) in both sexes was relatively higher than mineral apposition rate in trabecular bone, but that in the cortical bone also declined with age. This proportionately higher mineral apposition rate for cortical bone in women mirrors the findings of Recker *et al.*[28]

Subcortical osteoclasis in this study was relatively high before the age of 20 in both sexes; it fell in men with age, but in women it fell up to the age of 50 and then progressively increased. Cortical osteoclasis in men showed no real change with aging, but in women, during middle life, it increased significantly ($p = 0{\cdot}001$) and remained high throughout later life.

When suitable parameters were compared within groups, there was no significant difference between necropsy and biopsy specimens.

Discussion

The cases studied have provided a pool of histomorphometric data in our normal population which can be compared with that from elsewhere in the United Kingdom and worldwide. A general overview of our data shows that bone is actively turning over throughout life. Most of the parameters, measured or derived, in both sexes remained constant in early adulthood (20 to 40 years), but thereafter, there are distinctive changes with age. Bone volume falls significantly with age, the fall being greater in women.[29 30] Like the findings of other studies, initial bone volume was higher, and the decline in bone volume occurred earlier in women than men. In common with others,[3 8] the decline in bone volume in women noted here was first apparent after the age of 40 years. In contrast, a South African study, in white women noted an increase in bone volume up to the age of 60[5] before a decline was observed, suggestive of regional or methodological variations.

In general terms, the decline in the bone volume with age seems to be due to both a decrease in osteoblast activity and increased osteoclasis. The decreased osteoblast activity takes two major forms. There is a decrease in the proportion of bone surfaces on which new bone is being formed at any time, which is probably a reflection of a decreased number of active osteoblasts, and there is also a reduction in their activities, the rate of bone production by teams of osteoblasts, mineral apposition rate, activation frequency (Ac.f) and formation period (FP), the total amount of bone laid down by each team wall thickness being decreased. The absolute number of osteoclasts per unit volume of tissue increases with age and, as the mean bone volume per unit volume of tissue falls, there is an even greater increase in osteoclast number per unit volume of bone. This is hidden, to a certain extent, in our data because resorption parameters are described per unit surface, and as trabecular bone volume falls so relative bone surface area must increase. These data show that the increase in osteoclasis in women is much greater than in men.

The combined effect of decreased total osteoblast activity and increased osteoclasis is probably responsible for the fall in trabecular bone mass: the trabeculae become thinner, causing some to disappear and others to become fragmented. Riggs and colleagues[31] have suggested that excessive bone turnover with resorption activity marginally greater than formation, in the early postmenopausal period, is a cause of fragmentation and eventual loss of trabeculae. Parfitt *et al*[16] also propose that rapid perimenopausal bone loss is the important mechanism of trabecular bone loss in women. By examining older subjects as well, we have been able to show that this imbalance between bone formation and resorption is not restricted to the immediate postmenopausal period but continues with age. The proposed mechanism, however, adequately explains the increase in trabecular spacing we see with aging, both in men and women. This study also explains why the decrease in trabecular bone mass is more pronounced in postmenopausal women. However, many of our data do not show significant changes from decade to decade, some of which only become significant in people of advanced age.

Our data are in keeping with Parfitt's assertion that if uncoupling of osteoblast and osteoclast activity explains the decrease in bone volume with age, this alteration is of a relatively small degree, but because of its prolonged duration it can cause clinically important changes in bone mass. Because this also causes trabecular fragmentation, we predict that the strength of the bone would fall disproportionately with trabecular bone volume,[27 32 33] though this cannot be assessed histomorphometrically.

In the present study there was a significant fall in osteoid surface and osteoid volume with age and a proportionately smaller decrease in osteoid thickness. These results agree with the overall trend observed by Malluches *et al*,[3] but are at variance with those of other studies[5] where there was an increase in osteoid volume with age or little difference with age. In agreement with Vedi and Compston,[34] there was a wide range of values at any given age for osteoid surface and osteoid volume. The variation in results between studies may be explained partly by difficulties in objective evaluation of osteoid,[35–37] but perhaps racial differences may also be relevant. What can be stated with some certainty, however, from the assessment of osteoid in this study, is that there is no convincing evidence of relative

vitamin D deficiency in the elderly population of the north west of England.

The cortical parameters presented here are, by comparison with the trabecular parameters, relatively crude. Nevertheless, they show a generalised decrease in the total amount of cortical bone, both by a loss of bone within the cortex and erosion or "trabecularisation" of the cortex from the marrow surface. The resulting overall reduction in cortical bone increases progressively with age, thus increasing the risk of cortical fracture progressively in the aging population. Again, the fall is greater in women than in men. Our data are similar to the findings of Recker, in postmenopausal women with osteoporosis,[38] but the data against which ours can be judged are much less than for trabecular bone.

In conclusion, the demonstrated age and sex dependent variation in these static and dynamic parameters, and differences from other studies, including the study based in the South of England, confirm the need for large regional studies to provide a database of normal morphometric results for that population. Our data were deliberately restricted to one racial group (caucasian). Experience suggests that there may be a need for normal data to be accumulated within racial as well as regional groups.

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