Original Article

Effects of Restricted Calcium Intake on Bone and Maxillofacial Growth

Bone Mineral Content and Cephalometry

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ABSTRACT

Objective: To investigate the effects of a low calcium diet on maxillofacial development by evaluating Bone Mineral Content (BMC) in the lower alveolar bones, femurs, and tibias and by performing cephalometry on growing rats.

Materials and Methods: Thirty 5-week-old male Wistar rats were randomly divided into 3 groups; the control group (n = 10) was given standard diet for 6 weeks, the low calcium/standard diet group (n = 10) was given a calcium-restricted diet for the first 4 weeks, and then a standard diet for the following 2 weeks, and the low calcium diet group (n = 10) was given the calcium-restricted diet for 6 weeks. After the rats were euthanized, heads and legs were fixed and cephalometry was performed. Next, mandibles, femurs and tibias were digitally photographed and the BMC was evaluated using our newly developed software.

Results: The BMC was decreased in all of the bone samples from the two groups that received restricted calcium. In the low calcium/standard diet group, the BMC recovered the most in the tibias and least in the lower alveolar bones. Development of the mandibles in the anterior-posterior direction was accelerated, while that in the superior-inferior direction was inhibited in those rats.

Conclusion: The BMC reduction following calcium deficiency in the lower alveolar bone hardly recovers, so prevention is important. Development of the mandible in a superior-inferior direction is inhibited while that in an anterior-posterior direction is accelerated due to a calcium-restricted diet.

KEY WORDS: Calcium-restricted diet; Alveolar bone; Bone mineral content; Maxillofacial development; Growing rats

INTRODUCTION

According to the Ministry of Health, Labour and Welfare, Japanese calcium intake has been insufficient.[1] It can decrease bone mineral content (BMC) and peak bone mass (PBM). The PBM is reached at the end of puberty or at the beginning of adulthood in humans, and is one of the most important parameters to predict individual possibility of fracture.[2] Thus, for recommending a necessary means of prevention of fracture, an accurate evaluation of PBM is very useful.[3–5] In addition, in daily clinical practice when we think of alveolar bone treatment with implants, BMC is one of the very important parameters in making prognosis or postoperative evaluation. BMC is an essential parameter for the prevention, treatment and maintenance of periodontitis. Though there are comparisons of BMC among various bone types under the condition of insufficient calcium intake in adulthood, those in the growing period are rarely reported.

Once BMC is reduced, we have to recover it in order to enjoy a healthy life. In general, bone grafting and some medicines to increase the BMC are the major means of recovering BMC and bone volume.[6–9] In these treatments, evaluations of BMC is a valuable means for confirming bone reconstruction. BMC is generally evaluated by translating the opacity of the bone into that of an equivalent thickness of aluminum wedge, which is called microdensitometry.[10–12]

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Accepted: February 2007. Submitted: October 2006.

Insufficient calcium intake can affect maxillofacial growth, too.[13] Maxillofacial growth is often evaluated by cephalometric analysis. So, evaluation of BMC and cephalometric analysis are important means of evaluating the effect of insufficient calcium intake on skeletal development.

In previous evaluations of BMC using digital radiographs such as Dixel,[11] we could use only small imaging plates. Moreover, in cephalometric analysis, researchers conducting basic studies evaluated a great number of samples and manual analysis required much time. In the present study, we used a VISTA SCAN (Dürr Dental GmbH & Co. KG, Bietigheim-Bissingen, Germany) that was able to analyze larger animal bones and broader human regions (57 × 76 mm), and created a new computer program to analyze the results. Further, in order to save time and decrease workload, we created software for semi-automatic cephalometric analysis, too. Using those systems, we investigated the effects of a low calcium diet on BMC of lower alveolar bones, femurs and tibias, and on maxillofacial growth in growing male Wistar rats.

MATERIALS AND METHODS

Rats and Diets

We used thirty 5-week-old male Wistar rats (Crj:WI) (Charles River Laboratories Japan, Inc, Yokohama, Japan) and randomly divided them into 3 groups of 10 rats each. The control (Co) group was fed an AIN-93G standard diet (Oriental Yeast Co., Ltd., Tokyo, Japan) for 6 weeks. The low calcium/standard diet (LCSD) group was given a calcium-restricted AIN-93G diet containing 144 mg/100 g of calcium for the first 4 weeks, and then an AIN 93G standard diet for the following 2 weeks. The low calcium diet (LC) group was given the calcium-restricted AIN-93G diet for 6 weeks. All animals were allowed free access to the food and distilled water.

Each of the rats was housed individually. All the rats were euthanized, and then the heads and the legs were extracted and fixed. The experiments were carried out according to the Guidelines of Animal Research Center of Kyushu Dental College.

Radiography

Following fixation, the heads were cut into sagittal sections along the medial suture. Both right and left sections were used for the cephalometric analysis. Radiographs of the lateral view were taken. An ESM, which is a type of soft X-ray photographic device, was used (Softex Co., Ltd, Tokyo, Japan) at 30 kVp and 5 mA, with 70 cm between the focus and the surface, an exposure time of 90 seconds using soft X-ray film (Industrial X-ray film FR; Fuji Photo Film Co., Ltd, Tokyo, Japan).

Next, the mandibles were removed from the sectioned heads, and the mandibles, femurs, and tibias without soft tissues were subjected to digital radiography with a VISTA SCAN at 70 kVp and 7.0 mA, with 20 cm between the focus and the surface, and an exposure time of 0.40 seconds per imaging plate (Dürr Dental GmbH & Co. KG, Bietigheim-Bissingen, Germany). While obtaining the digital radiographs, we also took images of an aluminum wedge together with the bones to evaluate BMC by microdensitometry. The soft X-ray pictures were scanned (GT-X900; SEIKO EPSON CO, Tokyo, Japan) and stored in a personal computer (Dynabook AX/840LS; TOSHIBA CO, Tokyo, Japan), while the digital radiographs were directly stored in the same computer.

Bone Mineral Content

We created computer programs for evaluation of BMC and to perform cephalometric analysis using Visual Basic 6.0 (Microsoft Co, Seattle, U.S.A.) and Image Kit 7 (Microsoft Co, Seattle, U.S.A.). The digital radiographs consisted of 256 gradations, from 0 to 255. They were used as parameters of BMC when we evaluated BMC by microdensitometry. Microdensitometry is the means of evaluating BMC by translating it into the thickness of aluminum wedge equivalent which was taken in the radiograph together with the bone sample. By comparing mean gradations of pixels in the region of interest to the gradations of aluminum wedge equivalent, we can get BMC as a value of aluminum thickness. It is one of the major means of BMC evaluation.[11,12]

First, one of the radiographs was displayed on the screen. The scanning area on the aluminum wedge was fixed by a right-click. The area ranged from the ordinate of the right-click ±5 pixels (the area between green lines in Figure 1). After scanning, the correlation coefficient was calculated.

Second, both ends of the bone were fixed by left-clicks (red lines in Figure 1), after which the scanning began with the third left-click at any point above the bone. The scanning area ranged the mid-point of the fixed bone ends ±17 pixels (the blue area in Figure 1). In previous studies, a densitometer (PDS-15; KONICA MINOLTA HOLDINGS, INC, Tokyo, Japan) was used in evaluating BMC. However, that device has a slit size of 10 × 500 μm, which is too narrow to determine whether the scanning area is proper or singular. The scanning area ranged enough in our present study.

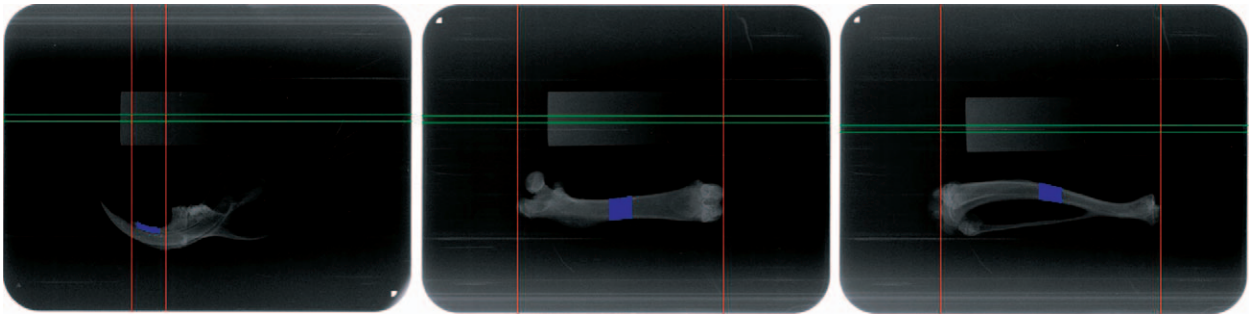

Figure 1. Representative display during evaluation of BMC in lower alveolar bone, femur, and tibia. Aluminum slope was scanned between green lines, and BMC was evaluated in the blue area. Red lines expressed both bone ends.

Cephalometry

A radiograph of the lateral view was displayed on the screen. Two directions of the coordinate axes were applied with the upper-left corner defined as the origin, and the downward direction along the Y axis and rightward direction along the X axis considered to be positive. There were 27 fixed points and 29 measurement items for length[14] (Table 1 and Figure 2).

Second, we plotted all the points except for U1′, U2′, L1′, L2′, and Co′, specifying each with the option button. The 22 points were stored in the computer and displayed on the screen at the same time (Figure 3). In this manner, we were able to obtain most of the length using a formula to get a distance between 2 points.

In order to obtain the distances of U1-U1′, U2-U2′, L1-L1′, L2-L2′, and Co-Co′, the computer program calculated formulae of lines A-N and Pg-Gn, and then used a formula to obtain the distance between a point and a line. Thus, we could obtain 5 measurement items, and could accomplish all of the measurements for our cephalometric analysis.

Reproducibility of software

Before evaluating all of the items, we needed to confirm the reproducibility of the results. We randomly chose 1 radiograph for each bone type and cephalometry, then evaluated BMC and made cephalometric analyses 3 times a day on 3 different days by one researcher. We evaluated the values according to the F-test by one-way analyses of variance (ANOVA).

Statistical Analyses

The results were analyzed statistically with Statistical Package for the Social Sciences Software (SPSS) version 13.0. We performed one-way ANOVA and made comparisons among pairs (Bonferroni correction) to examine the difference in data between the groups with the confidence level greater than 95%.

Table 1. Landmarks and Measurement Items Used for Cephalometric Analysis

Po:	Most posterior point on cranial valut
N:	Point the nasofrontal suture
A:	Most anterior point on nasal bone
E:	Intersection between frontal bone and most superior-anterior point of the posterior limit to the ethmoid bone
S:	Intersection between posterior border of basisphenoid and the tympanic bulla
Ba:	Most posterior and inferior point of occipital condyle
Pr:	Most inferior and anterior point on alveolar process of premaxilla
Bu:	Point on premaxilla between jawbone and lingual surface of upper incisors
Mu:	Point on intersection between maxillary bone and mesial surface of upper first molar
Iu:	Most prominent point between incisal edges of upper incisors
U1:	Point on mesial occulusal fossa of upper first molar
U1′:	Crossing point on A-N perpendicular to A-N from U1
U2:	Point on distal occulusal fossa of upper second molar
U2′:	Crossing point on A-N perpendicular to A-N from U2
Pg:	Point on most inferior contour of lower border of mandible, adjacent to incisors
Gn:	Point on most inferior contour of angular process of mandible
Go:	Most posterior point of angular process of mandible
Co:	Most posterosuperior point of condylar process
Co′:	Crossing point on Pg-Gn perpendicular to Pg-Gn from Co
Id:	Most inferior and anterior point on alveolar process of mandible
Bl:	Point on intersection between lingual surface of lower incisor and anteriormost part of lingual alveolar bone
Ml:	Point on intersection between the mandibular alveolar bone and mesial surface of first molar
Il:	Most prominent point between incisal edges of lower incisor
L1:	Point on mesial occulusal fossa of lower second molar
L1′:	Crossing point on Pg-Gn perpendicular to Pg-Gn from L1
L2:	Point on distal occulusal fossa of lower second molar
L2′:	Crossing point on Pg-Gn perpendicular to Pg-Gn from L2

RESULTS

All rats were weighed at the beginning of the experiment, the day that the diet of the LCSD group was changed to standard (at 4 weeks), and on the last day of the experiment (at 6 weeks). No significant differences were observed in increments of weight among the groups, as shown in Table 2.

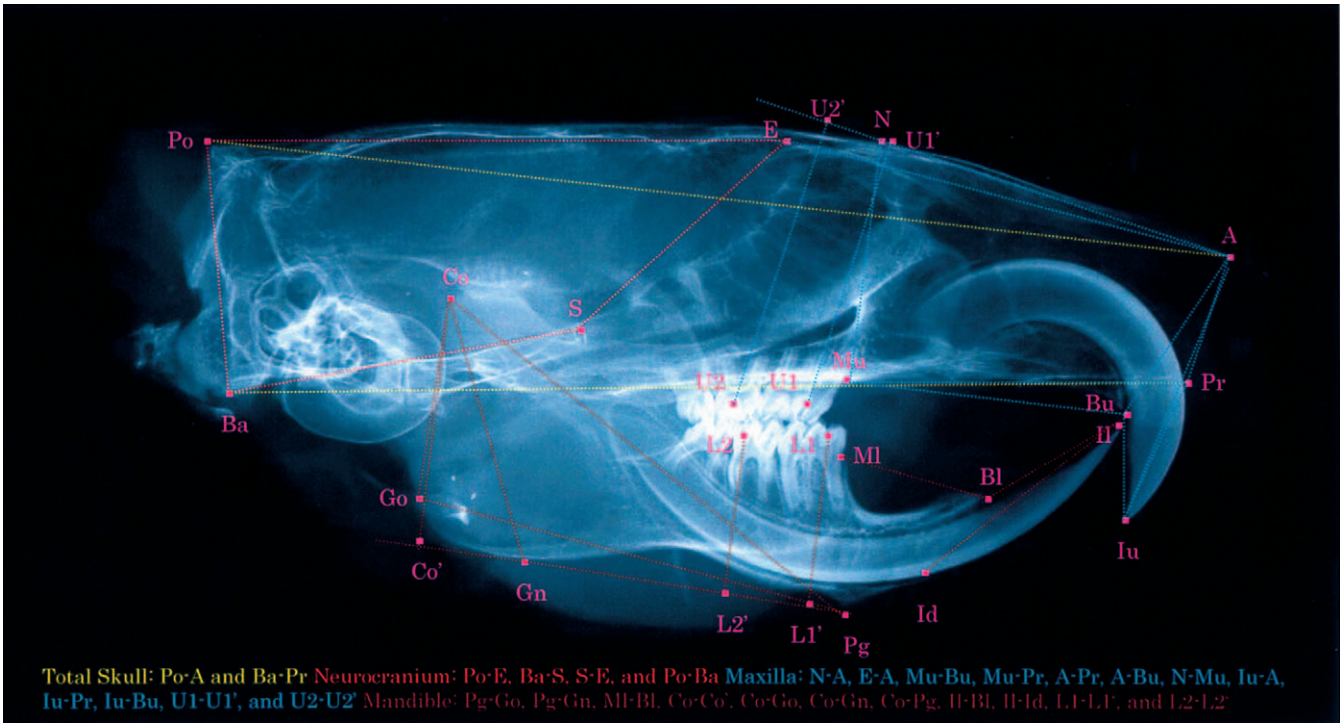

Figure 2. A landmark of 27 fixed points and 29 measurement items. We performed cephalometric analyses according to the landmark.

Table 2. Comparison of Weight Increments in First 4 Weeks and Following 2 Weeks of the Experiment (Unit: g)

	First 4 Weeks		Following 2 Weeks	
	Mean ± SD	Probability	Mean ± SD	Probability
Co	203.867 ± 22.318	—	44.344 ± 8.452	—
LCSD	202.610 ± 13.951	—	42.510 ± 6.248	—
LC	199.350 ± 22.779	—	37.230 ± 8.064	—

Statistically analyzed by one-way ANOVA. No significant difference was observed.

** $P = .01$, * $P = .05$.

For confirmation of the reproducibility of the results obtained with our computer software, we evaluated values according to the F-test by a one-way ANOVA as shown in Table 3, and we confirmed the reproducibility of our newly created computer programs.

For evaluation of BMC, we found significant differences; Co > LC ($P < .01$), Co > LCSD ($P < .01$), and LCSD > LC ($P < .05$) in the lower alveolar bones, Co > LC ($P < .01$), Co > LCSD ($P < .01$), and LCSD > LC ($P < .01$) in femurs, and Co > LC ($P < .01$) and LCSD > LC ($P < .01$) in the tibias (Table 4).

In cephalometric analysis, no significant differences were observed among the groups for the Total Skull and Neurocranium parameters. On the other hand, significant differences were observed in some of the other parameters; Co > LCSD ($P < .05$) in Mu-Pr, Co > LCSD ($P < .05$) and LC > LCSD ($P < .05$) in Iu-Pr, Co > LCSD ($P < .05$) in U1-U1′, and Co > LCSD ($P < .01$) in U2-U2′, which reflect size of Maxilla. Co > LCSD ($P < .01$) and Co > LC ($P < .01$) in Co-Go, Co > LCSD ($P < .05$) and Co > LC ($P < .01$) in Co-Gn, Co > LC ($P < .01$) in L1-L1′, and Co > LC ($P < .01$) in L2-L2′, which reflect growth in the superior-inferior direction of the mandible, as were Co > LCSD ($P < .05$) and LC > LCSD ($P < .05$) in Il-Bl, LC > Co ($P < .01$) in Pg-Gn, and LCSD > LC ($P < 0.05$) in Il-Id, which show growth in the anterior-posterior direction of mandible (Table 5).

DISCUSSION

It is generally known that BMC decreases due to a low calcium diet,[15–18] which was confirmed by our experimental results. BMC in the Co group was significantly higher than that in the LC group in the lower alveolar bones, femurs, and tibias. Previous studies have also reported the effects of a low calcium diet on various kinds of bones.[19,20]

Nevertheless, it was interesting that the recovery of BMC differed according to bone kind after changing from a low calcium diet to a standard one (Table 4). In the lower alveolar bones, recovery of BMC was moderate after changing from a low calcium diet to a standard one. On the other hand, in tibias, the BMC in the Co and LCSD groups was significantly higher than that in the LC group, while a significant difference was not observed between the Co and LCSD groups. Thus the BMC recovered completely because of

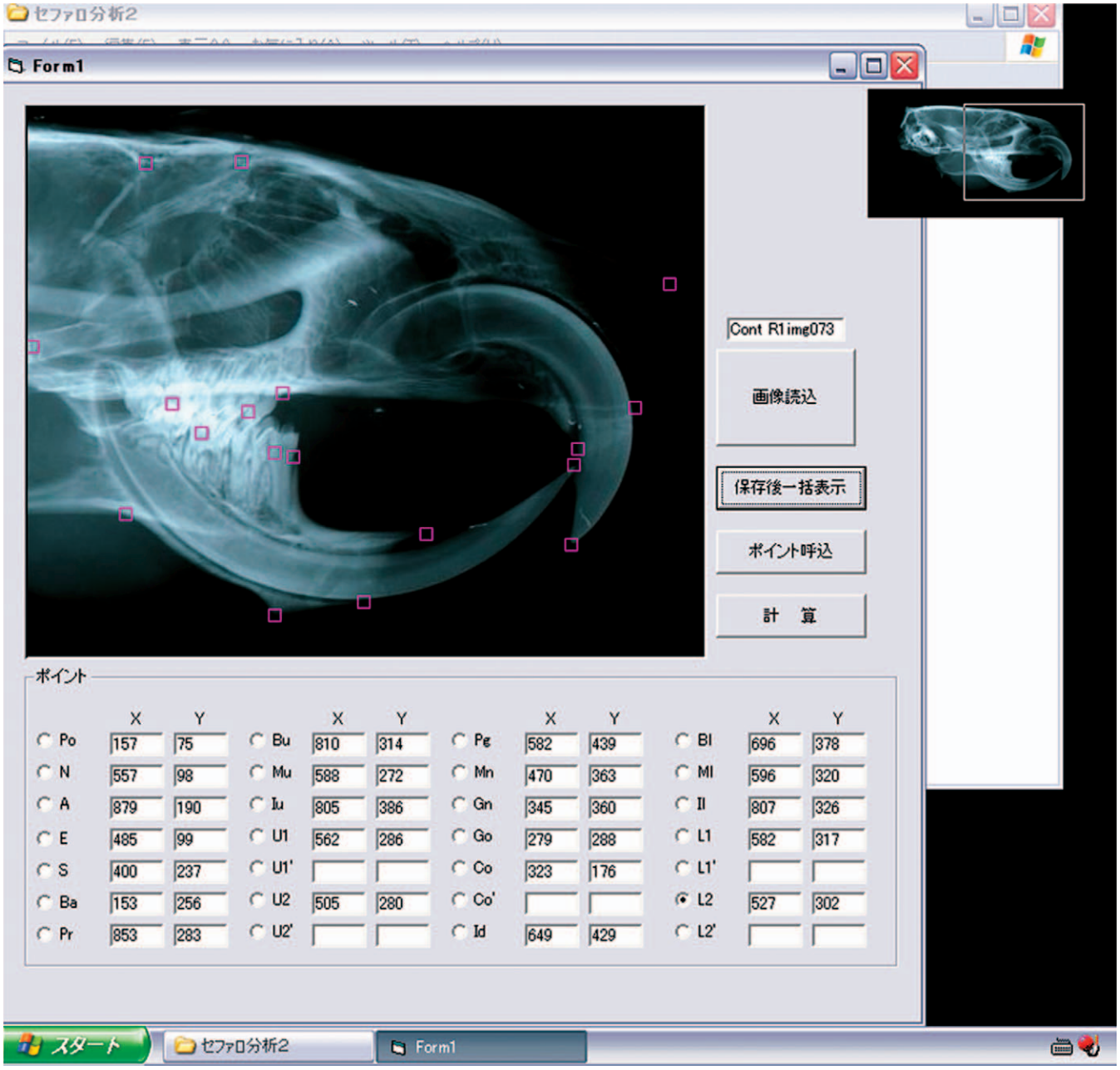

Figure 3. Representative display when determination of points has finished. After plotting the points, they were displayed on the screen at the same time with their coordinates.

changing from the low calcium diet into a standard one. In the femurs, recovery of BMC by changing from the low calcium diet to a standard one was greater than in the mandibles. Those results indicated that BMC in the mandibles and femurs recovered to some extent, though not completely, by changing from a low calcium diet to a standard one.

In the normal bone growth process, bone is deposited when there is sufficient calcium intake, however, when insufficient the bone remodeling process resorbs existing bone in order to maintain linear bone growth and periosteal expansion.[21,22] Further, an excess secretion of PTH due to secondary hyperparathyroidism might accelerate bone resorption in the present LC and LCSD groups. After changing from a low calcium diet to a standard one, we observed significant differences in the recovery of BMC according to the type of bone. In general, the lower alveolar bone is more sensitive to PTH than other bones,[23] which was considered to be the reason why BMC in the lower alveolar bones did not recover well by changing from the low calcium diet to a standard one. Also, the lower alveolar

Table 3. F-values for Confirmation of Reliability on our Software

Measurement Items	F-values
Po-A	1.557
Ba-Pr	0.836
Po-E	0.252
Ba-S	0.434
S-E	1.129
Po-Ba	3.138
N-A	3.621
E-A	0.105
Mu-Bu	2.143
Mu-Pr	0.662
A-Pr	0.429
A-Bu	0.776
N-Mu	1.718
Iu-A	0.004
Iu-Pr	0.625
Iu-Bu	0.034
U1-U1′	0.417
U2-U2′	0.010
Pg-Go	0.173
Pg-Gn	0.904
Ml-Bl	1.761
Co-Co′	0.058
Co-Go	0.001
Co-Gn	0.075
Co-Pg	0.483
Il-Bl	0.005
Il-Id	0.062
L1-L1′	4.589
L2-L2′	0.405
Mandible	0.412
Femur	0.041
Tibia	0.003

F_2^2 (0.05) = 19.0.

Measured values were evaluated according to F-test by one-way ANOVA.

bones are thought to have a relatively high turnover and such active bones tend to show bone loss, due to a small imbalance between bone resorption and formation.[23] We concluded that once BMC in the lower alveolar bones decrease, it rarely recovers completely, thus it is very important to prevent a reduction of BMC, especially in alveolar bones.

Our cephalometric analyses showed that the growth of mandibles in the superior-inferior direction was decreased significantly in the rats that consumed a calcium-restricted diet. It is known that bone formation and calcification are inhibited by a low calcium diet that continues for a long period. This is because resorption of pre-existing bone occurs in order to meet the calcium needs of longitudinal bone growth and periosteal expansion, resulting in reductions in bone size and weight.[24] Moreover, insufficient calcium intake could induce reduction of growth hormone, which resulted in reductions in bone size.[25] This was observed in the mandibles, which have greater growth potential.[26] On the other hand, we are not able to provide a satisfactory explanation as to why the growth of the mandible in an anterior-posterior direction was significantly increased in the rats that consumed the calcium-restricted diet. Values of LCSD in Iu-Pr, U1-U1′, U2-U2′, Il-Bl, and L2-L2′ were significantly lower than those of Co or LC. These parameters can be influenced by tooth contour, so the significant differences could be caused by constitutional tooth contour, habit, and other factors rather than calcium-restricted diet.

Though we allocated the groups randomly, the bone samples are small and we cannot know if they were initially the same. So, there may be a limitation in applying these data to smaller bone samples. Also, our software was made for 2 dimensional evaluations, but we hope to adapt it for 3 dimensional evaluations in the future.

We created new computer programs to evaluate BMC and to perform cephalometric analysis in our present study. Many researchers and general practitioners will be able to evaluate BMC with larger images with minimum exposure. In addition, much time can be saved and the workload reduced while performing cephalometric analyses. They can be revised on demand according to individual needs. Because of their usefulness, we hope that our computer programs will be utilized widely by many researchers and dental practitioners, and play an important role in basic studies, health promotion and treatment.

Table 4. Significant Differences in Comparisons of Bone Mineral Content of Alveolar Bones, Femurs, and Tibias (Unit: mmAl)

	Co		LCSD		LC	
	Mean ± SD	Probability	Mean ± SD	Probability	Mean ± SD	Probability
Alveolar Bone		Co-LCSD**		LCSD-Co**		LC-Co**
	1.256 ± 0.073	Co-LC**	1.125 ± 0.065	LCSD-LC*	1.058 ± 0.095	LC-LCSD*
Femur		Co-LCSD**		LCSD-Co**		LC-Co**
	1.234 ± 0.040	Co-LC**	1.200 ± 0.028	LCSD-LC**	1.139 ± 0.026	LC-LCSD**
Tibia						LC-Co**
	1.111 ± 0.065	Co-LC**	1.116 ± 0.035	LCSD-LC**	1.030 ± 0.031	LC-LCSD**

Statistically analyzed by one-way ANOVA and significant differences among the groups were searched by Bonferroni Correction.

** $P = .01$, * $P = .05$.

Table 5. Significant Differences in Changes in Linear Measurements of Craniofacial Skeletons (Unit: mm)

	Co		LCSD		LC	
	Mean ± SD	Probability	Mean ± SD	Probability	Mean ± SD	Probability
Maxilla						
Mu-Pr	17.753 ± 0.514	Co-LCSD*	17.383 ± 0.228	LCSD-Co*	17.661 ± 0.450	—
Iu-Pr				LCSD-Co*		
	7.814 ± 0.469	Co-LCSD*	7.384 ± 0.449	LCSD-LC*	7.789 ± 4.992	LC-LCSD*
U1-U1′	13.731 ± 0.824	Co-LCSD*	12.959 ± 0.740	LCSD-Co*	13.510 ± 0.948	—
U2-U2′	14.865 ± 0.647	Co-LCSD*	13.713 ± 0.865	LCSD-Co*	14.235 ± 0.940	—
Mandible						
Pg-Gn		Co-LCSD*				
	15.002 ± 1.200	Co-LC**	15.882 ± 0.734	LCSD-Co*	16.351 ± 1.071	LC-Co**
Co-Go		Co-LCSD**				
	9.568 ± 0.506	Co-LC**	8.850 ± 0.536	LCSD-Co**	8.812 ± 0.403	LC-Co**
Co-Gn		Co-LCSD*				
	13.603 ± 0.780	Co-LC**	13.019 ± 0.582	LCSD-Co*	12.595 ± 0.524	LC-Co**
Il-Bl				LCSD-Co*		
	7.668 ± 0.440	Co-LCSD**	7.326 ± 0.360	LCSD-LC*	7.677 ± 0.406	LC-LCSD*
Il-Id	12.295 ± 0.409	—	11.832 ± 0.827	LCSD-LC*	12.363 ± 0.557	LC-LCSD**
L1-L1′	7.539 ± 0.606	Co-LC**	7.237 ± 0.518	—	7.037 ± 0.345	LC-Co**
L2-L2′	7.023 ± 0.415	Co-LCSD**	6.523 ± 0.392	LCSD-Co**	6.661 ± 0.495	—

Statistically analyzed by one-way ANOVA and significant differences among the groups were searched by Bonferroni Correction.
** $P = .01$, * $P = .05$.

CONCLUSIONS

- Preventing BMC reduction in the lower alveolar bone is important and our computer programs can play an important role in detection.
- Development of the mandible in an anterior-posterior direction was accelerated following consumption of a low calcium diet, whereas that in the superior-inferior direction was inhibited.

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