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Effects of experimental occlusal hypofunction, and its recovery, on mandibular bone mineral density in rats

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SUMMARY The aim of this investigation was to examine the effects of experimental occlusal hypofunction, and recovery, on mandibular bone mineral density (BMD) using peripheral quantitative computed tomography.

A metal cap was inserted between the upper and lower incisors of 40 male Wistar rats (aged 6 weeks) to prevent the molars from biting. The rats were divided into two equal groups: 'hypofunction' and 'recovery' animals. In addition, there was a third group comprising 20 control animals. The recovery animals were anaesthetized at 4 weeks in order to remove the metal cap using pliers. The rats were killed under deep anaesthesia, after which the mandibles were immediately removed and fixed in 10 per cent neutral formalin. After 2, 4, 6, and 8 weeks, BMD was measured in the cancellous and cortical bone in the first molar region. Data were analysed using one-way analysis of variance.

At 6 and 8 weeks, in the hypofunction group, cancellous bone density decreased on the buccal and lingual sides, at the bifurcation of the root and at the root apex. In the recovery group, the density on the buccal and lingual sides had recovered to the normal levels, compared with the control group. However, density of the bifurcation of the root and the root apex recovered by only 30 and 50 per cent, respectively. At 6 and 8 weeks, cortical bone density in the hypofunction group had reduced in the lingual basal and lingual middle cortical bone areas. Cortical bone density in the lingual basal and lingual areas recovered to control group levels at 6 weeks.

Recovery of occlusal hypofunction may restore decreased BMD of both cancellous and cortical bone.

Introduction

A decrease in physical activity may lead to an increase in bone loss and in the incidence of osteoporotic fractures (Chilibeck *et al.*, 1995). As the jaws receive physical stimulation from biting and chewing, it is important to investigate the relationships between function and jawbone structure. Mechanical stress applied to bone influences bone volume and structure by controlling bone remodelling, and it is generally agreed that application of a sufficient degree of mechanical stress is necessary to maintain bone volume and structure (Steigman *et al.*, 1989; Maeda and Suzuki, 1990; Klemetti, 1996). In normal rats, extraction of teeth from the maxilla is associated with osteoporotic changes in the mandibular trabecular bone (von Wowern *et al.*, 1979). Therefore, occlusal function appears important in maintaining the volume and structure of the mandible.

Animal studies of occlusal hypofunction have investigated function of the periodontal ligament (PDL; Muramoto *et al.*, 2000; Kaneko *et al.*, 2001; Watarai *et al.*, 2004). Recently, it was reported that cancellous bone mineral density (BMD) in rat mandible decreased by occlusal hypofunction (Sato *et al.*, 2005). Therefore, occlusal hypofunction may affect not only PDL function but also jawbone structures. However, very little is known about the BMD of the jawbone after recovery of functional occlusion.

Thus, the effects of experimental occlusal hypofunction, and recovery, on mandibular BMD in the rat were investigated using peripheral quantitative computed tomography (pQCT).

Materials and methods

Sixty male Wistar rats, 6 weeks of age, were used in the study. To prevent mastication in the hypofunction group, 20 rats were prepared according to a modification of the method described by Warita *et al.* (2004), in which a metal cap was inserted between the upper and lower incisors to prevent the molars from biting (Figure 1). In the recovery group (20 rats), the metal cap was removed, and the occlusion re-established, after 4 weeks. A control group of 20 rats had a metal band applied around the cervical region of the upper and lower incisors to ensure that the rats would use the molars to bite. The rats received a standard laboratory powder diet (Oriental Yeast Co. Ltd., Tokyo, Japan) and water *ad libitum*. Four rats from each group were sacrificed under deep anaesthesia every 2 weeks (Figure 2). The cranial bones and femurs were removed immediately and prepared by fixing in 10 per cent neutral formalin.

BMD was measured in mandibular cancellous and cortical bone in the first molar region, using pQCT (XCT Research SA, Stratec Biomedical Systems AG, Birkenfeld,

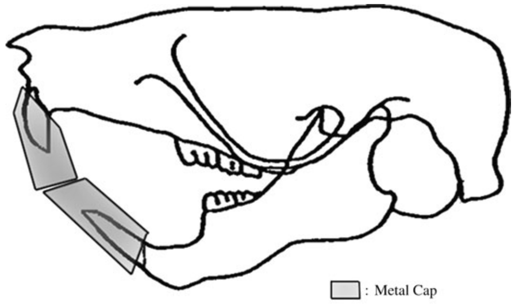

Figure 1 Metal cap inserted between the upper and lower incisors of the rat. Schematic diagram of experimental hypofunction.

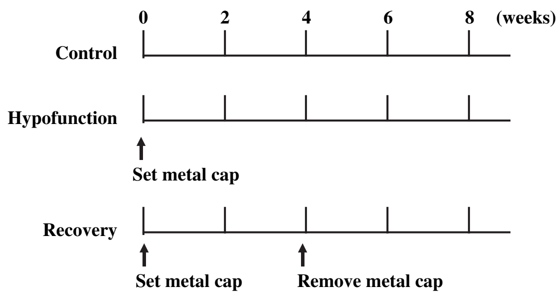

Figure 2 Time schedule of experiment. In the hypofunction group ($n=20$; four rats each every 2 weeks), the rats were sacrificed at 2, 4, 6, and 8 weeks following placement of the appliances. In the recovery group ($n=20$; four rats each every 2 weeks), the rats had the appliances removed after 4 weeks and were sacrificed at 2 and 4 weeks post-removal. Twenty untreated rats served as the controls ($n=20$; four rats each every 2 weeks).

Germany; Figure 3). Computed tomography (CT) scans were obtained with a scan time of 5 minutes, slice width of 0.5 mm, and a voxel size of 0.10 mm. All animals were maintained and used in accordance with the guidelines for the use of laboratory animals (ECA02-0002, Nihon University School of Dentistry at Matsudo).

Statistical methods

The data were analysed by one-way analysis of variance ($P < 0.05$). The BMD values are presented as means and standard deviations.

To investigate repeatability, the BMD measurements were repeated 2 weeks after the initial measurements. The mean difference between the first and second measurements, the standard error of the measurement, and the relative contribution of errors to the total observed variations for each variable were calculated using the following formula:

$$V_e = \frac{\sum (X_1 - X_2)^2}{2n},$$

where V_e is the error variance, X_1 and X_2 are the repeated measurements, and n is the sample size.

A small number of significant differences between the first and second measurements reflected the difficulties in measuring BMD. However, the contribution of the errors to the total variance was small, less than 10 per cent.

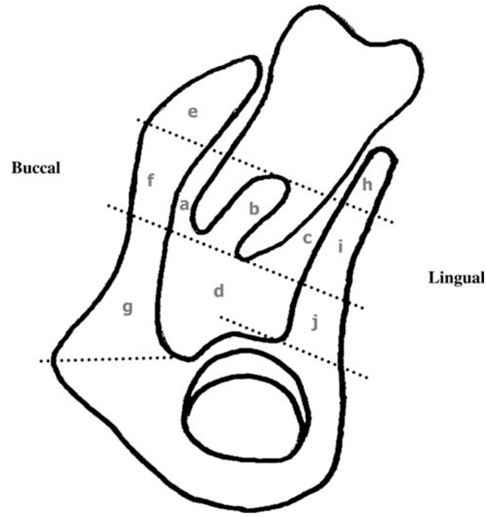

Figure 3 Cancellous and cortical bone measurement areas. a, buccal cancellous bone; b, cancellous bone; c, lingual cancellous bone; d, root apex cancellous bone; e, buccal cervix cortical bone; f, buccal middle cortical bone; g, buccal basal cortical bone; h, lingal cervix cortical bone; i, lingual middle cortical bone; and j, lingual basal cortical bone.

Results

Cancellous bone

When compared with the control group, cancellous BMD began to decline from 4 weeks in the hypofunction group. By weeks 6 and 8, cancellous bone density had declined by 11.6 and 14.0 per cent on the buccal side, 16.7 and 18.4 per cent on the lingual side, 12.3 and 19.1 per cent at the bifurcation of the root, and 25.6 and 12.3 per cent at the root apex, respectively ($P < 0.01$ for all values). In the recovery group, density on the buccal and lingual sides recovered to the control levels by week 6. However, density on the bifurcation of the root and root apex did not recover to control levels (Figure 4a; $P < 0.01$).

Cortical bone and the femur

At weeks 6 and 8, cortical bone density in the hypofunction group had decreased by 12.2 and 14.4 per cent at the lingual basal area and 8.0 and 7.8 per cent at the lingual central area ($P < 0.01$ for all values). No significant differences between the hypofunction and control groups were observed on the buccal side (Figure 4a–d). In the recovery group, the lingual basal and lingual middle cortical bone recovered to control levels between 4 and 6 weeks.

No significant differences between the recovery and control groups were observed on the lingual or buccal sides at week 8 (Figure 4e–j).

To examine the effect of occlusal hypofunction on BMD on the whole body, the density was also measured in the

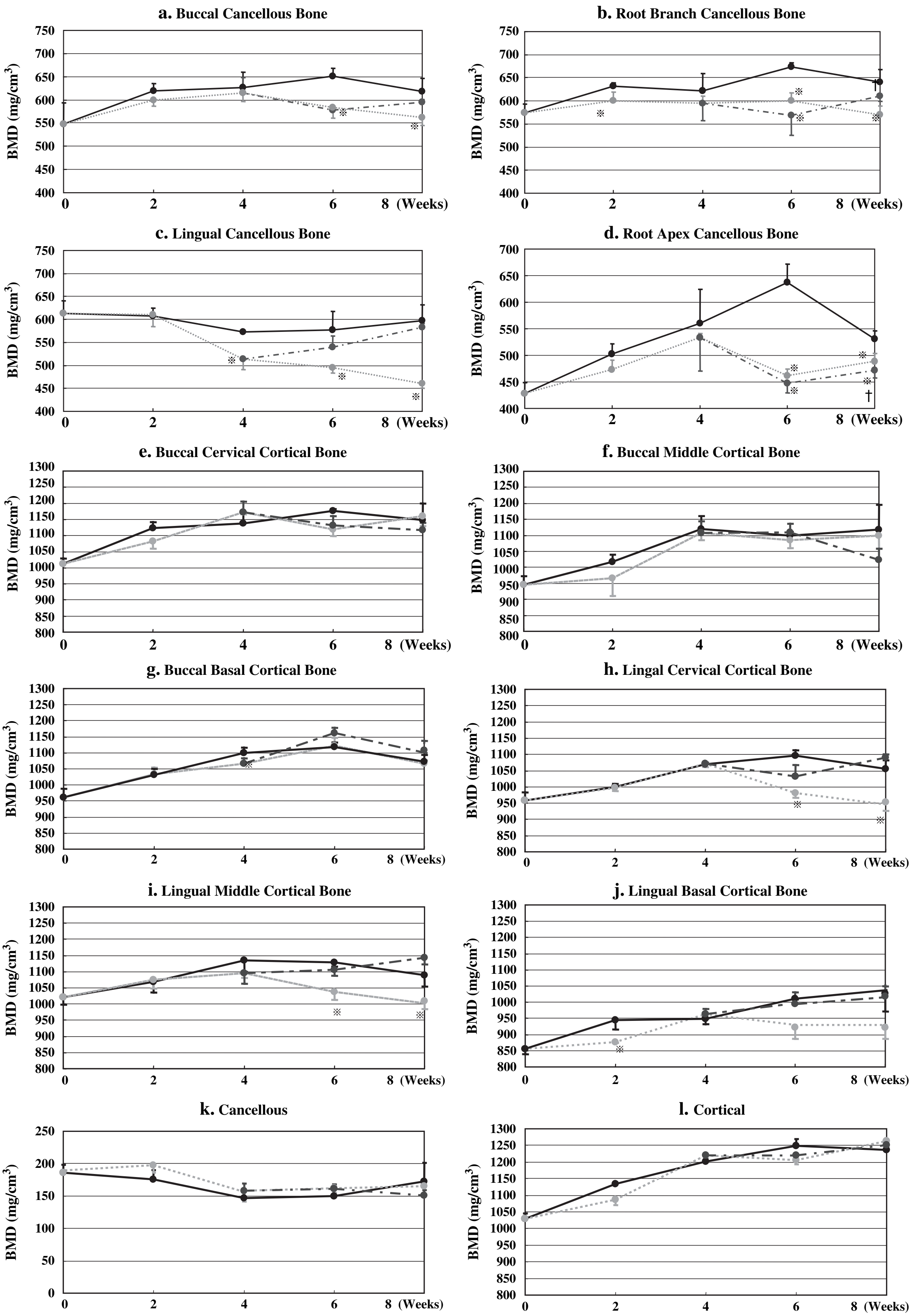
a. Buccal Cancellous Bone
b. Root Branch Cancellous Bone
c. Lingual Cancellous Bone
d. Root Apex Cancellous Bone
e. Buccal Cervical Cortical Bone
f. Buccal Middle Cortical Bone
g. Buccal Basal Cortical Bone
h. Lingal Cervical Cortical Bone
i. Lingual Middle Cortical Bone
j. Lingual Basal Cortical Bone
k. Cancellous
l. Cortical
BMD (mg/cm3)
0 2 4 6 8 (Weeks)

Table 1 The rate of bone mineral density in cancellous, cortical, and femur bone.

	Weeks 2	4	6	8
Buccal cancellous bone				
Control (%)	100.0	100.0	100.0	100.0
Hypofunction (%)	96.8	93.3	88.4	86.0
Recovery (%)	—	—	88.7	96.2
Root branch cancellous bone				
Control (%)	100.0	100.0	100.0	100.0
Hypofunction (%)	94.9	95.4	87.7	80.9
Recovery (%)	—	—	85.2	90.4
Lingual cancellous bone				
Control (%)	100.0	100.0	100.0	100.0
Hypofunction (%)	100.6	89.6	83.3	81.6
Recovery (%)	—	—	93.5	97.6
Root apex cancellous bone				
Control (%)	100.0	100.0	100.0	100.0
Hypofunction (%)	94.2	95.1	74.4	87.7
Recovery (%)	—	—	70.2	89.0
Buccal cervical cortical bone				
Control (%)	100.0	100.0	100.0	100.0
Hypofunction (%)	96.4	103.0	95.2	101.0
Recovery (%)	—	—	96.2	97.2
Buccal middle cortical bone				
Control (%)	100.0	100.0	100.0	100.0
Hypofunction (%)	95.0	98.9	98.8	98.4
Recovery (%)	—	—	101.0	91.6
Buccal basal cortical bone				
Control (%)	100.0	100.0	100.0	100.0
Hypofunction (%)	100.2	96.9	100.3	99.4
Recovery (%)	—	—	103.9	109.3
Lingual cervical cortical bone				
Control (%)	100.0	100.0	100.0	100.0
Hypofunction (%)	98.8	100.2	89.5	89.5
Recovery (%)	—	—	94.1	103.4
Lingual middle cortical bone				
Control (%)	100.0	100.0	100.0	100.0
Hypofunction (%)	100.7	96.6	92.0	92.2
Recovery (%)	—	—	97.9	105.0
Lingual basal cortical bone				
Control (%)	100.0	100.0	100.0	100.0
Hypofunction (%)	92.9	100.8	87.8	85.6
Recovery (%)	—	—	98.8	98.6
Femur cancellous bone				
Control (%)	100.0	100.0	100.0	100.0
Hypofunction (%)	110.7	107.8	108.8	96.2
Recovery (%)	—	—	108.0	87.4
Femur cortical bone				
Control (%)	100.0	100.0	100.0	100.0
Hypofunction (%)	96.0	101.5	96.6	102.1
Recovery (%)	—	—	97.7	101.2

The increase and decrease rate when control 100% was assumed.

intermediate portion of the femur. No changes in density were seen in the cancellous or cortical bone (Figure 4d–k). These results are summarized in Table 1.

Discussion

BMD determination has been widely applied for the prevention, diagnosis, and treatment of osteoporosis, with dual-energy X-ray used as the standard method. However, the results from this method express BMD as the area of bone density (grams per square centimetre). In contrast, determination by CT enables measurement of density per unit volume, and the results can be used to differentiate between cancellous and cortical bone.

In this study, loss of masticatory function resulted in a reduction in bone density of 38.1 per cent at the root apex of cancellous bone and 16.7 per cent in cancellous bone on the lingual side. Furthermore, in cortical bone on the lingual side, density reduced by 7.8–14.4 per cent (Figure 4a,b). Rats have a chipping-type bite mode, which loads a larger force in the vertical than in the horizontal direction; this is different from the grinding type in herbivores. As the rat molar inclines to the lingual side, the bite force is loaded more on the root apex and lingual side than on the buccal side.

Mechanical stimulation of alveolar bone during mastication is crucial to the underlying bone structure. Bresin *et al.* (1994) found that reduced masticatory function caused by the consumption of a soft diet resulted in a decreased width of the alveolar bone in the molar region. Another study (Kiliaridis *et al.*, 1996) showed decreased radiographic alveolar bone mass in the molar region. In addition, a reduction in the amount of cancellous bone below the mandibular first molar has been observed with other experimentally induced functional disturbances (von Wowern *et al.*, 1979). Masticatory muscle function is a determinant of the amount and density of cortical and cancellous bone (Bresin *et al.*, 1999). The reduction of intermittent forces applied to the alveolar bone during mastication result in a reduction of bone density, accompanied by decreased trabecular bone volume and thickness (Mavropoulos *et al.*, 2004). Maki *et al.* (2003) reported differences in bone volume on either side in a temporomandibular joint dysfunction model accompanied by right and left asymmetry in rats. These findings were thought to be due to changes in the local mechanical environment, i.e. an absence or decrease in occlusal load transferred to the alveolar bone during biting. Therefore, it is appears that good functional occlusion is likely to be crucial in maintaining the volume and structure of the mandible.

In contrast, in the recovery group, the cancellous bone density on the buccal and lingual sides and the cortical bone density on the lingual side recovered to control levels.

Figure 4 Peripheral quantitative computed tomograph results for bone mineral density (mg/cm^3) of mandibular cancellous bone (a–d), of mandibular cortical bone (e–j), and of femur cancellous and cortical bone (k–l). Solid line: control group; broken line: hypofunction group; dash-dotted line: recovery group. (a–j) Significantly different from control group: $^*P < 0.01$; significantly different from hypofunction group: $^{\dagger}P < 0.01$; (k–l) no significant difference between control and hypofunction groups.

However, the cancellous bone density at the bifurcation of the root and root apex recovered to only 30 and 50 per cent of the control level, respectively (Figure 4a). Eui-Seok *et al.* (2002) examined the effect of occlusal hypofunction, and its recovery, on the periodontal tissues of the rat molar using a similar experimental model. They demonstrated that the amount of ED-1, a general marker for monocytes, macrophages, dendritic cells, and osteoclasts in the rat, recovered to control level at 3 days. Weinreb *et al.* (1997) investigated the short-term recuperation of bone mass during skeletal reloading, after a period of unloading, in young rats, and found that both cancellous and cortical bone mass was reduced in the unloaded groups and was accompanied by the production of hypomineralized bone. Furthermore, changes in tibial cancellous bone mass by its recovery during reloading were more pronounced, but followed a similar pattern and normalized by 2 weeks. These findings and the results of the current study suggest that recovery of occlusal hypofunction recovered allows recovery of not only periodontal function but also of BMD. However, the root and root apex cancellous bone did not recover to the control group levels, since stress distribution inside the bone is not concentrated near the root, a longer period of recovery may be required.

Conclusions

Occlusal hypofunction results in a decrease in BMD of both cancellous and cortical bone. This decreased density, with the exception of the bifurcation of the root and the root apex in cancellous bone, recovers to control levels when normal function is restored.

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