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Influence of sex hormone disturbances on the internal structure of the mandible in newborn mice

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SUMMARY It has not yet been clarified how sex hormones affect craniofacial bone development immediately after birth. The purpose of this study was to examine the effects of sex hormone deficiency on craniofacial bone development immediately after birth, in terms of the internal structure of the mandible in newborn mice with orchiectomy (ORX) and ovariectomy (OVX). ORX, OVX and a sham-operation were performed on 40 five-day-old C57BL/6J mice. Eight weeks after surgery, each mandible was subjected to histomorphometric analysis of trabecular (Tr) and cortical (Ct) bone mineral density (BMD) by peripheral quantitative computed tomography (pQCT).

In the experimental groups, a significant reduction in BMD was found in comparison with the control groups. In histomorphometric analysis, the number of tartrate-resistant acid phosphatase (TRAP)-positive cells in the condyle and the thickness of the condylar cartilage layer was significantly greater in the experimental mice than in the controls. Trabecular bone volume of the condyle measured on azocarmine-aniline blue (AZAN) sections was significantly less in the experimental mice than in the controls. These results indicate that mandibular growth is inhibited by sex hormone disturbances and the relevant internal structures changed. The findings show that sex hormones are one of the key determinants of mandibular growth and development immediately after birth.

Introduction

In orthodontic treatment, teeth move in the bone of the maxilla and mandible. Tooth movement is also influenced by the condition of the internal structure of the maxilla and mandible. Although sex hormones play an important role in maintenance of bone volume, a reduction causes osteoporosis. It has been demonstrated that ovariectomy (OVX) and orchiectomy (ORX) induce condylar bone loss, and that oestrogen and androgen are effective in the prevention of bone loss during adolescence (Fujita *et al.*, 2001). Oestrogen and androgen stimulate differentiation of the brain during embryogenesis (Swerdloff *et al.*, 1992; Quadros *et al.*, 2002); however, it has not yet been clarified how sex hormones affect craniofacial bone development immediately after birth.

Craniofacial growth shows great variation among individuals, and mandibular growth is related to various factors, such as growth hormones (Hwang and Cha, 2004), growth factors (Delatte *et al.*, 2004), heredity (Oshikawa *et al.*, 2004), and mechanical stress (Bresin *et al.*, 1999). The effects of sex hormones on bone and muscle development are greater than those of genetic or environmental factors (Morishima *et al.*, 1995). It has recently been reported, in an experimental study, that the suppression of sex hormone secretion during the pubertal growth phase inhibits craniofacial growth, particulary mandibular growth, and results in reduced craniofacial development (Fujita *et al.*, 2004).

The purpose of this study was to examine the effects of sex hormone deficiency on craniofacial bone development immediately after birth, in terms of the internal structure of the mandible in newborn mice with ORX and OVX.

Materials and methods

Animals

Forty C57BL/6J 5-day-old mice (Jackson Laboratory, Bar Harbor, Maine, USA) were used in this experiment. The mice were divided equally into two experimental groups with ORX and OVX, and the corresponding sham-operation (control) groups. Under general anaesthesia with sodium pentobarbital, using a stereoscopic microscope (SZX9, Olympus Optical Co., Tokyo, Japan) 20 male and 20 female mice underwent ORX, OVX and the corresponding sham-operation five days after birth. All mice were sacrificed 8 weeks after surgery. The body weight was measured every four days (data given in Fujita *et al.*, 2004). The animals were treated under the ethical regulations defined by the Ethics Committee, Hiroshima University Faculty of Dentistry.

Peripheral quantitative computed tomography (pQCT) measurements

After removing the surrounding soft tissue, the mandible was immersed in 70 per cent ethanol. Each mandible was

then subjected to analysis of trabecular (Tr) and cortical (Ct) bone mineral density (BMD) by pQCT (XCT Research SA+, Norlandstratec, Pforzheim, Germany). Each mandible was scanned by three slices passing through a region 0.1 mm anterior and 0.1 mm posterior to the mesial root of the first molar. After scanning, the mandible was represented by 0.26 mm-thick cross sections, using a voxel size of 0.06 mm. The threshold used for cortical bone measurements was 690 mg/cm^3 with a separation mode of 1.

Histomorphometric analysis

The mandibular condyles were fixed in 4 per cent formaldehyde, decalcified in EDTA (pH 7.4) for 2 weeks, dehydrated in an ascending ethanol series (70, 80, 90, 95, 99, 100 per cent), embedded in paraffin, and cut into 7 μm thick frontal sections. The sections were stained with tartrate-resistant acid phosphatase (TRAP), haematoxylin-eosin (H-E), and azocarmine-aniline blue (AZAN) for histological observation using an optical microscope (BH2-RFCA, Olympus Optical Co.).

The TRAP-stained sections were used to count the number of osteoclasts in the condylar head, and the H-E stained sections to measure the thickness of the condylar cartilage layers. The articular cartilage layers were divided into fibrous (articular), proliferative (chondrogenic), and maturative/hypertrophic (cartilaginous) zones. The sections stained with AZAN were used for histomorphometric analysis, which was performed in the subchondral area of the condyle, using the image analysis program NIH Image 1.59 (National Institutes of Health, Bethesda, Maryland, USA). On the sections passing through the centre of the mandibular condyle, the number of TRAP-positive cells was counted. For quantification of Tr bone volume, the area was measured on the frontal sections, and the means were calculated.

Statistical analysis

Analysis of variances (ANOVA) and pairwise comparisons (Fisher) were performed to examine the differences in values measured among the four groups with a confidence level greater than 95 per cent.

Results

Analysis of BMD

In the pQCT scan, Tr-BMD and Ct-BMD of the mandible were significantly lower in the experimental mice than in the controls. Although Tr-BMD in the ORX mice was similar to that in the OVX mice, Ct-BMD was significantly lower in the ORX than in the OVX mice (Figures 1a and b).

Number of TRAP-positive cells

The number of TRAP-positive cells in the condyle was significantly greater in the experimental mice than in the controls. No significant differences in the number of TRAP-positive cells were found between ORX and OVX mice (Figure 2a).

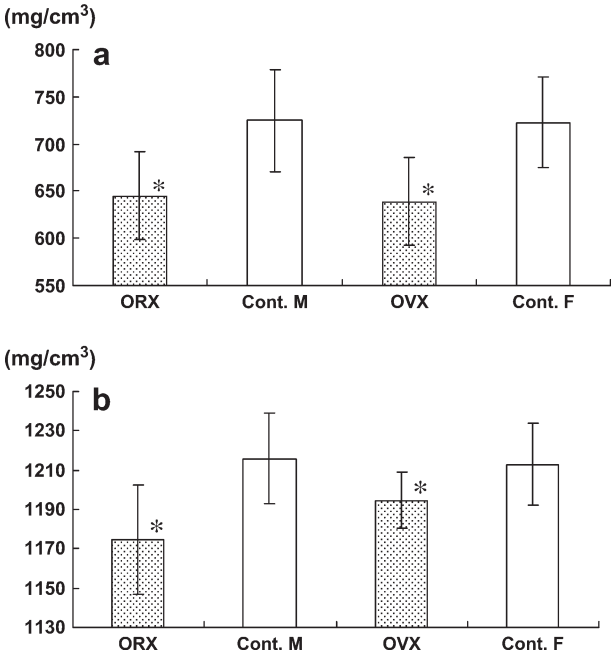

Figure 1 Trabecular bone mineral density (a) and cortical bone mineral density (b) of the mandible analysed by peripheral quantitative computed tomography in orchiectomy (ORX) and ovariectomy (OVX) mice, and male and female controls. * indicates significant difference from controls ($P < 0.05$).

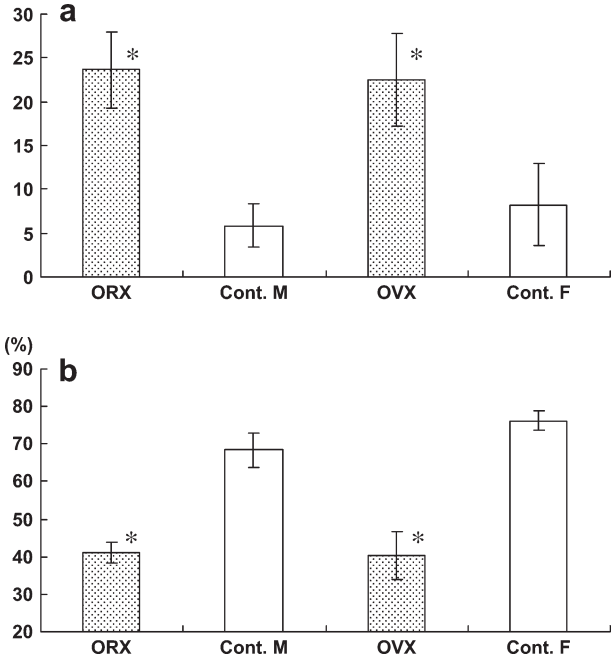

Figure 2 The number of tartrate-resistant acid phosphatase positive cells (a) and trabecular bone volume (b) in orchiectomy (ORX) and ovariectomy (OVX) mice, and male and female controls. * indicates significant difference from controls ($P < 0.05$).

Trabecular bone volume

Tr bone volume of the condyle, measured on AZAN sections, was significantly less in the experimental mice than in the controls. The Tr bone volume of the condyle had a negative correlation with the number of TRAP-positive cells. Tr bone volume was similar in the ORX and OVX mice (Figures 2b and 3).

Thickness of the condylar cartilage layers

The total thickness of the articular cartilage layers was significantly greater in the experimental mice than in the controls. The ORX and OVX groups exhibited 1.7- and 2.1-fold larger values, respectively. Among the cartilage layers, the thickness of the proliferative and maturative/hypertrophic layers were significantly different between the experimental and control mice (Table 1).

Discussion

In this experiment, the influence of sex hormone on the internal structure of the mandible immediately after birth was examined. An awareness of how the maxilla and mandible grow immediately after birth is of interest for

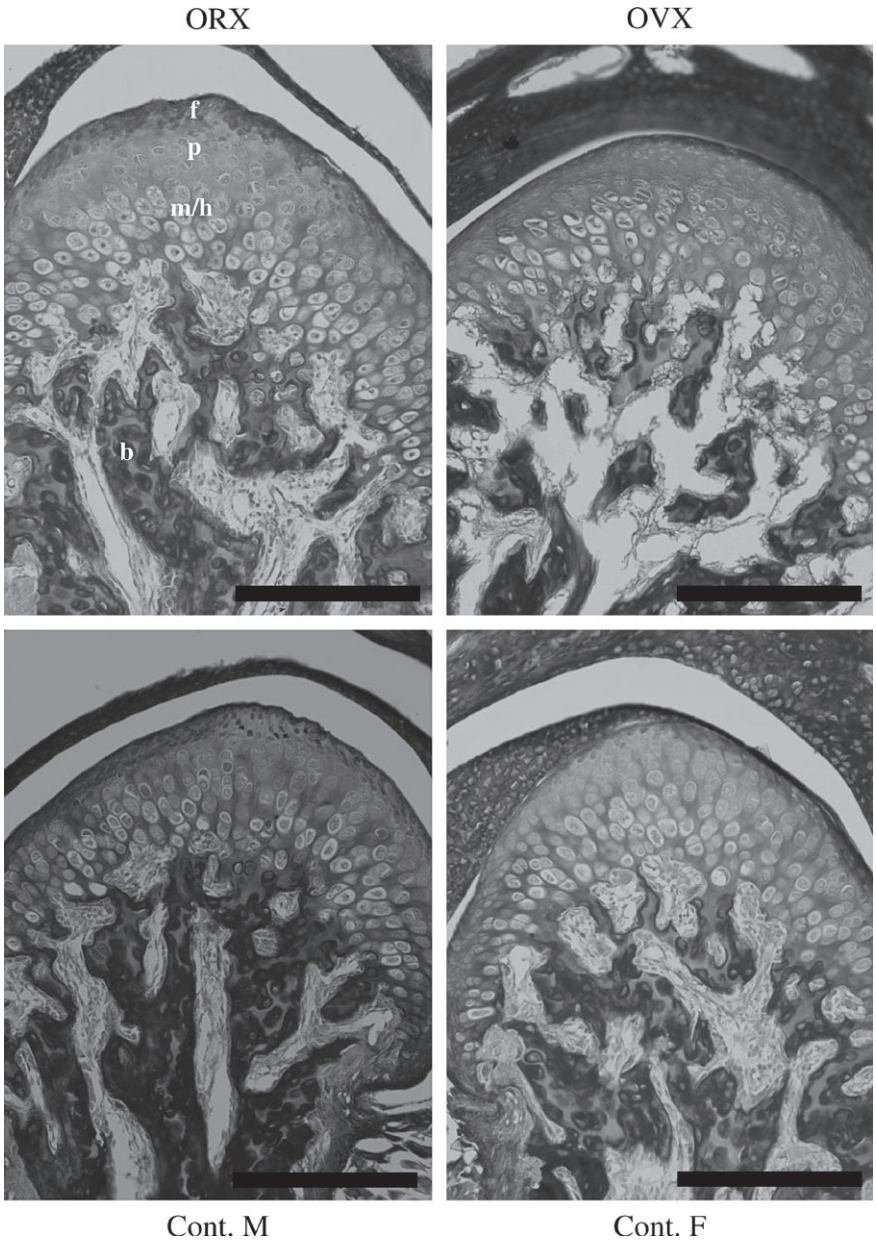

Figure 3 Photomicrographs of the condyles of ovariectomy (OVX) and orchiectomy (ORX) mice, and male and female controls, 8 weeks after surgery. Bars = 200 μm. Azocarmine-aniline blue staining, ×200 magnification. f, fibrous layer; p, proliferative layer; m/h, maturative/hypertrophic layer; b, bone.

Table 1 Thickness (μm) of the condylar cartilage layers in ovariectomy (OVX) and orchiectomy (ORX) mice, and male and female control groups.

Cartilage layer	ORX	Cont. M	OVX	Cont. F
Total	35.35 ± 0.77*	21.22 ± 0.61	45.57 ± 0.67*	21.92 ± 0.85
Fibrous	2.6 ± 0.15	2.1 ± 0.26	2.3 ± 0.25	1.5 ± 0.32
Proliferative	9.68 ± 0.46*	5.86 ± 0.42	15.67 ± 0.63*	6.13 ± 0.67
Maturative/hypertrophic	23.25 ± 0.74*	13.26 ± 0.48	27.6 ± 0.98*	14.29 ± 1.22

*indicates significant difference from controls ($P < 0.05$).

orthodontists. It was assumed that sex hormones were closely related to craniofacial growth because oestrogen and androgen are mostly secreted from the ovary and orchis and the function of sex hormones is activated in adolescence when bone growth is at its highest. Thus, ORX and OVX mice were used in this investigation.

It has previously been reported, in a morphometric study using cephalometric analysis, that the disturbance of sex hormone secretion immediately after birth affects craniofacial growth (Fujita *et al.*, 2004). In the present investigation, the histomorphometric changes were further examined, using pQCT, in terms of the internal structure of the mandible in ORX and OVX mice immediately after birth. This analysis showed that mandibular Tr-BMD and Ct-BMD were significantly lower in the experimental mice than in the controls. Although a decrease of BMD in patients with osteoporosis (Takagi *et al.*, 1995) and in mice (Omi and Ezawa, 1995; Gaumet-Meunier *et al.*, 2000) has been mainly found in Tr bone, in this study of ORX and OVX mice immediately after birth, Ct-BMD was significantly reduced analogous to the Tr bone. In these experiments, adult animals were used. There is limited information about changes in the internal structure of the mandible, measured by pQCT, immediately after birth.

The mandibular condyle is a centre for mandibular growth; however, growth of the mandible is not determined only by cartilaginous but also membranous growth (Berraquero *et al.*, 1992). Ct-BMD reduction was found to be greater in the ORX than in the OVX mice in this study, and skeletal growth of ORX mice was inhibited more than that of OVX mice in a previous investigation (Fujita *et al.*, 2004). Bone growth has been shown to be related to cortical bone density (Maki *et al.*, 2000). This may be a reason why sex hormone secretion blockage immediately after birth causes poor development. These speculations, derived from the present findings, will hopefully be confirmed in future studies.

It is currently accepted that ORX and OVX enhances the turnover of long bones such as the femur or tibia. This phenomenon has also been demonstrated in the mandibular condyle (Fujita *et al.*, 2001). However, the precise mechanism that causes this phenomenon remains to be elucidated. Furthermore, the influences on condylar modelling immediately after birth and subsequent growth still remain unclear.

With respect to bone remodelling affected by OVX, various studies have been carried out. Wronski *et al.* (1988) reported that the initial phase of rapid bone loss in the tibia of OVX rats was coincident with the maximal increase in bone turnover, and then both the bone loss and turnover decreased. Androgens stimulate normal skeletal development during puberty (Johansen *et al.*, 1988), and the delay of puberty in humans has been associated with a lower peak in bone mass (Finkelstein *et al.*, 1992). In the present study, the blockage of sex hormone secretion immediately after birth promoted bone resorption, and it was confirmed that Tr bone volume decreased. This finding is similar to the result of a previous experiment using eight-week-old mice (Fujita *et al.*, 2001).

The influence of sex hormones on bone growth after adolescence is well documented. OVX increases hypertrophic cartilage, and the total amount of growth plate cartilage in OVX animals is decreased by oestradiol (Turner *et al.*, 1994). In the present study, the experimental group exhibited significant differences in the thickness of the proliferative and maturative/hypertrophic layers from the controls, and Tr bone volume was less than that in the control groups. Therefore, sex hormone deficiency may disturb endochondral ossification. These results also indicate that sex hormones alter condylar remodelling, leading to degenerative changes in the temporomandibular joint.

These findings demonstrate that obstruction of the secretion of sex hormone causes changes in the internal structure of the condyle. In addition to previous morphometric results with lateral cephalograms (Fujita *et al.*, 2004), the present study has demonstrated the influence of sex hormones on bone growth immediately after birth, and that sex hormones substantially influence craniofacial growth in newborn mice.

Conclusions

Mandibular growth is inhibited by sex hormone disturbances and the relevant internal structures changed. These findings indicate that sex hormones are one of the key determinants of mandibular growth and development immediately after birth.

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References

Berraquero R, Palacios J, Rodriguez J I 1992 The role of the condylar cartilage in mandibular growth. A study in thanatophoric dysplasia. American Journal of Orthodontics and Dentofacial Orthopedics 102: 220–226

Bresin A, Kiliaridis S, Strid K G 1999 Effect of masticatory function on the internal bone structure in the mandible of the growing rat. European Journal of Oral Sciences 107: 35–44

Delatte M, Von den Hoff J W, Maltha J C, Kuijpers-Jagtman A M 2004 Growth stimulation of mandibular condyles and femoral heads of newborn rats by IGF-I. Archives of Oral Biology 49: 165–175

Finkelstein J S, Neer R M, Biller B M, Crawford J D, Klibanski A 1992 Osteopenia in men with a history of delayed puberty. New England Journal of Medicine 326: 600–604

Fujita T, Kawata T, Tokimasa C, Tanne K 2001 Influence of oestrogen and androgen on modelling of the mandibular condylar bone in ovariectomized and orchiectomized growing mice. Archives of Oral Biology 46: 57–65

Fujita T *et al.* 2004 Effects of sex hormone disturbances on craniofacial growth in newborn mice. Journal of Dental Research 83: 250–254

Gaumet-Meunier N *et al.* 2000 Gonadal steroids and bone metabolism in young castrated male rats. Calcified Tissue International 66: 470–475

Hwang C J, Cha J Y 2004 Orthodontic treatment with growth hormone therapy in a girl of short stature. American Journal of Orthodontics and Dentofacial Orthopedics 126: 118–126

Johansen J S *et al.* 1988 Serum bone Gla-protein as a marker of bone growth in children and adolescents: correlation with age, height, serum insulin-like growth factor I, and serum testosterone. Journal of Clinical Endocrinology and Metabolism 67: 273–278

Maki K, Miller A, Okano T, Shibasaki Y 2000 Changes in cortical bone mineralization in the developing mandible: a three-dimensional quantitative computed tomography study. Journal of Bone and Mineral Research 15: 700–709

Morishima A, Grumbach M M, Simpson E R, Fisher C, Qin K 1995 Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. Journal of Clinical Endocrinology and Metabolism 80: 3689–3698

Omi N, Ezawa I 1995 The effect of ovariectomy on bone metabolism in rats. Bone 17: 163–168

Oshikawa M, Sugano N, Ishigaki R, Ito K 2004 Gene expression in the developing rat mandible: a gene array study. Archives of Oral Biology 49: 325–329

Quadros P S, Pfau J L, Goldstein A Y, De Vries G J, Wagner C K 2002 Sex differences in progesterone receptor expression: a potential mechanism for estradiol-mediated sexual differentiation. Endocrinology 143: 3727–3739

Swerdloff R S, Wang C, Hines M, Gorski R 1992 Effect of androgens on the brain and other organs during development and aging. Psychoneuroendocrinology 17: 375–383

Takagi Y, Fujii Y, Miyauchi A, Goto B, Takahashi K, Fujita T 1995 Transmenopausal change of trabecular bone density and structural pattern assessed by peripheral quantitative computed tomography in Japanese women. Journal of Bone and Mineral Research 10: 1830–1834

Turner R T, Evans G L, Wakley G K 1994 Reduced chondroclast differentiation results in increased cancellous bone volume in estrogen-treated growing rats. Endocrinology 134: 461–466

Wronski T J, Cintron M, Dann L M 1988 Temporal relationship between bone loss and increased bone turnover in ovariectomized rats. Calcified Tissue International 43: 179–183