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Mineralisation density of human mandibular bone: quantitative backscattered electron image analysis

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ABSTRACT

This study examined the tissue level mineralisation density distribution in mandibles from 88 adult humans. Mandibles (19–96 y) were sectioned vertically in midline (MID), mental foramen (MF), and third molar (M3) regions. Surgical fragments from M3 were obtained from individuals aged 16–38 y. All specimens were cleaned, embedded in PMMA, micromilled and examined by digital 20 kV backscattered electron (BSE) stereology. Quantitation was based on rescaling image histograms to the signal range between a monobrominated (0) and a monoiodinated (255) dimethacrylate resin standard. Mineralisation density increased with age ($r = 0.70$; $P < 0.0001$): the mean for 39 individuals aged between 16 and 50 y was significantly lower ($P < 0.0001$) than for 35 individuals over 51 y (mean ($\pm$S.E.M.): 158.20 (1.63) and 174.71 (1.27) normalised grey level units respectively). There was good correlation in mean mineralisation density between different sites in the same mandible, but MID was significantly less highly mineralised than the other sites: MID 173.90, MF 177.34, M3 177.11 ($P < 0.002$ and 0.01 for MF and M3 respectively; paired *t* test), as was the alveolar bone density when compared with the bone of the inferior cortex (e.g. MID: 171.13 (1.53) and 174.46 (1.14) $P < 0.0001$). No sex difference was found. Partially dentate mandibles generally had regions of higher mineralisation than fully dentate and edentulous mandibles. The lowest density bone occurred at the alveolar crest anteriorly and superolingually at M3, matching sites of net resorption following tooth loss. Highest densities were found inferolingually at MID, inferiorly at MF and buccally at M3, matching the sites thought to experience the highest functional strains. This stresses the importance that local factors may have in the remodelling of the edentulous mandible. Morphology showed that there is a preponderance of highly mineralised cement lines, and of packets containing dead, mineralised, osteocytes.

Key words: Osteoporosis; bone tissue density fractionation; ageing; osteocyte cell death.

INTRODUCTION

A greater understanding of the factors affecting the turnover of mandibular bone is of relevance to most areas of dentistry. Of major current interest is the relative importance that local and systemic factors have in influencing mandibular residual ridge reduction. However, before it is possible to study any association between bone turnover of the mandible with the turnover at other sites in the skeleton it is necessary to establish first the changes that occur within the bone with ageing and with tooth loss.

The main constituents of bone tissue are protein (mainly type I collagen), mineral and water. The degree to which the mineral may be substituted for water is variable (Richelle, 1964) and tends to increase with the increasing age of any particular packet of bone. Since bone is constantly being turned over even in the adult skeleton, the tissue consists of regions with different levels of mineralisation. Thus a measure of mean mineralisation density can give an indication of the rate of bone turnover and provide longitudinal historical information in the absence of experimental labelling.

It is possible to study the relative quantities of bone with differing degrees of mineralisation by density

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gradient fractionation (Grynpas et al. 1986), but the fact that the bone is powdered removes the opportunity for detailed investigation in the histological context, such as is available in microradiography. The latter requires slices of constant thickness for any attempts at quantitation, but these are difficult to prepare, and the typical 100 μm section contains too great a volume of tissue to provide images of high volumetric resolution. Quantitative backscattered electron imaging analysis in a scanning electron microscope (BSE–SEM), as used in this study, employs bulk specimens but samples only a thin (0.5–1 μm) surface layer (Boyde et al. 1993; Howell & Boyde, 1994).

This study addressed the questions whether mandibular mineralisation density varies with age or with sex, whether different regions of the mandible show different levels of mineralisation which might account for the different patterns of bone loss seen at different sites in the mandible, and whether the mineralisation density varies between dentate, edentate and partially dentate mandibles.

MATERIALS AND METHODS

Human mandibles (19–96 y), mainly of dissecting room origin, were sectioned into 2 mm thick vertical plane-parallel slices; these were selected from the midline, 2–6 mm posterior to the mental foramen and from the region of the third molar (Tables 1, 2). In addition, pieces of fresh mandibular bone from a further 38 individuals (16–38 y) were obtained from operations for the removal of unerupted or partially erupted third molar teeth. These fragments were produced by the 'lingual split' technique in which a chisel is used to cleave off a portion of lingual cortical plate (the lingual tuberosity; Edwards 1954) to provide a path of removal for the third molar. The whole mandibular cross section regions are abbreviated as midline MID, the mental foramen MF, and the third molar M3: the smaller surgical specimens equivalent to M3 are denoted as 'M3LS'. A total of 88 individuals was studied.

All specimens were cleaned in an enzyme detergent solution (Terg-A-Zyme, Alconox Inc. New York, NY, USA), washed in distilled water and dehydrated in ethanol, as described by Kingsmill & Boyde (1998). They were then transferred from 100% ethanol to a minimum of 2 changes of freshly distilled methylmethacrylate (MMA) and embedded by placing into distilled MMA activated by the addition of 1 g azo-iso-butyronitrile, polymerised at 40 °C. The embedded material was then prepared for examination using digital backscattered electron (BSE) stereology.

Block surface preparation

The poly(methylmethacrylate) (PMMA) embedded specimens were sectioned on a band saw and ground on wet carborundum paper to expose the specimen at the surface of the block, the face of which was then finished to a flat surface using a Polycut E micromiller (Reichert-Jung, Germany; Boyde 1984). The base of the block was made parallel to the block face both to reduce the need for refocusing at different regions within the same specimen and to eliminate tilt of the surface within each field.

Mounting the specimens

The specimens were mounted onto a square raft of an aluminium alloy 80 mm × 80 mm using small pieces of double sided adhesive carbon tape. To eliminate charging, a continuous ring of conducting carbon paint was first traced around the sides of each block immediately adjacent to the face of interest. This track was continued down the sides of the block to make contact with the carbon tape attachments. Once the raft was full of specimens, a layer of evaporated carbon was applied.

Microscopy

The distribution of mineralisation densities within the mineralised tissues was determined by BSE–SEM and digital image analysis using a digital scanning microscope (Zeiss DSM 962) with automation functions controlled by an IBAS computer (Kontron Elektronik, Munich, Germany; Boyde et al. 1995*a*, *b*; Boyde & Jones, 1996). The microscope was equipped with an annular solid state BSE detector (KE Electronics, Toft, Cambridge, UK). Fields of view were chosen on the specimens with the microscope operating at ×2000 and TV scan rate in secondary electron mode. With whole bone cross sections, fields from all aspects of the bone were selected, taking care not to allow adjacent fields to overlap. For analytical imaging, the field was a 2.7 mm square containing 512 × 512 pixels. Under these conditions discrete measuring points recur at 5.27 μm intervals and constitute a dense stereological grid. The working distance was constant at 17 mm to maintain constant specimen detector geometry: focus setting was achieved by moving the specimen stage in the Z axis. The time taken for selection of all the fields allowed the filament and electronics to reach a stable operating temperature.

Filament current saturation was ensured by reference to the BSE signal. Fields were captured automatically using a slow repeating scan in BSE mode at an accelerating voltage of 20 kV. Each image took approximately 30 s to capture.

The standards consisted of 2 monosubstituted halogenated dimethacrylates (Davy, 1994; $C_{22}H_{25}O_{10}Br$ ('monobrom'), mean BSE coefficient according to the procedure given by Lloyd (1987) = 0.1159 to $C_{22}H_{25}O_{10}I$ ('monoiod'), mean BSE coefficient 0.1519), BSE signal levels which span the range found for normal bone (Boyde et al. 1995*a*). A field containing both standards was imaged at the beginning of each run, after the tenth, and each succeeding 20 images, and again after the last field had been recorded.

Data analysis

The images were edited to eliminate any artefactual features. The histograms of the edited images were stretched to 256 grey levels covering the range of BSE grey levels between monobrom and monoiod. To correct for any instrumental drift during the run, each image was stretched by linear interpolation between the values from the 2 consecutive standard fields between which it was taken.

The mean, standard deviation and median of each stretched image histogram were recorded, the values given lying on a scale where 0 represents black (monobrom) and 255 represents peak white (monoiod), the highest mineralisation density considered (155 on this scale corresponds to a density of 2.0 g/ml; Howell et al. 1998).

To facilitate further analysis, the histogram bins were pooled (Boyde et al. 1995*b*). Three different steps of fractionation were used. The data were separated into 16 or 8 equally sized bins to analyse the different histogram shapes and distributions. To present the data in this paper, we used 4 unequally sized bins that correspond to bone tissue with low (grey levels 1–149), medium ('med' 150–174), high (175–199) and very high ('vhigh' 200–255) mineralisation level to match the scheme used by Boyde et al. (1995*b*). This last scheme reflects the biological situation: low density bone is that which has been newly added and relatively recently mineralised; medium density bone has reached a normal mineralisation plateau; whilst high density bone has been maturing for long enough to reach values that are not commonly found in younger bones. Very high density bone may either be very old, or cement line matrix, or contain mineralised osteocyte lacunae and canaliculi.

Since there was often more than one image from each region of interest, pooled mean values were calculated after checking the standard deviations for similarity. The 2-sample *t* test was used to compare columns of data, Student's paired *t* test to reveal trends between different sites within an individual, and Pearson's linear correlation coefficient in assessing the relationship of one variable to another. Unless otherwise stated, the mean data and the standard error of the mean (±S.E.M.) are presented in the graphs and tables.

Analysis with sex and age

The younger age groups were best represented in the lingual split surgical material. For this reason, closely corresponding fields were selected from the whole bone cross sections; in edentulous mandibles this was taken as the region of the mylohyoid ridge. This was necessary because the mean mineralisation density measurements differed around the cortex.

Analysis with site

The data were compared between different points on the slices and between different locations within the mandible. Thus the inferior, buccal, alveolar and lingual cortices and the trabecular zones of the slices were compared, as were the third molar, the mental foramen and the midline regions. The term 'alveolar' is used here to denote the most superior aspect of the mandibular body, taken as the crest of the ridge in edentulous regions.

Analysis for dental status

For dental status, the third molar and mental foramen regions were separated into dentate, partially dentate (having anterior teeth and no more than one molar tooth) and edentate. All the partially dentate mandibles had anterior teeth, so that the midline specimens were only separated into locally-dentate and edentate groups. Specimens which were too small to determine how many teeth would have been present were omitted from the analysis of dental status.

Morphological imaging

The same specimens were further studied, also using BSE–SEM, to record relevant morphological detail over a range of magnifications. Here, the more important criterion was to produce high quality images, rather than high quality measurement data, and a 1024×1024 pixel matrix was used, recording the images directly on the Zeiss DSM 962 system (Figs 3–5).

Bone apparent density against mineralisation density

The mineralisation density measurements obtained in this experiment were tested for a relationship with the apparent density measurements obtained for matching mental foramen and midline regions of the mandible as determined previously. (The apparent density was determined by weighing the slices and dividing by a volume calculated as the product of section thickness and the mean area of the 2 sides of the section: Kingsmill & Boyde, 1998.)

RESULTS

A total of 893 mandibular analytical images was recorded and archived on optical disks: 317 from the third molar, 328 from the mental foramen and 248 from the midline regions. Of the specimens representing the lingual tuberosity site, one was severely cracked as a result of the surgery and was eliminated, leaving a total of 75 individuals ranging in age from 16 to 92 y. The 38 specimens from individuals under 40 y of age were all by-products of minor oral surgical procedures. Two specimens (48 and 49 y) were collected postmortem, and 10 specimens from individuals between 54 and 75 y were from major surgical resections. The remainder were from dissecting rooms. Further details of the sample are shown in Table 1.

The standard deviations (S.D.) in the histograms of the images fell in a narrow range (24.2–35.3). This meant that the images showed similar distributions of grey levels, thus justifying grouping of the data for further analysis.

Variation with sex

There were no significant differences in the mean mineralisation density measurements between the sexes (Table 1), even if only those individuals over the age of 50 y were compared.

Table 1. *Number, age, sex distribution and mean mineralisation density of M3LS mandibular specimens*

	Male	Female	All
n	43	32	75
Mean age (y)	52	45	49
Median age	60	29	48
Age range	18–88	16–92	16–92
Mean grey level in Br–I range	167.51	164.04	166.00
S.E.M.	1.85	2.70	1.42

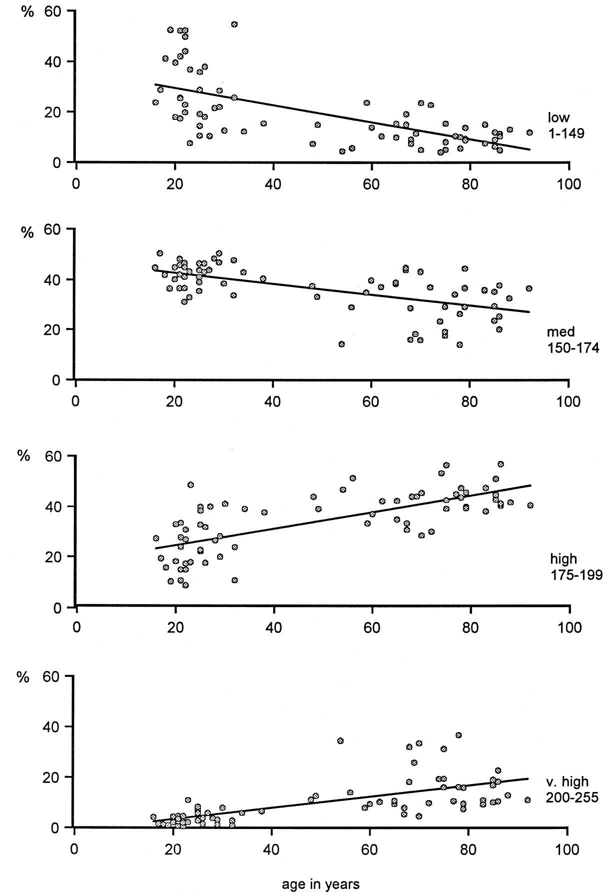

Fig. 1. Variation in percentage occupation of 4 unequal mineralisation density fractions with age – M3LS site only.

Variation with age

The mean mineralisation densities of the lingual tuberosity of the mandible showed interindividual spread. However, there is a clear increase in the mean mineralisation density with age (with a regression equation of the form $y = 0.33x + 149.92$, where $y =$ density and $x =$ age), the correlation ($r = 0.70$) being highly significant ($P < 0.0001$). The mean mineralisation density for the 39 individuals 16–50 y of age was significantly lower ($P < 0.0001$) than for the 35 individuals of 51–92 y, means ± S.E.M. being 158.20 ± 1.63 and 174.71 ± 1.27, respectively. If the younger subgroup is further divided to examine the differences in the lower age brackets, a significant difference ($P < 0.05$) was seen between age range 15–24 (mean ± S.E.M., 153.92 ± 2.31, $n = 7$) and 25–34 (mean ± S.E.M., 160.73 ± 2.03, n = 17), but no significant change was seen above 40 y of age.

The 4 bin data (Fig. 1) show how the rising mean mineralisation density is accounted for by a smaller percentage of bone falling into the lower 2 bins with

Table 2. *Mean and* S.E.M. *of the mean mineralisation densities of the mandibular groups*

	Number of individuals	Mean grey level in Br–I range	S.E.M.
M3 group			
Inferior	31	177.75	0.90
Buccal	32	179.93	0.95
Alveolar	25	177.55	1.43
Lingual	30	175.82	1.40
Trabecular	14	173.01	2.31
MF group			
Inferior	40	180.06	0.62
Buccal	41	177.11	0.71
Alveolar	41	174.30	0.77
Lingual	41	178.08	0.69
Trabecular	23	173.82	1.22
MID group			
Inferior	27	174.46	1.14
Buccal	28	173.05	1.42
Alveolar	28	171.13	1.53
Lingual	28	174.58	1.23
Trabecular	25	173.78	1.28

Table 3. *Mean* (S.E.M.) *BSE signal intensity values* (*in Br–I range*) *for edentate, partially dentate and dentate mandibles*

	Edentate	Partially dentate	Dentate
M3 group	(n = 18)	(n = 7)	(n = 6)
Inferior	176.65 (1.01)	180.52 (2.06)	174.09 (1.18)
Buccal	178.26 (1.24)	183.43 (1.62)	178.20 (1.52)
Alveolar	176.84 (2.62)	179.08 (1.65)	177.99 (0.79)
Lingual	173.91 (1.77)	181.59 (2.66)	172.10 (0.66)
Trabecular	168.18 (1.87)	177.64 (4.05)	182.00 (0.00)
MF group	n = 19	n = 6	n = 9
Inferior	179.47 (1.04)	181.71 (0.94)	179.46 (1.38)
Buccal	176.51 (1.17)	179.29 (0.95)	176.11 (1.71)
Alveolar	173.14 (1.04)	178.48 (2.18)	172.90 (1.88)
Lingual	176.92 (0.84)	182.70 (1.32)	177.64 (1.81)
Trabecular	172.17 (1.49)	177.64 (0.00)	175.47 (2.57)
MID group	n = 15		n = 13
Inferior	172.40 (2.39)	— —	176.10 (0.56)
Buccal	169.59 (2.58)	— —	176.05 (0.97)
Alveolar	167.37 (2.81)	— —	174.38 (0.97)
Lingual	171.94 (2.34)	— —	176.87 (0.76)
Trabecular	171.04 (2.32)	— —	175.94 (1.17)

increasing age. Correlations between mean mineralisation density and age for the whole sample are:

bin 1 (low) 1–149 $y = 0.34x + 36.19$ $r = -0.65$
bin 2 (med) 150–174 $y = 0.22x + 46.97$ $r = -0.60$
bin 3 (high) 175–199 $y = 0.33x + 18.06$ $r = 0.71$
bin 4 (vhigh) 200–255 $y = 0.22x + -1.19$ $r = 0.65$

all being highly significant ($P < 0.0001$).

Analysis by site

Third molar (*M3*) *slices*. The mean mineralisation densities of the different sites were calculated from 32 whole cross sections from the posterior body of the mandible. The mean age was 74.88 ranging from 48–92 y (Table 2). Student's paired t tests reveal that, for each individual, the buccal bone is significantly more highly mineralised than all the other sites. More details are shown in Tables 4 and 5. No site showed a significant correlation between mean mineralisation density and age or sex. This is consistent with the previous finding that there is only an increase in mineralisation density up to the age of around 40 y.

Mental foramen (*MF*) *slices*. In the region of the mental foramen, data from 41 mandibles were analysed. The ages ranged from 48–96 y with a mean of 73.79 y: there were 24 males (mean age 70.75, range 48–88) and 15 females (mean age 77.31, range 50–96). There was no significant differences in age or mean mineralisation density between the sexes. The mean and S.E.M. values are shown in Table 2.

Paired t tests showed the mean mineralisation density of the inferior cortex to be significantly greater than that at all other sites ($P < 0.0001$, except the lingual where $P < 0.001$, Table 4). Pearson's linear correlation coefficient showed the mineralisation density of the inferior cortex to be correlated with that for the buccal ($r = 0.66$, $P < 0.0001$), alveolar ($r = 0.64$, $P < 0.0001$) and lingual ($r = 0.66$, $P < 0.0001$) but not the trabecular sites. The mandibular trabeculae usually make up so small a proportion of this region that often no trabecular field was recorded (hence n = 23).

The buccal region had a higher mean mineralisation density than the alveolar and trabecular regions ($P < 0.001$ and $P < 0.005$) and these were positively correlated ($r = 0.61$, $P < 0.0001$, and $r = 0.46$, $P < 0.05$). The mean mineralisation density of the buccal region did not differ significantly from that of the lingual site and was positively correlated ($r = 0.61$, $P < 0.0001$). The mean lingual value was greater than for the alveolar ($P < 0.0001$, $r = 0.59$ and $P < 0.0001$) and trabecular regions ($P < 0.01$), but not correlated. The alveolar and trabecular regions were weakly correlated ($r = 0.57$, $P < 0.01$) and were not significantly different.

At the mental foramen site, the bone from the inferior cortex therefore generally consists of more highly mineralised bone than the lingual and buccal cortices, which in turn are more highly mineralised

Table 4. *Student's paired* t *tests of differences between zones of the 3 mandibular regions*

	Buccal	Alveolar	Lingual	Trabecular
M3 group				
Inferior against	0.01	ns	ns	0.02
Buccal against	—	0.05	0.001	0.001
Alveolar against	—	—	0.02	ns
Lingual against	—	—	—	0.0001
MF group				
Inferior against	0.0001	0.0001	0.001	0.0001
Buccal against	—	0.001	ns	0.005
Alveolar against	—	—	0.0001	0.01
Lingual against	—	—	—	0.01
MID group				
Inferior against	0.05	0.0001	ns	ns
Buccal against	—	0.01	0.05	ns
Alveolar against	—	—	0.001	0.01
Lingual against	—	—	—	ns

ns, not significant.

Table 5. *Pearson's linear correlation coefficient* r *values between zones of the 3 mandibular regions*

	Buccal	Alveolar	Lingual	Trabecular
M3 group				
Inferior with	0.66	ns	0.67	ns
Buccal with	—	0.70	0.70	0.64
Alveolar with	—	—	0.64	ns
Lingual with	—	—	—	0.79
MF group	—	—	—	—
Inferior with	0.66	0.64	0.66	ns
Buccal with	—	0.61	0.61	0.46
Alveolar with	—	—	0.59	0.57
Lingual with	—	—	—	ns
MID group	—	—	—	—
Inferior with	0.87	0.91	0.87	0.77
Buccal with	—	0.90	0.87	0.83
Alveolar with	—	—	0.85	0.82
Lingual with	—	—	—	ns

than the alveolar and trabecular regions: all, except the lingual with the trabecular region, are correlated.

Apart from a weak negative correlation for the trabecular site ($r = -0.49$, $P < 0.05$; $y = -0.22x + 190.49$), none of the sites showed a relationship between the mineralisation density and age of the individual.

Midline (*MID*) *slices*. A total of 28 mandibular midlines were analysed (mean age 73.79, range 19–92 y). The 10 females were significantly older ($P < 0.05$) than the males (mean age 80.60, range 70–92: mean age 70.00, range 19–86) (see Tables 2, 4, 5).

Relationship between sites of the same mandible

Mandibles with more than one site represented (i.e., M3, MF, MID) were cross correlated. There was

Table 6. *Student's paired* t *tests and Pearson's linear correlation coefficients between MID, MF and M3 sites of the same mandible*

Inferior cortex	n	Paired t test	r	$P <$
MID with MF	22	0.0001	0.49	0.05
MID with M3	19	0.02	0.36	ns
MF with M3	25	ns	0.31	ns
M3 and MF greater than MID				
Buccal cortex				
MID with MF	23	0.001	0.49	0.02
MID with M3	21	0.0001	0.46	0.05
MF with M3	26	ns	ns	ns
M3 and MF greater than MID				
Alveolar cortex				
MID with MF	23	ns	ns	ns
MID with M3	17	0.001	ns	ns
MF with M3	20	0.05	ns	ns
M3 greater than MF and MID				
Lingual cortex				
MID with MF	23	0.05	ns	ns
MID with M3	20	ns	ns	ns
MF with M3	25	0.02	0.55	0.005
MF greater than MID and M3				
Medulla				
MID with MF	11	ns	0.74	0.01
MID with M3	9	ns	ns	ns
MF with M3	9	ns	ns	ns

good correlation in the mean mineralisation density between different sites in the same mandible. Taking an overall mean value for each whole slice, the midline was significantly less highly mineralised than the other sites (MID 173.90, MF 177.34, M3 177.11, $P < 0.002$ and 0.01 for MF and M3 respectively; paired t test). Looking in greater detail (Table 6), the mean mineralisation density of the inferior and buccal cortices was significantly greater at the third molar and the mental foramen sites than at the midline. The density of the alveolar bone was greatest at the third molar site, whilst that for the lingual cortex was greatest at the mental foramen site. There was no significant difference in the mineralisation density of the trabecular region between sites (see Fig. 2 for summary diagrams showing the pattern of the mineralisation density variation around the mandible).

Relationship with dental status

The results are shown in Table 3. In the third molar region, the lingual cortex of partially dentate mandibles was significantly more highly mineralised than in either edentulous ($P < 0.05$) or dentate mandibles

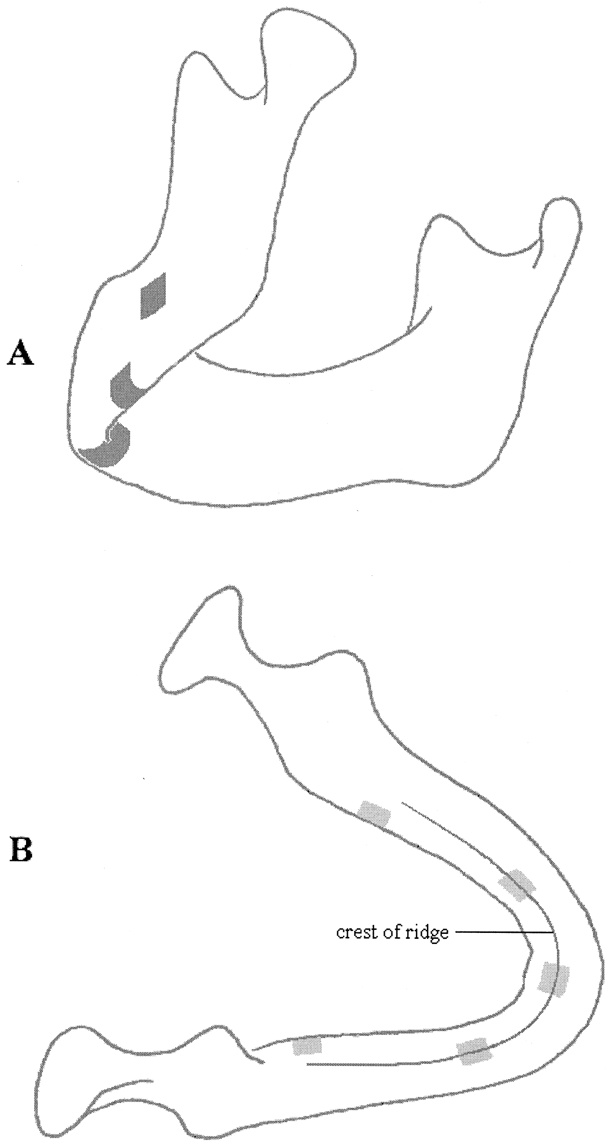

Fig. 2. Diagrams showing the sites with the highest (*a*, oblique view) and lowest (*b*, plan view) mean mineralisation densities found in the present study. (*a*) the sites of highest mineralisation density lie inferolingually at the midline, inferiorly at the mental foramen region and buccally at the posterior site; (*b*) the site with the lowest mineralisation density corresponds to the alveolar crest in the anterior mandible, but shifts towards the lingual tuberosity posteriorly.

($P < 0.02$). This was also true of the inferior cortex of partially dentate compared with dentate mandibles ($P < 0.05$). Dentate individuals were significantly younger than the partially dentate in this sample (mean ages of 63.80 and 78.57 respectively, $P < 0.05$).

In the mental foramen region the partially dentate values were significantly greater than the edentulous at both the alveolar and the lingual regions ($P < 0.05$ and $P < 0.005$). Dentate mandibles showed no significant differences from either the edentulous or the partially dentate.

As noted above, the partially dentate group was included with the dentate group at the midline. There was a difference in the alveolar value between these 2 groups which just reached significance ($P < 0.05$), the partially dentate value being the higher. However, the numbers in the groups were very small (n = 7 and 6 respectively). The same level of significance ($P < 0.05$) was found between the buccal and the alveolar sites when comparing mandibles with and without teeth.

Bone apparent density against mineralisation density

There was no significant correlation between the bone apparent density as measured in g/ml and the level of mineralisation as determined from the backscattered electron analysis for the mandibular (MF and MID regions $r = 0.30$, n = 38 and 27 respectively).

Morphological observations

BSE–SEM imaging demonstrates well the variation in mineralisation densities that occur in bone (Figs 3–5). At all sites examined, the mandible was seen to consist largely of secondary osteonal bone (Fig. 3*a*, *b*). The diameters of the haversian canals in the buccal cortex tended to be greater than in the lingual cortex as reported by Atkinson & Woodhead (1968), von Wowern & Stoltze (1980), Jäger et al. (1990) and Kingsmill & Boyde (1998).

Higher degrees of mineralisation are seen in the cement lines (Fig. 3*c*, *d*), the appositional lamellar bone (Fig. 3*a*), interstitial lamellar bone, regions with mineralised osteocyte lacunae (Fig. 4*a*–*c*) or mineralised haversian canals (Fig. 4*d*) and at the sites of woven bone formation. Regions with less dense bone occurred at bony prominences (Fig. 3*b*) which may correspond to sites of muscle attachment.

Mineralised osteocyte lacunae are a frequent finding in the ageing mandible (Pudwill & Wentz, 1975), and extensive regions may be affected (Fig. 4*a*, *b*). In addition, the canaliculi were often completely mineralised (Atkinson & Hallsworth, 1983), even when this was not so evident in the lacunae proper. The mineralisation of blood vessel (haversian) canals was also a fairly frequent finding (Fig. 4*d*), but here the surrounding osteocyte lacunae did not always appear to be mineralised. Mineralisation within haversian canals was seen in both the alveolar and the basal bone (no attempt was made to quantify at which site or in which individuals it was more common).

Extensive areas of bone with extrinsic Sharpey

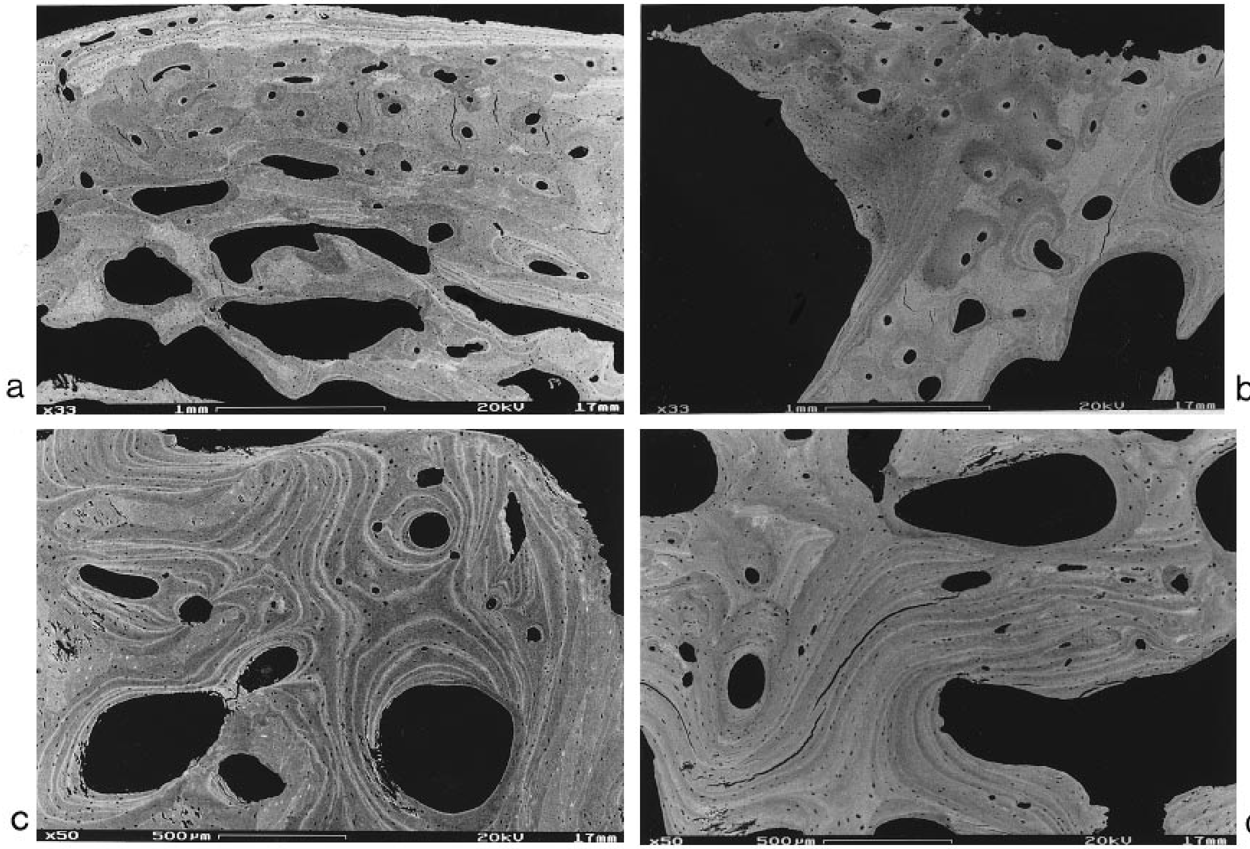

Fig. 3. 20 kV, BSE-SEM micrographs of micromilled, PMMA-embedded blocks of bone from the mandibular midline. The brightness of the image is dependent upon the mean electron backscattering coefficient of the tissue. Gain and contrast levels have been adjusted such that PMMA in the background is black and the most highly mineralised bone is nearly white. (*a*, *b*) Two images typical of mandibular bone, showing the large proportion of the cortex that may be taken up by secondary osteonal bone, in both the interstitial lamellar bone fraction is lower than the osteonal bone fraction. In *a*, inferior cortex from a 67-y-old male, highly mineralised circumferential lamellae which have not been replaced by secondary osteons can be seen at the top of the field. The mineralisation density fraction histogram for the analytical image corresponding to this particular field had 5.75 % falling in the low (1–149), 39.51 % in the medium (150–174), 46.35 % in the high (175–199), and 8.38 % falling in the very high (200–255) range. The mean mineralisation density (on the scale of monobrominated standard = 0, monoiodinated standard = 255) for the field was 174.53. (*b*) 78-y-old male, showing a bony prominence on the labial aspect of the mandibular midline with a lower mineralisation density than the surrounding bone. This may be the site of attachment for the mentalis muscle. Bone may be less highly mineralised here because the bone would have had to remodel more rapidly than the adjacent bone whilst the muscle attachment moved inferiorly with the recession of the ridge, or because the turnover is higher here as a result of the functional stimulus of the attachment (either on the bone itself or on the blood supply). The mineralisation levels determined from the corresponding analytical image in this image were lower than at other sites of the mandible: 39.90 % low, 40.43 % medium, 17.76 % high, and 1.90 % very high. The mean grey level value was 153.26. (*c*, *d*) Highly mineralised cement lines were seen in some mandibles, particularly at the midline (Atkinson & Hallsworth, 1983). Here they occurred in the cancellous regions and contributed to the high mean mineralisation density values obtained for this site, although occasionally the mineralisation levels in the interstitial bone reached a higher value. Both are from females, *c* aged 82 y and *d* aged 78 y. Note that the cement lines are arranged around the marrow spaces: these probably represent prolonged resting periods. The 4 bin histogram for the analytical image from *d* had 7.74 % falling in the low (1–149), 29.08 % in the medium (150–174), 48.89 % in the high (175–199), and 14.16 % falling in the very high (200–255) range. The mean grey level for the whole field was fairly high at 176.87.

fibres with typical nonmineralised centres were seen particularly in the (fundus) areas beneath the tooth apices (Fig. 5*a*, *b*). Sharpey fibre bone was not seen to the same extent in edentulous mandibles. At higher resolution, the bone appears porous where mineralisation of the osteocytes and their lacunae has not occurred (Fig. 5*c*, *d*), and the collagen fibre arrays within the bone can be seen. Fig. 5*e*, *f* demonstrates the continuous change in collagen fibril orientation through and between lamellae (Boyde, 1972). The apparently exceptional widths of the lamellae relate to the oblique tangential section to the lamellar planes in these fields.

DISCUSSION

The present study was undertaken to determine how the relative mineralisation density of the mandible changes with age, sex and location. The effect of dental status was also investigated to see if it gives any insight into the effect that function may have upon bone turnover.

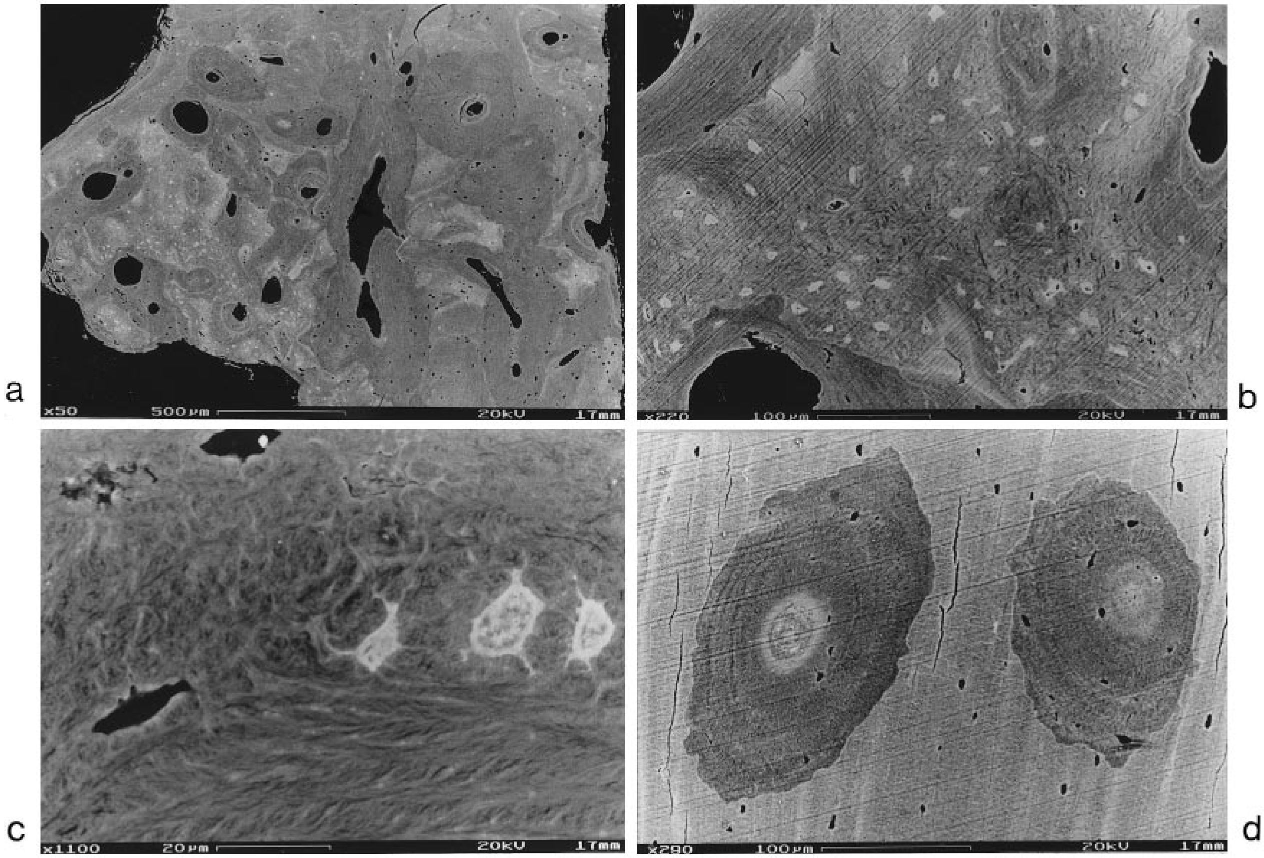

Fig. 4. (*a*) 20 kV BSE–SEM located towards the crest in the buccal cortex in the mental foramen region of a 79-y-old male. This image shows the extensive areas of bone that may be occupied by mineralised osteocyte lacunae (white spots). There are several infilled and mineralised haversian canals. The bone surrounding most of the mineralised lacunae is old, as indicated by its whiter appearance than the adjacent vital osteons. In the corresponding analytical field, the division into 4 bins gave 6.90% low, 34.09% medium, 46.67% high, and 12.19% very high. The mean grey level value was 176.55. (*b*) The same site as in *a* at a higher magnification indicating that each white spot represents a mineralised osteocyte. Parallel scratches are due to imperfect micromilling. (*c*) High resolution BSE–SEM of bone in the midline of the mandible of a 69-y-old male (labial cortex near to ridge crest) with osteocytic lacunae which have undergone differing stages of mineralisation. A mineralised 'pearl' can be seen in the lacuna at the top of the image. By contrast, the lacuna to the bottom left of the image shows no signs of mineralisation, although the canaliculi surrounding it are mineralised. In the lower part of bone in the image, some mineralised canaliculi have been cut obliquely. (*d*) 20 kV BSE–SEM of buccal cortex near ridge crest of mental foramen region of the mandible of a 79-y-old male showing the mineralisation of haversian canals. The canaliculi of the osteocytes in the interstitial bone are mineralised: it would be difficult to envisage how the osteocytes concerned could survive.

Mineralisation density

The largest age series was available for the bone lingual to the site of the mandibular third molar (M3LS), and this site gave the best demonstration of an increase in mineralisation density with age. This was substantiated by the results found for the midline. The findings reflect the clinical perception that tooth extraction is more difficult in the elderly: better mineralised bone is more resistant to bending (Currey 1969).

The whole bone slices showed that different aspects of the mandibular cross section have different mean mineralisation densities. This agrees with the findings of previous studies (Manson & Lucas, 1962; Atkinson & Woodhead, 1968; von Wowern & Stoltze, 1977; Jäger et al. 1990) which found larger haversian canal sizes, indicative of at least the resorption phase of remodelling, in the alveolar and buccal cortices, especially in the elderly. However, using quantitative microradiography, Hobson & Beynon (1988) found no difference in the mineral density between buccal and lingual sites in 6 mandibles, but their technique of measurement was not as sensitive as the one described here.

In general, the present study found that the mineralisation density tended to reduce the more superior the site sampled on the mandible, with the alveolar portion having the lowest density at the mental foramen and midline regions, in agreement with Landini (1991). In the M3 region, it was the superolingual aspect of the slice that had the lowest

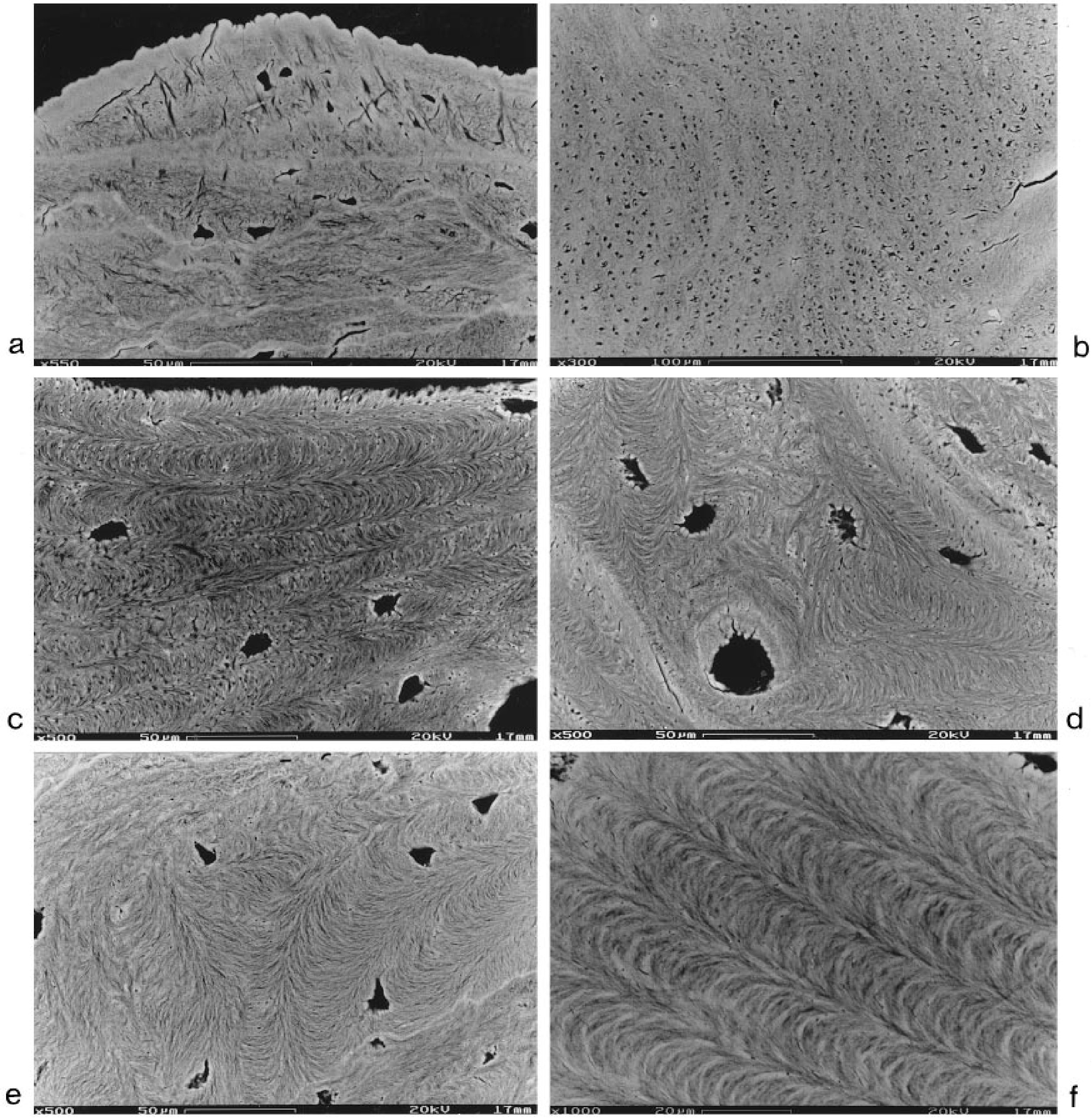

Fig. 5. (*a*, *b*) 20 kV BSE–SEM micrographs of mental foramen regions of 2 dentate mandibles, showing the large areas of bone near the tooth sockets which contain large numbers of Sharpey's fibres. (*a*) The fibres are seen in longitudinal section in the socket side of the alveolar bone proper on the buccal aspect of the tooth of this 86-y-old old male. Note the reversal line halfway down the field. There is a thin surface layer on the bone that seems to lack any structural features, but elsewhere intrinsic fibres can be seen. (*b*) Fibres in transverse or oblique section at the fundus of a tooth socket (57-y-old dentate male). The fibres were much more numerous here than at any other site around the tooth. The nonmineralised fibre cores show as dark stellate features. (*c*–*f*) Higher magnification images showing osteocytes, their canaliculi and collagen orientation patterns. (*c*) 20 kV BSE–SEM of the bone beneath the apex of a central incisor of a 92-y-old female. Note the patent osteocyte canaliculi cut in cross section. (*d*) 20 kV BSE–SEM of trabecular bone near the base of the mandibular midline of an 82-y-old female, showing large numbers of nonmineralised osteocyte lacunae and canaliculi (compare with Fig. 4*c*). (*e*) 74-y-old male midline and (*f*) 78-y-old female mental foramen region: interstitial lamellar bone near to the alveolar crest, showing the collagen fibre arrangement within the lamellae.

density. It is interesting to note that these sites of lowest density match the sites known to undergo the greatest net resorption following tooth loss.

Conversely, the regions of highest density were at the lingual/inferior region at the midline, the inferior cortex at the mental foramen region and the buccal cortex at the most posterior site sampled. These sites of high density correspond to where the highest principal strains have been predicted to occur during biting from finite element analysis models (Korioth et al. 1992), and where the bone is stiffer (Schwartz-Dabney & Dechow, 1997).

A possible relationship between mineralisation density and function was also found when allowance was made for the dental status of the individual. In the posterior mandible, the partially dentate had higher mean mineralisation densities of the lingual cortex than either dentate or edentate individuals. Bone of the lower border cortex of the partially dentate was also more mineralised than in the dentate (the latter were younger, but the mean age of each was well above that at which the mean mineralisation density had reached a plateau). Partially dentate individuals can still generate high strains with their remaining teeth (Helkimo et al. 1976) and the highest compressional forces are experienced on the lower border during biting (van Buskirk et al. 1988). However, the extraction of teeth reduces the torsional rigidity of the mandible (Daegling et al. 1992) and the bone reduces in cross sectional area. Therefore, in order to withstand the same loads, there must be some improvement in the mechanical properties of the bone. This must either be achieved through a consolidation of the bone (Kingsmill & Boyde, 1998), or via a change in its mineralisation (as was seen here). Both events, however, probably occur independently because no relationship was found between the apparent density and the mineralisation density (i.e. bone quantity acts independently from bone quality).

Correlation between the mineralisation density of the 5 zones at each of the 3 regions studied was good, except for the alveolar and trabecular regions. However, the latter particularly was often represented by a very small amount of bone, showing a large standard deviation in the mineralisation density measurements. In considering the 3 different regions of the mandible, there was also a positive correlation in mean mineralisation density between the regions, with adjacent regions (i.e., MID with MF, and MF with M3) having closer associations. Thus the mean mineralisation densities at the inferior, buccal and trabecular sites of the midline were correlated with the respective sites at the mental foramen region, and the third molar and mental foramen regions were correlated over the buccal and lingual cortices. Only at the buccal site were values at all regions correlated; it was the density in the buccal region of the mandible that Klemetti et al. (1993) found to correlate best with postcranial bone density.

Morphological observations

The images of mandibular bone show that a large proportion of the cortex may be taken up by secondary osteonal bone; this contrasts with the conclusions of Lautenbach (1972) that the mandible contained very few haversian systems. The mineralisation of the haversian systems probably accounts for the 'filling defects' seen by Atkinson & Hallsworth (1983) in their study of PMMA casts of spaces in mandibular bone. This mineralisation, and that of the osteocytic lacunae, would not be evident on conventional demineralised histological sections.

Regions with less dense bone were found at the site of some bony prominences which were thought to be sites of muscle attachment. The bone may be less dense here may be because: (1) this site is having to remodel more rapidly than the rest as the muscle attachment moves inferiorly as the ridge recedes; (2) turnover is at a more normal level here due to the functional stimulus of the attachment, with the other regions becoming increasingly hypermineralised; (3) that remodelling here has occurred at a time when full mineralisation could not be realised for some reason; or (4) the activity of the muscles of the floor of mouth and tongue may change upon becoming edentulous (Klemetti et al. 1994).

The highly mineralised cement lines seen particularly in the mandibular midline of some elderly individuals probably delineate prolonged resting periods as the mandible becomes increasingly consolidated by the continued apposition of bone encroaching on the marrow space. This would account for the increasing apparent density that was seen to occur in the mandible with increasing age at this site (Kingsmill & Boyde, 1998).

Summary of findings

1. There is an increase in mandibular mineralisation density with age, but no difference was found between the sexes.
2. The pattern of lower mineralisation density in the mandible seems to match the pattern of bone loss that follows loss of teeth.
3. The regions of highest mineralisation density mirror the sites thought to experience the highest strains.
4. The mandible undergoes alterations in its net mineralisation level on becoming partially dentate, and these probably counter the reduction in torsional rigidity following loss of the posterior teeth.
5. Several features are evident in ageing mandibular bone, such as the mineralisation of osteocytes and haversian canals, which may have an influence on local turnover and the ability of the mandible to adapt to new loading situations.

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