An experimental study on the effect of UNICAL® on debilitated mandibles of rats in growth stage—using indicators of bone density and cephalometric analysis—

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Abstract We examined the effect of UNICAL® a calcium supplement with a high absorption rate, on debilitated mandibles of rats in the growth stage using cephalometric analysis and peripheral quantitative computed tomography (pQCT).
I. Cephalometric analysis results
We implemented coordinates analysis and true measurement for the cephalometric analysis. Compared with the control group, the low calcium/UNICAL® diet group showed significantly greater values in many indicators, whereas the low calcium diet group showed significantly lower values in only a few indicators. There were no significant differences seen between the low calcium/standard diet group and the control.
II. Mandibular analysis results
Compared with the control group, the low calcium diet and low calcium/standard diet group were significantly different in most indicators, whereas the low calcium/UNICAL® diet group showed no significant difference, except for trabecular bone density.
We concluded that UNICAL® is an effective calcium supplement that is able to aid in the recovery of a debilitated mandible, which can result from insufficient calcium intake during the growth period.

Key words
Calcium,
Cephalometric analysis,
Mandible,
pQCT,
UNICAL®

Introduction
Osteoporosis is characterized by decreases in bone mass and ultrastructural changes, which lead to fragile bones and a greater risk of fracture. One key factor related to osteoporosis is bone density, which is affected by hereditary factors, as well as such post-natal factors as exercise and nutrition. Peak bone mass is achieved after the adolescent growth spurt, while in menopause bone mass decreases in a striking manner and then more slowly with advanced age. Two means of prevention against osteoporosis are increasing bone mass during growth and reducing age-related bone loss by exercise or drug therapy. It has also been reported that if calcium intake during the growth stage is inadequate, calcium in serum will decrease and result in bone debility. Thus, it is important to take in enough calcium during the growth stage.

Many methods have been applied to obtain objective information regarding bone structure. Micro densitometry (MD) is used to measure bone density; however, the analysis is only in two dimensions. Thus it is impossible to distinguish the difference between cortical bone and trabecular bone. On the other hand, peripheral quantitative computed tomography (pQCT), employed in the present study, is able to provide bone density information in three dimensions, which allows for the distinction between
cortical and trabecular bone.

Various studies have shown the effects of vitamin D₃, calcitriol, and ipriflavone on debilitated bone. However, there is no report known of the effect of dietary therapy on debilitated mandibles that are a result of insufficient calcium intake in the growth stage. In the present study, we investigated the effects of UNICAL®, a calcium supplement with a high absorption rate, on debilitated mandibles in rats during the growth stage.

### Materials and methods

Twenty 5-week-old Wistar male rats, each weighing approximately 125 g, which we considered to correspond to preschool children, were used. They were randomly divided into 4 groups, with 5 rats in each group. The control group rats were fed a standard diet with tap water for 7 weeks. Rats in the low calcium group were fed a low calcium diet (30% of the calcium of the standard diet) and tap water for 7 weeks. Those in the low calcium/diet group were fed the same low calcium diet and tap water for 3 weeks, and then the standard diet and tap water for the next 4 weeks. Rats in the low calcium/UNICAL® diet group were fed the low calcium diet and tap water for 3 weeks, and then a UNICAL® supplemented standard diet and tap water for the next 4 weeks. All rats had access to food and water freely. All diets were produced Oriental Yeast, Tokyo, Japan (Table 1). The UNICAL® diet is made from calcium citrate prepared from sea urchin shells along with added chondroitin. Calcium citrate is obtained by baking sea urchine shells at more than 1000°C until they change into CaO, after which citrate is added.

After 7 weeks, all rats were killed under deep anesthesia, and the mandibles were removed and fixed in 10% neutral buffered formalin. Two weeks later, the samples were washed with tap water and all soft tissue was removed before drying.

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

### Body weight

The body weight of each rat was recorded once a week.

### Cephalometry

The dried mandibles were separated into two parts, left and right, equivalently and then placed onto X-ray film with the lingual surface facing the film, allowing the mental foramen to be in focus. Soft X-ray picture were taken with CSM (ESM-2, Softex, Japan) and Fuji Softex film (FG, Fujifilm) at 28kVp, 6mA, 60 second exposure, and a focus-to-film distance of 60cm. The X-ray pictures were

### Table 1 The origin of the mineral mixture (mg/100g)

<table>
<thead>
<tr>
<th></th>
<th>Standard diet</th>
<th>Low calcium diet</th>
<th>UNICAL® diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>480</td>
<td>144</td>
<td>1697</td>
</tr>
<tr>
<td>P</td>
<td>650</td>
<td>612</td>
<td>612</td>
</tr>
<tr>
<td>Mg</td>
<td>87</td>
<td>87</td>
<td>111</td>
</tr>
<tr>
<td>Na</td>
<td>220</td>
<td>293</td>
<td>221</td>
</tr>
<tr>
<td>K</td>
<td>440</td>
<td>746</td>
<td>412</td>
</tr>
<tr>
<td>Fe</td>
<td>32</td>
<td>32</td>
<td>30</td>
</tr>
<tr>
<td>Cu</td>
<td>0.46</td>
<td>0.5</td>
<td>0.43</td>
</tr>
<tr>
<td>Zn</td>
<td>3.4</td>
<td>3.0</td>
<td>3.18</td>
</tr>
<tr>
<td>Mn</td>
<td>1.6</td>
<td>2.6</td>
<td>1.5</td>
</tr>
<tr>
<td>I</td>
<td>0.46</td>
<td>0.3</td>
<td>0.43</td>
</tr>
<tr>
<td>Cl</td>
<td>170</td>
<td>174</td>
<td>158.95</td>
</tr>
</tbody>
</table>

Fig. 1 Reference points for coordinate analysis of lateral cephalograms

Cr: The most posterior point of coronoid process.
CC: The most distant point of the line Cd-Cr.
Cd: The most posterior point of condylar head.
GC: The deepest point of the line Go-Cd.
Go: Gonion.
Ag: Ante-gonion.
MA: The deepest point of the outer margin of bone which connects Me and Ag.
Me: Menton.
Id: Infradentale (labial side)
In: Edge of the lower incisor
Id': Infradentale (lingual side)
AF: The deepest point of the outer margin of bone which connects Al and Id.
Al: The highest point of the mesial alveolar bone at the lower first molar
M1: The highest point of the mesiobuccal cusp of the lower first molar
M2: The highest point of the central cusp of the lower third molar
Fig. 2 Reference points and items for linear measurements of rat mandibular length

- Bi: The alveolar base of the lower incisor
- Cd-Id: Length of mandible
- Go-Id: Length of mandibular body
- Al-Id': Length of lingual side of a alveolar bone
- Id'-M2: Length of the lower dental arch
- Cd-Bi: Distance from condyle head to the alveolar base of the lower incisor

Fig. 3 Reference points and items for linear measurements of rat mandibular height

- Cd-Go: Height of mandibular ramus
- Cd-Ag: Height of condylar head
- Cr-Ag: Height of coronoid process
- Al-Mc: Height of the central portion of alveolar bone

Fig. 4 Analysis image of mandible

Mandibular measurement (Peripheral quantitative computed tomography)

The XCT research SA modeler (Stratec Medizintechnik GmbH, Pforzheim, Germany) for animals was used. Around the root apex of the first molar, 3 slices with a pixel size of 0.08 mm and height of 0.46 mm were measured, and the one with the longest root was used for image analysis (Fig. 4).

(1) Cortical bone analysis

Cortical bone was defined as bone with a density of more than 690 mg/cm³ and the region around it was enclosed manually. We determined cortical bone density (mg/cm³), cortical bone cross-sectional area (mm²), and cortical bone mineral content (mg/mm).

(2) Trabecular bone analysis

The region of trabecular bone was defined manually. Trabecular bone density (mg/cm³), trabecular bone cross-sectional area (mm²), and trabecular bone mineral content (mg/mm) were each measured.

For all the results, a t-test was used to determine statistically significant differences between the experimental groups and control group. All statistical tests were conducted at the alpha = 0.05 level.

Result

I. Body weight

No significant differences in body weight were found between any of the groups.
Table 2  The result of coordinate analysis (mm, mean ± S.D.)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Low calcium diet group</th>
<th>Low calcium and standard diet group</th>
<th>Low calcium and UNICAL® diet group</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>Y</td>
<td>X</td>
<td>Y</td>
<td>X</td>
</tr>
<tr>
<td>Cr</td>
<td>63.7±0.5</td>
<td>42.9±0.2</td>
<td>59.3±2.0**</td>
<td>39.5±1.8*</td>
</tr>
<tr>
<td>CC</td>
<td>61.1±2.0</td>
<td>32.2±0.5</td>
<td>63.3±3.3</td>
<td>28.2±3.4</td>
</tr>
<tr>
<td>Cd</td>
<td>87.3±3.1</td>
<td>30.2±0.7</td>
<td>88.0±2.6</td>
<td>28.3±0.8*</td>
</tr>
<tr>
<td>Gc</td>
<td>76.6±1.8</td>
<td>87.1±1.1</td>
<td>77.4±1.5</td>
<td>6.7±1.7</td>
</tr>
<tr>
<td>Go</td>
<td>92.7±1.9</td>
<td>-6.5±1.6</td>
<td>93.5±2.7</td>
<td>-6.6±2.4</td>
</tr>
<tr>
<td>Ag</td>
<td>69.7±3.9</td>
<td>-16.7±1.3</td>
<td>71.2±5.6</td>
<td>-17.7±0.7</td>
</tr>
<tr>
<td>Me</td>
<td>-5.1±1.9</td>
<td>-16.7±0.7</td>
<td>-6.8±0.9</td>
<td>-17.2±0.8</td>
</tr>
<tr>
<td>td</td>
<td>-22.4±2.6</td>
<td>-8.5±1.4</td>
<td>-20.3±1.5</td>
<td>-10.4±0.9</td>
</tr>
<tr>
<td>In</td>
<td>-47.8±2.3</td>
<td>31.5±2.2</td>
<td>-50.9±0.6</td>
<td>31.4±1.5</td>
</tr>
<tr>
<td>td*</td>
<td>-26.5±1.0</td>
<td>11.1±1.1</td>
<td>-27.3±0.7</td>
<td>10.0±1.1</td>
</tr>
<tr>
<td>Al</td>
<td>-4.6±1.8</td>
<td>3.0±0.9</td>
<td>-6.1±0.1</td>
<td>2.0±0.3</td>
</tr>
<tr>
<td>Al</td>
<td>4.2±0.9</td>
<td>10.1±1.6</td>
<td>4.3±1.3</td>
<td>9.2±1.9</td>
</tr>
<tr>
<td>M₀</td>
<td>5.5±1.4</td>
<td>19.5±0.6</td>
<td>6.0±1.4</td>
<td>18.2±0.6</td>
</tr>
<tr>
<td>M₁</td>
<td>20.8±1.2</td>
<td>19.8±0.8</td>
<td>21.9±1.3</td>
<td>18.9±0.7</td>
</tr>
<tr>
<td>MA</td>
<td>35.7±1.3</td>
<td>9.6±2.3</td>
<td>36.7±1.7</td>
<td>10.0±2.2</td>
</tr>
</tbody>
</table>

*: Compared with control group, P<0.05
**: Compared with control group, P<0.01

Table 3 The result of true measurement (mm, mean ± S.D.)

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Low calcium diet group</th>
<th>Low calcium and standard diet group</th>
<th>Low calcium and UNICAL® diet group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Id</td>
<td>115.1±2.1</td>
<td>114.7±1.0</td>
<td>116.5±2.7</td>
<td>132.1±3.0**</td>
</tr>
<tr>
<td>Cd</td>
<td>114.5±1.2</td>
<td>113.4±0.5</td>
<td>114.8±2.8</td>
<td>129.7±4.4**</td>
</tr>
<tr>
<td>Id*</td>
<td>30.0±0.7</td>
<td>31.7±1.5</td>
<td>31.3±0.8</td>
<td>35.0±1.7**</td>
</tr>
<tr>
<td>Id-M₀</td>
<td>48.0±0.9</td>
<td>49.9±1.8</td>
<td>49.5±1.0</td>
<td>56.2±1.1**</td>
</tr>
<tr>
<td>Bi-Cd</td>
<td>33.6±2.3</td>
<td>31.2±2.7</td>
<td>36.4±2.4</td>
<td>40.3±2.0**</td>
</tr>
<tr>
<td>Cd-Go</td>
<td>35.9±1.0</td>
<td>35.2±1.5</td>
<td>35.7±1.6</td>
<td>38.1±2.5</td>
</tr>
<tr>
<td>Cd-Ag</td>
<td>49.5±1.8</td>
<td>48.2±1.0</td>
<td>49.5±2.3</td>
<td>55.3±3.1*</td>
</tr>
<tr>
<td>Cr-Ag</td>
<td>59.2±0.3</td>
<td>57.0±1.3*</td>
<td>57.1±2.7</td>
<td>64.9±0.8**</td>
</tr>
<tr>
<td>Af-Me</td>
<td>19.6±0.1</td>
<td>19.8±0.9</td>
<td>19.0±0.5</td>
<td>21.6±0.6**</td>
</tr>
</tbody>
</table>

*: Compared with control group, P<0.05
**: Compared with control group, P<0.01

II. Cephalometric analysis

A. Coordinates analysis

Significant differences were found between the experimental groups and the control group, as shown in Table 2. Compared with the control group, the low calcium/UNICAL® diet group showed significantly greater values for many of the indicators. Further, the low calcium diet group showed significantly lower values in only a few indicators, while there were no significant differences found between the low calcium/standard diet group and the control.

B. True height and length

Results of true height and length measurements showed significant differences between the control group and experimental groups, as shown in Table 3. Most indicators in the low calcium/UNICAL® diet group showed a significantly greater value than
Table 4: The result of mandible (mean ± S.D.)

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Low calcium diet group</th>
<th>Low calcium and standard diet group</th>
<th>Low calcium and UNICAL® diet group</th>
</tr>
</thead>
<tbody>
<tr>
<td>trabecular bone density (mg/cm³)</td>
<td>533.5 ± 34.3</td>
<td>238.5 ± 90.8**</td>
<td>310.3 ± 61.1**</td>
<td>443.8 ± 39.8*</td>
</tr>
<tr>
<td>trabecular bone cross-sectional area (mm²)</td>
<td>2.5 ± 0.8</td>
<td>2.6 ± 0.3</td>
<td>2.9 ± 0.5</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>trabecular bone mineral content (mg/mm)</td>
<td>1.3 ± 0.3</td>
<td>0.6 ± 0.3**</td>
<td>0.9 ± 0.1*</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>cortical bone density (mg/cm³)</td>
<td>1251.6 ± 23.1</td>
<td>1173.1 ± 30.7*</td>
<td>1203.5 ± 11.9*</td>
<td>1231.5 ± 24.8</td>
</tr>
<tr>
<td>cortical bone cross-sectional area (mm²)</td>
<td>4.5 ± 0.4</td>
<td>3.5 ± 0.6*</td>
<td>3.6 ± 0.3*</td>
<td>4.6 ± 0.4</td>
</tr>
<tr>
<td>cortical bone mineral content (mg/mm)</td>
<td>5.6 ± 0.4</td>
<td>4.2 ± 0.8*</td>
<td>4.3 ± 0.4**</td>
<td>5.6 ± 0.5</td>
</tr>
</tbody>
</table>

*: Compared with control group, P < 0.05
**: Compared with control group, P < 0.01

III. The results of mandible

pQCT results are shown in Table 4. Compared with the control group, the low calcium diet and low calcium/standard diet groups differed significantly for most indicators, whereas the low calcium/UNICAL® diet group showed no significant difference, except for trabecular bone density.

Discussion

There are currently several kinds of calcium supplements available; however, the absorbability of each is different. In our previous studies, various calcium supplements were used in therapy for debilitated bones, such as calcium-rich sardine powder and ipriflavone, and the results were reported. In the present study, the UNICAL® diet, which is made from sea urchin shells and has increased absorption materials added, was used because of its high absorbability and satisfactory effects on the recovery of debilitated mandibular condyles have been reported. Further, the UNICAL® diet contains many kinds of mineral elements besides calcium such as zinc and magnesium. It was reported that zinc promotes ossification, while magnesium has been the focus of recent studies because insufficient intake is thought to be a cause of many diseases and can also interfere with the metabolism of calcium and bone.

We studied 5-week-old Wistar male rats, which were considered to correspond to preschool children, in order to observe the effect of the UNICAL® diet on debilitated mandibles. pQCT and cephalometric analysis were employed to observe bone structure changes.

I. Body weight

No significant differences in body weight were found between any of the rat groups. This result suggests that the calcium present in bone is in storage. Thus, when calcium intake is insufficient for a period of time, the calcium stored in bone provides a buffer and if calcium is sufficiently supplied again within a short amount of time, bone metabolism and body weight increase will not be affected.

II. Cephalometric analysis

Development of the face and skull is affected by the function and development of adjacent structures. A functional matrix that takes functional factors and environmental factors into account has been advocated by Moss et al. On the other hand, Scott proposed that the development of the face and skull is mainly determined by inherited factors and that environmental factors, except for those caused by surgical means, have no impact.

We used cephalometric analysis to measure and determine mandibular morphology. The factors related to bone development include inherited, postnatal, and environmental factors, as well their combined effect. In the present study, we intended to study the effect of the UNICAL® diet not only on mandible morphology but also on the repair of debilitated bone. Compared with the control group, the low calcium diet group showed a significantly lower both X and Y value for Cr, only Y for Cd value and the true length of Cr-Ag, which suggested that
low levels of calcium had an inhibitory effect on the development of the mandible. Further, we found that the coronoid process and condylar head were also influenced largely by insufficient calcium intake; however, the other points of the mandible were not influenced significantly.

In the low calcium/standard diet group, no significant difference was found in the results of coordinate analysis of true measurement. In the present experimental protocol, calcium intake was insufficient in developing rats that were models of humans from infant to school age, but was normal in adolescence. However, no cephalometric changes were noted. Therefore, we concluded that sufficient calcium intake during adolescence is important, as this is the stage when growth and development of the mandible are the most remarkable.

Compared with the control group, the low calcium/UNICAL® diet group showed significantly greater values in all true length indicators of Cr-Ag, Al-Me, and Cd-Ag, as well as in Cr, CC, Ag, In, Al, and MA. The UNICAL® diet contains not only calcium, zinc, and magnesium, but also chondroitin, such as D-glucuronic acid mucopoly saccharide and N-acetyl-D-galactosamine mucopolysaccharide, which are thought to promote calcium absorption in the intestine. Moreover, it contains many other kinds of minerals related to bone growth. Since the diet was changed during adolescence, in which bone growth and development are the most striking, the most satisfactory results were seen in the low calcium/UNICAL® group and it was suggested that dietary therapy with UNICAL® could recover the negative impact of insufficient calcium intake during school age growth years. Further, it has been shown that the absorption rate of UNICAL® in the intestine is 1.34 times that of milk and 5.25 times that of calcium from eggshells.

III. Mandibular analysis
Bone mineral content measurement is a popular means to evaluate bone and a variety of methods are employed, such as dual energy X-ray absorptiometry (DXA), quantitative ultrasound (QUS), quantitative computed tomography (QCT), and pQCT. In the past, we used micro densitometry (MD) to measure mandibular bone mineral content. MD is simple and easy to perform, however, it can only demonstrate the structure of bone in two dimensions, so bone mineral content is recorded as g/cm², which is different from true bone mineral content. Further, the same bone mineral content from different sites will show a different value. In contrast, pQCT is able to measure peripheral bone mineral content in three dimensions, which allows the measurement of true bone mineral content, and distinction between trabecular bone and cortical bone as well as other indicators of bone structure. pQCT was developed as a small-field, high-resolution extension of the existing QCT system, to measure the peripheral skeleton with a substantial improvement of image definition. Currently available pQCT machines perform transverse scans over a wide range of sizes and regions of interest.

When compared with the control group, the low calcium/standard diet group showed significantly lower values for all indicators except for trabecular bone cross-sectional area, whereas the low calcium/UNICAL® diet group had no significant differences except for trabecular bone density. These results suggest that the standard diet could not allow for recovery of bone structure changes or bone mineral content, while the UNICAL® diet provided for recovery of most of those indicators.

The basic multicellular units in trabecular bone respond to low calcium intake early and constantly, while the response in cortical bone declines soon. As reasons for that difference, differences in metabolism rate and anatomy between cortical bone and trabecular bone have been suggested, while the material used has also been mentioned. However, since the exact reason is not yet known, further research is required. In the study, the low calcium/UNICAL® diet group showed significantly lower trabecular bone density than the control group, which may have been because the insufficient calcium intake could not be recovered easily in trabecular bone.

It has been reported that a high calcium diet could not help a debilitated mandible to recover completely; however, the bone reconstruction was promoted and the number of trabeculae increased. We consider that our relatively satisfactory results demonstrated that UNICAL® is a high calcium supplement with a high absorption rate.

In conclusion, significantly lower trabecular bone density was found in the low calcium diet/standard diet, and low calcium/UNICAL® diet groups, though the latter was higher than the former. We concluded that the UNICAL® diet has beneficial effects, however, the damage toward bone density caused by insufficient calcium intake before ado-
lescence could not be recovered completely by UNICAL® alone.

We reported a summary of this study in the 40th Japan Pediatric Dentistry Congress (June 2002, Chiba, Japan).

References


