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The effect of oral ipriflavone on the rat mandible during growth

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SUMMARY Different types of ipriflavone (IF) have been reported to be effective when used as a remedy for bone loss due to osteoporosis. However, no information is available regarding the relationship between IF and jaw bone structure. The aim of this study was to examine the effect of IF on rat mandibles during the growth stage. Thirty-two 5-week-old Wistar male rats were divided into four groups. The control group was fed a standard diet, group A received a low calcium diet (calcium content 30 per cent of the standard diet) for 6 weeks, and the other two groups were fed a low calcium diet for 3 weeks and then a standard diet without IF (group B) or with IF (group C) for 3 weeks. In addition, distilled water was provided for all groups. The effects of IF on mandibular size and bone mineral content were investigated, using lateral cephalometric analysis and peripheral quantitative computed tomography (pQCT).

For mandibular length, the control group showed a significantly higher value than groups A and B ($P < 0.01$, $P < 0.05$, respectively), while group C demonstrated a significantly higher value than group A ($P < 0.01$). In addition, the control group and group C showed significantly higher values for mandibular ramus height than group A ($P < 0.01$). However, bone mineral density in trabecular bone was significantly higher in the control group than in the other groups ($P < 0.01$) and bone mineral density in cortical bone was significantly higher in the control group than groups A, B and C ($P < 0.01$, $P < 0.01$, $P < 0.05$, respectively). Bone mineral density in both trabecular and cortical bone was significantly higher in group C than in groups A and B ($P < 0.01$, $P < 0.05$, respectively). These results indicate that complete recovery from calcium deficiency to the level of the control group may not be attainable, even though IF enhances calcium absorption to act on bone cells and promote bone construction. The importance of calcium intake in the early stages of development was confirmed. These findings also suggest an effect of IF on jaw bone structure.

Introduction

As the average age of the general population advances, increasing incidence of osteoporosis may lead to a variety of social problems. Thus, many approaches have been suggested for its prevention and treatment. An additional concern is that calcium intake in general is low in Japan (Department of Health and Medicine, Ministry of Health and Welfare, 2001). Increasing attention has also been directed to the influences of systemic bone debilitation in the dental field, for example during bone reparation after placement of an implant, or bony changes as a result of periodontitis. Ipriflavone (IF) has been used in Japan since 1988 to inhibit bone resorption, as well as a non-hormonal drug for osteoporosis (Agnusdei *et al.*, 1995; Ushiroyama *et al.*, 1995). It has been reported that IF has a variety of other effects, such as inhibiting the activity of osteoclasts and enhancing the activity of osteoblasts (Tsuda *et al.*, 1986; Ushiroyama *et al.*, 1995).

While it is considered important to understand the relationships between dietary composition and jaw bone structure, only a few investigations have been conducted on mandibular osseous tissue (Qin *et al.*, 1998; Maki *et al.*, 2002).

It has previously been reported that the mandible of rats became fragile when fed a low calcium diet for 3 weeks in early development (Zhang *et al.*, 1998). The aims of the present study were to attempt to clarify the extent of recovery of mandibles in a fragile condition due to a low calcium diet intake during growth with an IF-supplemented standard diet and to examine the effects of IF intake on mandibular size and also on the bone mineral density of mandibular osseous tissue.

Materials and methods

This study was approved by the committee for the use of laboratory animals of Kyushu Dental College, Japan.

Thirty-two 5-week-old Wistar male rats, each weighing approximately 125 g, considered to correspond with development in pre-school children, were divided randomly into four groups. The control group was fed a standard diet, while group A was given a low calcium diet (30 per cent of the standard diet) for 6 weeks, group B a low calcium diet for 3 weeks and then a standard diet for 3 weeks, and group C a low calcium diet for 3 weeks and then a standard diet supplemented with IF for 3 weeks (Table 1). Each group was also given distilled water. After 6 weeks, all rats were killed under deep anaesthesia, after which the cranial bones were immediately removed and fixed in 10 per cent neutral formalin.

Table 1 The composition of the experimental diets (mg/100 g).

	Standard diet	Low calcium diet
Calcium	480	144
Phosphorus	650	612
Magnesium	87	87
Sodium	220	293
Potassium	440	440
Iron	32	32
Copper	0.46	0.5
Manganese	1.6	2.6
Iodine	0.46	0.3
Chlorine	170	174

Lateral cephalometric analysis

After fixation, the cranial bones were divided along the median suture from the parietal bone to the mandible, and the soft tissue around the alveolar part of the left side of the mandible was carefully detached to expose the mental foramen. The median sagittal face of the left side of the head was mounted to contact the film surface, with the mental foramen set immediately under the focus. Soft radiographs were taken with a CSM (ESM-2, Sotex, Tokyo, Japan) using Fuji Softex film (FG, Fuji Film, Tokyo, Japan) at 28 kVp and 6 mA, with a 60 second exposure and a focus-to-film distance of 60 cm.

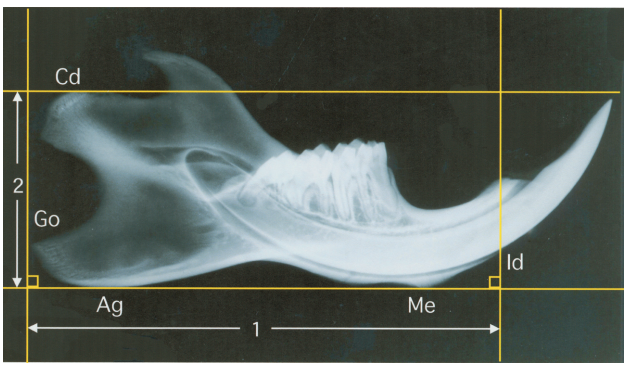

Figure 1 To determine the length of the mandible a tangent line was drawn through menton (Me) and antegonion (Ag) and two lines perpendicular to it were drawn through the rear and front edges of the mandible, with a parallel tangent line drawn through the top point of the condyle. 1, length of the mandible; 2, height of the mandibular ramus; Cd, the most superior point of the condylar head; Go, gonion; Id, infradentale (labial side).

In order to determine the length of the mandible and the height of the mandibular ramus, a tangent was drawn through menton (Me) and antegonion (Ag), and a parallel line through the top of the condyle. Two perpendicular lines were then drawn through the anterior and posterior edges of the mandible (Figure 1). The radiographs were printed at ×5 magnification before the measurements were undertaken. Therefore, all measurements in Table 2 are five times the actual size. All measurements were obtained with a calliper with an accuracy of 0.05 mm.

Bone density

Using peripheral quantitative computed tomography (pQCT; XCT Research, model SA, Stratec-Medizinteecnik Gmbh, Pforzheim, Germany), the bone samples were centrally located between the scanner unit source and the detector with the aid of a support, which produced a preliminary view and a tomographic scan that were shown on the screen (Figure 2). The mandibular bone was scanned around the centre of the mandibular first molar mesial root at three different positions with three different slices, each with an interval of 0.1 mm. Three slices consisting of trabecular and cortical components with a voxel size of 0.08 mm and a height of 0.26 mm were chosen and measured. Cortical bone was defined as that with a density of more than the threshold value (690 mg/cm^3). The region it enclosed was manually determined and the density then measured (mg/cm^3). The region of trabecular bone was defined manually, after which trabecular bone density (mg/cm^3) was measured.

For all the results, a *t*-test was used to determine statistically significant differences between the four groups (significance $P < 0.05$).

The reproducibility of the pQCT measurements and cephalometric analysis were assessed in the three rat mandibles on five occasions. The coefficient of variation, including the repositioning error, was 0.65 per cent for cortical bone density, 5.14 per cent for trabecular bone density, 2.35 per cent for the length of the mandible and 3.12 per cent for the height of the mandibular ramus.

Table 2 The length of the mandible and the height of the mandibular ramus (mm). Note that all measurements are ×5 the actual size.

	Length of the mandible		Height of the mandibular ramus	
Control	117.60 ± 3.41	]**	60.07 ± 1.91	]**
Group A	112.52 ± 2.20	]*	56.44 ± 2.11	]*
Group B	114.54 ± 2.71	]**	58.74 ± 2.14	]**
Group C	116.21 ± 3.70		60.02 ± 1.25	

*$P < 0.05$; **$P < 0.01$.

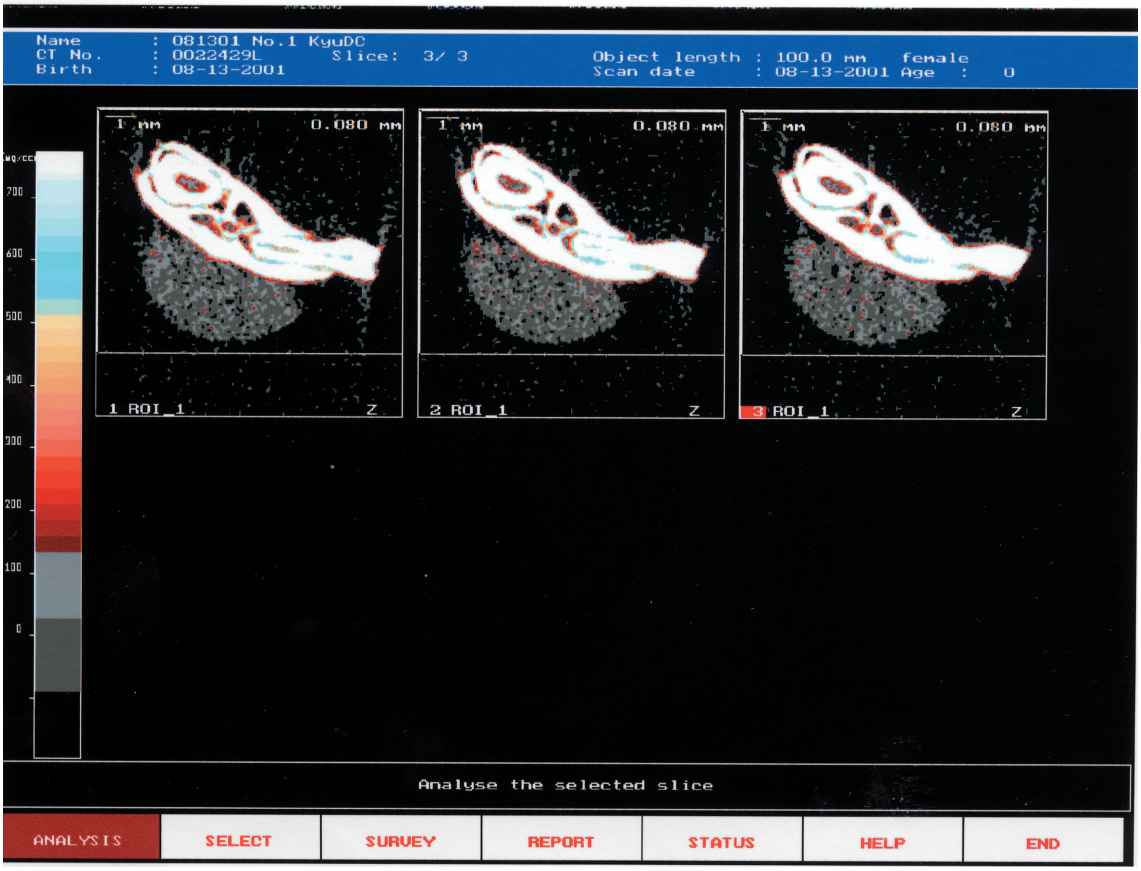

Figure 2 Peripheral quantitative computed tomography slices. The mandibular bones were scanned around the centre of the mandibular first molar mesial root at three different positions at an interval of 0.1 mm.

Results

The results of the lateral cephalometric analyses and bone mineral density measurements, together with the significant differences, are shown in Tables 2 and 3. For mandibular length, the control group showed a significantly higher value than groups A and B ($P < 0.01$, $P < 0.05$, respectively), while group C showed a significantly higher value than group A ($P < 0.01$). In addition, the control group and group C showed significantly higher values for mandibular ramus height than group A ($P < 0.01$) (Table 2).

Bone mineral density in trabecular bone was significantly higher in the control group than the other groups ($P < 0.01$) and bone mineral density in cortical bone was significantly higher in the control group than groups A, B and C ($P < 0.01$, $P < 0.01$, $P < 0.05$, respectively). Bone mineral density in both trabecular and cortical bone was significantly higher in group C than in groups A and B ($P < 0.01$, $P < 0.05$, respectively) (Table 3).

Discussion

Calcium is an essential nutrient for normal growth and development, as an adequate amount in the diet helps to build the skeleton and prevent skeletal disorders during childhood and adolescence (Matkovic

Table 3 Bone mineral density of mandibular trabecular and cortical bone using peripheral quantitative computed tomography (mg/cm^3).

	Trabecular bone density		Cortical bone density	
Control	532.81 ± 39.51	]**]**]**	1253.71 ± 22.52	]**]**]**]*
Group A	242.5 ± 75.30	]*]**	1157.51 ± 28.51	]**]**
Group B	309.5 ± 53.24	]**	1201.50 ± 19.98	]**
Group C	376.21 ± 51.23	]*	1222.54 ± 17.52	]*

*$P < 0.05$; **$P < 0.01$.

et al., 1990; Matkovic, 1991, 1992). In addition, a strong bone structure in young adulthood is one of the most important factors for preventing osteoporosis and associated fractures later in life (Tato *et al.*, 1996; Marchiguano, 1997).

The effects of IF (from soya beans) on bone, acting as an inducer of isoflavone, have been studied (Agnusdei *et al.*, 1995; Ushiroyama *et al.*, 1995), but it was considered important to investigate the relationship between dietary composition and mandibular structure, as there is no known evidence regarding the effect of IF on the structure of the mandible. It has been shown that IF is highly effective for bone production in long bones, such as the femur and hind limb (Yamazaki *et al.*, 1986; Foldes *et al.*, 1988). However, it has previously been reported that the action of nutrients on osteogenesis is different in the mandible compared with other bones (Maki *et al.*, 2002). Therefore, further investigation was considered necessary to compare the effects of IF on the mandible with those on other bones, such as the femur and tibia, under the same conditions.

It is important to ensure the reproducibility of these methods. Therefore, the coefficient of variation was calculated, which was found to be less than 0.05, confirming that this method was reliable.

In the present study, the low calcium diet group showed a significantly shorter mandibular body and ramus than the other three groups. Kiliaridis *et al.* (1985) studied rat mandibles during the growth stage and reported that the skeletal units of the mandible affected by calcium deficiency were the angle, condylar, and coronoid processes as well as the corpus, which was mainly due to a reduction in the sagittal dimension. In rats, sagittal growth is much greater than vertical growth and remodelling follows the V-principle (Duterloo and Vilamann, 1978). In the present study, while the height of the mandibular ramus recovered in the low calcium and standard diet groups to the level of the control group, the length of the mandible did not completely recover. The findings for the low calcium and standard diet with IF group (group C) were not significantly different from the control group.

It has been reported that IF has a variety of effects, such as inhibiting the activity of osteoclasts and enhancing the activity of osteoblasts (Tsuda *et al.*, 1986; Ushiroyama *et al.*, 1995). Therefore, on the basis of these findings, the alveolar crest of the mandible, where membranous ossification was dominant, was nearly restored to the level of the control group by IF therapy. Additional studies are required in the future to investigate the relationships between IF and endochondral ossification and chondrocytes.

Many approaches have been attempted for the quantitative determination of minerals in bone (Carter and Hayes, 1976; Ladizesky *et al.*, 2000), with bone mineral density determination widely applied to the prevention, diagnosis and treatment of osteoporosis. However, a quantitative determination of minerals in the mandible is difficult because it includes the teeth. Of the various methods used, dual-energy X-ray (DXA) has become the standard analysis. However, DXA expresses bone mineral density as the area of bone density (g/cm), because it is only possible to obtain information about the bone when condensed in a dimensional manner. On the other hand, bone mineral density calculated as the area of bone density determined by pQCT enables the measurement of density per unit volume, and can also differentiate between cortical and trabecular bone.

In the present study, bone mineral density in both cortical and trabecular bone was significantly higher in the control group than in the three experimental groups and bone mineral density in both cortical and trabecular bone was significantly higher in group C than in the other experimental groups. Notably, a greater effect was exerted on the trabecular bone, probably due to faster bone metabolic turnover in trabecular than in cortical bone.

Conclusions

The present investigation attempted to clarify the extent of recovery of rat mandibles in a fragile condition due to a low calcium diet intake during early development by an IF-supplemented standard diet. When calcium intake is inadequate in the early stages of development, it is difficult to recover to normal levels, as seen in this study even when sufficient calcium was given later. This tendency was especially marked in bone mineral density and in the effects on mandibular length and ramus height. As a result, complete recovery from calcium deficiency, to the same level as in the control group, might not be attainable even though IF enhances calcium absorption from the small intestine to act on bone cells and promote bone production. Therefore, the importance of calcium intake in early development is confirmed and the effect of IF on the structure of the jaw bone is suggested. Additional studies are needed to further compare the effect of IF on other long bones such as the tibia.

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