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The effects of food consistency on maxillary growth in rats

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SUMMARY The effect of food consistency on the bone appositional pattern at the growth site in the palatal region of the maxillary complex in growing rats was examined by quantitative analysis employing bone histomorphometry.

Sixty inbred male rats aged 14 days in the weaning period were divided into two groups. One group was fed a conventional solid diet in addition to milk, while the other received the same diet but in liquid form in addition to milk. They were weaned at 21 days of age. Vital staining was employed to enable a longitudinal recording of bone apposition.

In rats fed a liquid diet, the amount of bone apposition on the occlusal surface of the palate was reduced in the region between the first molars, but was increased in the region between the third molars, indicating a more anteriorly directed growth rotation of the palate. The width and ossification rate of the synchondrosis of the midpalatal suture was smaller. Furthermore, lateral growth of the maxilla was inhibited considerably in the distal area.

In conclusion, this study shows that food consistency affects the bone appositional pattern at the growth site in the palatal region of the maxillary complex. The results also suggest that the difference in the growth pattern in the upper viscerocranium induced by different food consistencies is caused not only by a difference in mechanical force of the masticatory muscles acting on the muscle insertion areas but also by a difference in the growth pattern in the region which receives occlusal loading.

Introduction

Previous investigations have demonstrated that the physical consistency of food influences craniofacial growth and morphology in growing animals. Changes in the loading of the bones induced by different food consistencies may affect not only the local bone in areas of muscle insertion (Engström *et al.*, 1986) but also the bone in areas where no direct muscle insertion exists, in the bone morphology and internal structure (Bouvier and Hylander, 1981; Kiliaridis, 1989; Yamada and Kimmel, 1991; Bresin *et al.*, 1994).

In the upper viscerocranium, it was reported that in rats fed a soft diet the growth pattern of the upper viscerocranium was orientated in a more orthocranial direction compared with rats fed a hard diet (Kiliaridis *et al.*, 1985). Since subsequent studies confirmed decreased bone apposition in bones at areas of muscle insertion and their adjacent area (Engström *et al.*, 1986), changes in fibre size and composition (Kiliaridis *et al.*, 1988) and in contractive capacity of the masticatory muscles (Kiliaridis and Shyu, 1988), it is believed that the underlying mechanism for the alterations in the upper viscerocranium growth pattern is a change in bone remodelling at areas of muscle insertion due to the low forces during muscle contraction.

At the same time, investigations had been directed to the effects of food consistency on the growth of the palatal region of the maxillary complex. Some researchers have reported that growing rats fed a soft diet have a significantly narrower maxillary arch breadth (Watt and Williams, 1951; Moore, 1965; Beecher and Corruccini, 1981), while others have reported little change in the breadth of the maxillary arch (Bouvier and Hylander, 1984; Bouvier and Zimny, 1987); thus, a consensus on the effect of food consistency on the palatal region of the

maxillary complex has not yet been established. Even if changes occur in this region, they are unlikely to be due to a direct effect of muscular contraction on bone growth because this region is not an area of muscle insertion. Since this region is an area which receives occlusal loading, and is situated near the neurocranium, growth and development of this region may be affected by occlusal loading and/or growth of the neurocranium. However, no study has been reported on the effect of food consistency on bone growth in the palatal region of the maxillary complex. In order to analyse the mechanism underlying the changes in craniofacial morphology and growth pattern induced by food consistency, it is essential to investigate the growth in this region in detail.

Bone histomorphometry is a useful technique for assessing the amount of bone apposition (Takahashi, 1983). This method is mainly used in studies of metabolic bone diseases or in the analyses of drug effects on bone, but it is possible to assess the difference in the amount of bone growth quantitatively by modifying the measurement methods. Bone histomorphometry was employed in this study to investigate the effects of food consistency on the bone appositional pattern at the growth site in the palatal region of the maxillary complex in growing rats.

Materials and methods

Sixty male inbred Fischer rats (F344/DuCrj, Charles River Japan, Inc., Yokohama, Japan) were used. Young rats usually begin to wean at around 14 days of age and begin to chew the food provided for the nurse rat in addition to the milk. In the preliminary experiment, it was observed that the upper and lower incisors were already erupted at 12 days of age. The eruption of lower first molars was observed at 16 days of age, upper first and lower second at 17 days of age, and upper second at 18 days of age. It was also observed that rats lick and gnaw solid food at 13 days of age. Consequently, feeding experiments were started at 14 days of age. The animals were randomly divided into two groups, each consisting of 30 young rats and three nurse rats. One group received a conventional pellet diet (LABO MR Stock, Nihon Nosan Kogyo, KK, Yokohama, Japan) with water supplied from a bottle, and was designated the 'control' group (solid diet group). The other group received the same diet ground into powder (20 μm) using a grinder (COSMOMISER-II, Nara Kikai Seisakusho, Inc., Tokyo, Japan) and mixed with four parts (w/w) of water as in previous studies (Kim, 1990; Kuroe, 1991), and was designated the 'test' group (liquid diet group). All animals were fed *ad libitum*, and were weaned at 21 days of age. Vital staining was employed to record bone apposition during the experimental period. It was performed after all teeth occluded completely (40 days of age; Schour and Massler, 1967), and was chosen as the late experimental period to avoid the resorption of the labelled line by the bone drift. Tetracycline hydrochloride (20 mg/kg body wt) and dotite calsein (8 mg/kg body wt; Wako Pure Chemical Industries Ltd, Osaka, Japan) were injected at the age of 49 and 55 days respectively. The rats aged 57 days at the end of the experimental feeding period were killed by i.p. injection of an overdose of phenobarbital sodium (Nembutal® injection 40 mg/kg body wt; Dainipponseiyaku, Inc., Osaka, Japan) and decapitated. After the specimens were fixed in 70 per cent alcohol, dental arch measurements and bone histomorphometry were conducted as described below.

Body weight and body length measurements

To evaluate constitutional growth, animals were weighed and the body length was measured under diethyl ether anaesthesia every 7 days at a fixed time. This inhalation anaesthesia was performed at a low level of anaesthetic depth, and was efficacious for only a few minutes, so that its effect, if any, appeared to be negligible on the animals' general growth. Rats aged 14 days were too young to tolerate the anaesthesia, so were weighed without anaesthesia. Body weight was measured using an electronic balance (CB-600, Shinko Denshi, Inc., Tokyo, Japan) and body length, including the tail, was measured with a metric ruler graduated in millimetres up to 0.5 mm.

Dental arch measurements

Although several researchers have reported the effect of food consistency on the morphology of

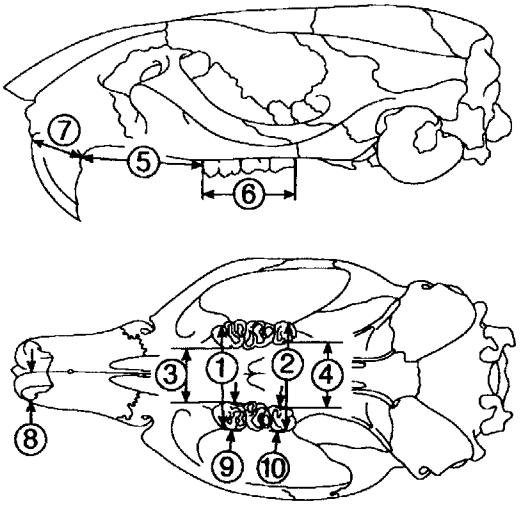

Figure 1 Dental arch measurements. (1) IMWB1: distance between the most external cervical points of the mesiobuccal cusps of the upper first molars. (2) IMWB3: distance between the most external cervical points of the distobuccal cusps of the upper third molars. (3) IMWL1: distance between the most internal cervical points of the mesiolingual cusps of the upper first molars. (4) IMWL3: distance between the most internal cervical points of the distolingual cusps of the upper third molars. (5) DIM1: distance between the most distal cervical point of the upper right incisor and the most mesial cervical point of the upper right first molar. (6) LMD: distance between the most mesial cervical point of the upper right first molar and the most distal cervical point of the upper right third molar. (7) LLI: labiolingual distance of the upper right incisor at cervical points. (8) MDI: mesiodistal distance of the upper right incisor at cervical points. (9) BLM1: buccolingual distance of the upper right first molar at cervical points. (10) BLM3: buccolingual distance of the upper right third molar at cervical points.

the maxillary dental arch, some of their results were contradictory. To double-check this, the linear dimensions of the dentition were measured. In this study, the linear measurements of dentition (Fig. 1) included the distance between the upper first molars (IMWB1, IMWL1), the distance between the upper third molars (IMWB3, IMWL3) and the distance between the upper incisor and the upper first molar (DIM1). As morphological changes in the teeth may affect these measurements, additional measurements were made for confirmation; these included the labiolingual distance of the upper incisor (LLI), the mesiodistal distance of the upper incisor (MDI), the distance between the upper first molar and the upper third molar (LMD), the buccolingual distance of the upper first molar (BLM1), and the buccolingual distance of the upper third molar (BLM3). Specimens fixed in 70 per cent alcohol were used. The measurements were made using electronic digital calipers (MAX-CAL 950–101, Japan Micrometer MFG. Co. Ltd, Hyogo, Japan). Each variable was measured three times and their mean value was recorded. Some of the data were used in bone histomorphometry.

Bone histomorphometry

The quantitative measurement of bone apposition at the growth site in the palatal region of the maxillary complex was performed by modified bone histomorphometry.

Reproducibility of section-cutting is important in histomorphometrical measurements. For that purpose, a newly devised stereotaxic fixation apparatus was used to establish reference planes in specimens fixed in 70 per cent alcohol (Fig. 2). This stereotaxic fixation apparatus was specially designed to enable the insertion of two metal wires in fixed positions on the specimen. The specimen, with wires inserted, was held by a cutting machine vice which had been newly devised for the stereotaxic fixation apparatus. In this way the specimen was always positioned in a fixed orientation, enabling section-cutting at fixed directions. Sections of the first and third molar area were cut in a plane parallel to the plane of the maxillary molar cusps and perpendicular to the sagittal plane. Sections of the premaxillary–maxillary suture were cut in a plane perpendicular to the plane of the maxillary molar cusps and parallel to the sagittal plane.

The specimens were stained with Villanueva bone stain, dehydrated in alcohol and embedded in methyl methacrylate by standard methods (Konno and Takahashi, 1981). They were sectioned by a cutting machine (Cutting Unit, EXAKT Atparatebau, Norderstedt, Germany) at regulated positions (Fig. 3). Sections cut at a thickness of 100–150 μm were planed down to 30 μm using two ground glass plates by a modified method of Honma and Wakamatsu (1983).

These undecalcified sections were photographed under a fluorescent microscope (BHS-RFC, Olympus Optical Co., Tokyo, Japan). An excitation filter (20BP490) and barrier filters

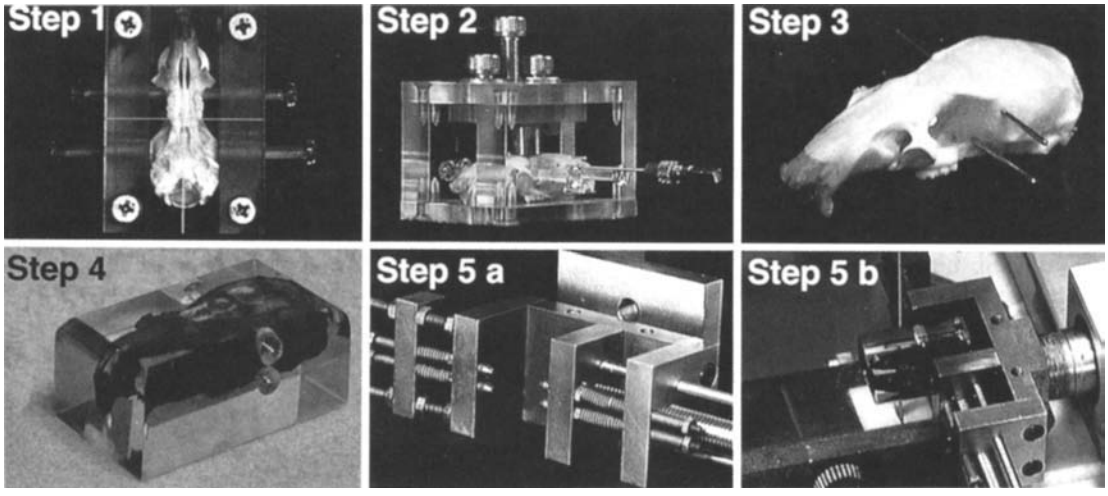

Figure 2 Determination of reference planes by means of a stereotaxic fixation apparatus. (1) The specimen was positioned as follows: (i) the cusps of the maxillary molars were in contact with the internal base of the stereotaxic fixation apparatus; (ii) the midline of the specimen, i.e. the line between the mesial contact point of the upper incisors and the midpoint of the line drawn between the distolingual cusps of the upper third molars, was lined up with the reference midline of the stereotaxic fixation apparatus; (iii) the distal surface of the maxillary third molar was placed on the reference line perpendicular to the reference midline of the stereotaxic fixation apparatus. (2) Two holes were drilled through the specimen, using two pairs of holes on the side face of the stereotaxic fixation apparatus as a guide. (3) The specimen was taken out of the stereotaxic fixation apparatus, and two wires were inserted into the holes of the specimen. The specimen was then dehydrated, infiltrated and embedded in the methyl methacrylate. (4) The methyl methacrylate around the wires was trimmed to expose the ends of the wires. (5) The ends of the wires were held with the specially designed vice (a), and the specimen was sectioned with a cutting machine at regulated positions (b).

(17AFC and 17O515; Olympus) were used to allow the passage of light at wavelengths of 435–490 nm.

Photographs were taken at the following magnifications. (i) The first and third molar area: microscopic magnification ×31.25, photographic magnification ×4.58. (ii) Premaxillary–maxillary suture: microscopic magnification ×31.25, photographic magnification ×3.82.

The images of the photographs were loaded into a computer (Macintosh IIvx, Apple Computer, Inc., Cupertino, CA, USA) by means of a digitizer (SD-421A, Wacom, Kitasaitama, Japan). The variables shown in Table 1 and Figure 4 were computed using an image analysis program (NIH Image 1.44, public domain software). The vector of bone growth was divided into various components and measured. To standardize the measurement area between groups and between specimens, reference lines which determine the measurement area were used (see Fig. 4). Measurement variables were those used in conventional bone histomorphometry. However, in suture measurement, a new variable was established to measure the distance between bones (distance between bones = sutural area/sutural perimeter, see Table 1). These were divided into primary and secondary variables, primary variables being measured directly and secondary variables being calculated from primary variables.

Statistical analysis

Differences in growth and morphological parameters between the control and test groups were evaluated by the unpaired *t*-test using a statistical analysis program (STATISTICA, StatSoft, Inc., Tulsa, OK, USA). For a variable of dental arch or bone histomorphometric measurement showing significant difference between the test and control groups, a ratio of the mean value in the test group to the mean value in the control group was calculated. As this ratio was calculated taking the mean value in the control group as 1, the smaller the ratio below 1, the greater the growth inhibition; and the bigger

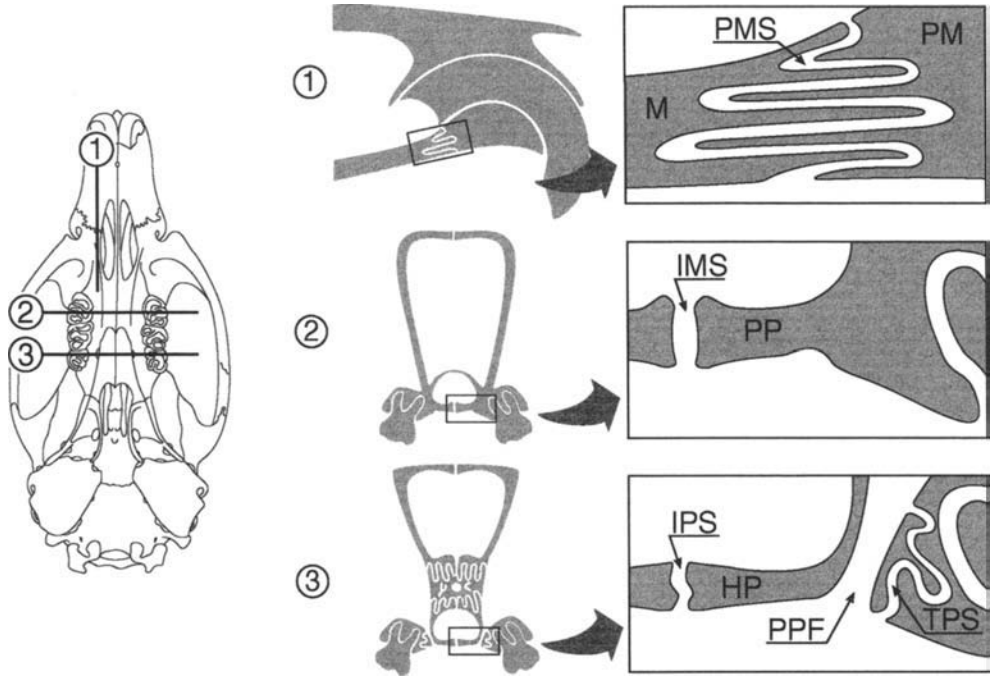

Figure 3 Measurement areas in bone histomorphometry. (1) Premaxillary–maxillary suture (PMS): located lateral to the anterior palatine foramen. M, maxilla; PM, premaxilla. (2) The first molar area: intermaxillary suture (IMS) and the palatal process of maxilla (PP) corresponding to the mesiodistal centre of the upper first molar. (3) The third molar area: interpalatine suture (IPS), horizontal plate of palatine bone (HP) and transverse palatine suture (TPS) at the upper third molar adjacent to the posterior palatine foramen (PPF).

the ratio above 1, the greater the growth promotion in the test group.

Analysis of the difference between the right and left measurements

Some of the dental arch and bone histomorphometrical measurements were made unilaterally. To examine the significance of differences between the right and left measurements, 10 samples were randomly selected and the differences between the right and left sides were compared by a paired *t*-test.

Error of the method

Errors in dental arch measurements were examined by double recording of 10 specimens; errors in bone histomorphometrical measurements were monitored by measuring the photographs from 10 rats. The measurements were analysed using the formula $Se^2 = \Sigma d^2/2n$ where d is the difference between two measurements (Dahlberg, 1948).

Results

Four animals died during the course of the experiment. As a result the number of animals in each group was 28 at the end of the experimental period.

No significant differences were found between the right and left measurements in all the variables of dental arch and histomorphometrical measurements which were measured unilaterally.

Error of the method

The errors in dental arch and bone histomorphometrical measurements are given in Table 2. The average method error was 0.026 mm for the dental arch measurements. Among the histomorphometrical variables, SPpms and SPtps showed larger errors, but as the percentages of the error variance of these variables to the total variance were small (0.03 and 0.07 per cent respectively), the data of these

Table 1 The variables used for the quantitative analysis of the amount of growth at the growth site in the palatal region of the maxillary complex

Variable		Description (unit)
CORTICAL BONE		
Palatal process of maxilla	Horizontal plate of palatine bone	
*Primary variables**		
OSApp	OSAhp	osteoid seam area (μm^2)
CBApp	CBAhp	calcified bone area (μm^2)
ILApp	ILAhp	interlabelling area (μm^2)
DRLpp	DRLhp	distance between reference lines (μm)
Secondary variables		
OSWpp	OSWhp	osteiod seam width; OSW=OSA/DRL (μm)
CBWpp	CBWhp	calcified bone width; CBW=CBA/DRL (μm)
ILDpp	ILDhp	interlabelling distance; ILD=ILA/DRL (μm)
BARpp	BARhp	bone appositional rate; BAR=ILD/labelling time*** (μm/day)
BALpp	BALhp	bone appositional lag time; BAL=OSW/BAR (day)
SYNCHONDROSIS		
Intermaxillary suture	Interpalatine suture	
*Primary variables**		
ILDims	ILDips	interlabelling distance; average of the measurement points ($W_1,...,W_n$), ILD = $\Sigma W_n/N$*** (μm)
SAims	SAips	synchondrosis area; area of the synchondrosis enclosed with the two reference lines which establish the synchondrosis area (μm^2)
SLims	SLips	synchondrosis length; distance between the two reference lines which establish the synchondrosis area (μm)
Secondary variables		
ORSims	ORSips	ossification rate of synchondrosis; ORS = ILD/labelling time** (μm/day)
DBims	DBips	distance between bones; DB = SA/SL (μm)
SUTURE		
Premaxillary–maxillary suture	Transverse palatine suture	
*Primary variables**		
FLApms	FLAtps	first labelled area; area enclosed with the first labelled lines (tetracycline hydrochloride) on each bone of the suture and two reference lines which were tangential to the upper and lower border of the labelling lines (μm^2)
SLApms	SLAtps	second labelled area; area enclosed with the second labelled lines (dotite calsein) on each bone of the suture and two reference lines which were tangential to the upper and lower border of the labelling lines (μm^2)
SPpms	SPtps	sutural perimeter; mean length of the sutural surface of each bone of the suture within the reference lines which establish the sutural area (μm)
SApms	SAtps	sutural area; area of the suture enclosed with the two reference lines which establish the sutural area (μm^2)
DRLpms	DRLtps	distance between reference lines; distance between two reference lines which were tangential to the upper and lower border of the labelling lines (μm)

Table 1 *Continued*

Secondary variables		
ILApms	ILAtps	interlabelling area; ILA = FLA – SLA (μm^2)
ILDpms	ILDtps	interlabelling distance; ILD = ILA/DRL (μm)
SGRpms	SGRtps	sutural growth rate; GRS = ILD/labelling time** (μm/day)
DBpms	DBtps	distance between bones; DB = SA/SP (μm)

*Primary variables were measured within the measurement areas defined by the reference lines.
**Labelling time = (1/2) number of labelling days + number of interval days.
***The labelled lines of cartilaginous ossification were discontinuous. Hypothetical lines were drawn in the gaps of the discontinuous lines. Interlabelling distances were measured on horizontal lines drawn at fixed distances apart. Measurements were made on the real labelling lines as far as possible.

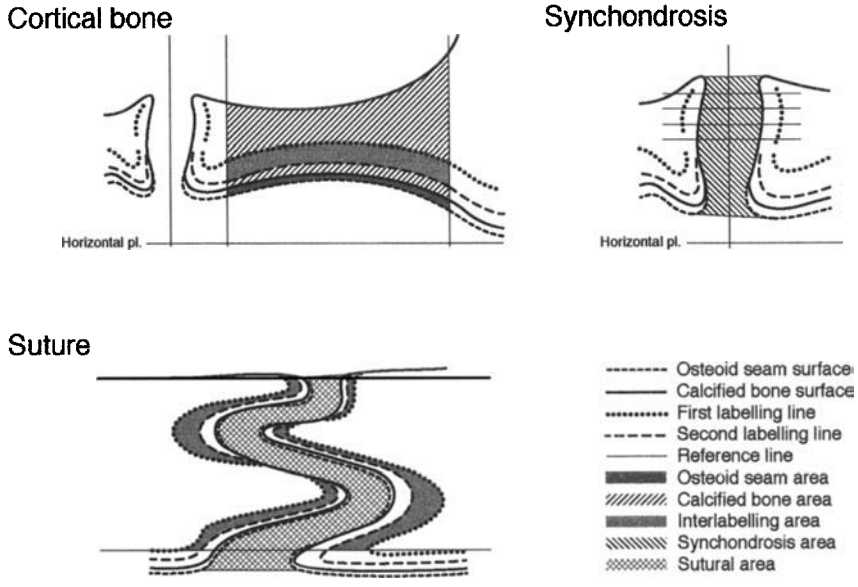

Figure 4 Reference lines and sites of histomorphological measurement in the palatal region of the maxillary complex. Cortical bone: the growth direction was taken as the direction perpendicular to the horizontal plane. Reference lines parallel to the growth direction were used. In order to standardize the measurement in both groups, the measurement areas were set as follows. The first molar area: (1) the mean value of the lingual width of maxillary molar dentition (IMWL1) for each group was halved; (2) on the horizontal plane, at the positions corresponding to distances of 15 and 75 per cent of the calculated value from the centre of the synchondrosis, two reference lines were drawn parallel to the growth direction. The third molar area: (1) the mean value of the lingual width of maxillary molar dentition (IMWL3) for each group was halved; (2) on the horizontal plane, at the positions corresponding to distances of 5 and 30 per cent of the calculated value from the centre of the synchondrosis, two reference lines were drawn parallel to the growth direction. Variables were measured and calculated within the areas defined by the above-mentioned lines. Synchondrosis: the growth direction was taken as a direction parallel to the horizontal plane. To establish the synchondrosis area, two reference lines were employed connecting two edges of each bone at upper and lower side. To measure the synchondrosis length, a reference line was drawn perpendicular to the growth direction at the centre of the synchondrosis. Interlabelling distance was measured on the horizontal line which was drawn at an interval of 50 μm from the intermaxillary suture (the first molar area), and on the horizontal line drawn at an interval of 25 μm from the interpalatine suture (the third molar area). Suture: the growth direction was taken as the long axis of the bones of the suture. Two reference lines which were tangential to the upper and lower border of the labelling lines and also parallel to the growth direction were used. To establish the sutural area, two more reference lines that joined the upper and lower edge of each bone of the suture were used.

Table 2 Errors of the method

Variable	*n*	Se^2	*N*	SD_{tot}^2	Se^2 in % of SD_{tot}^2	Se	Unit
DENTAL ARCH MEASUREMENTS							
IMWB1	10	1.03E–03	56	1.89E–02	5.45	0.032	mm
IMWB3	10	1.35E–03	56	3.83E–02	3.51	0.037	mm
IMWL1	10	1.07E–03	56	1.66E–02	6.45	0.033	mm
IMWL3	10	1.20E–03	56	3.47E–02	3.44	0.035	mm
DIM1	10	1.01E–03	56	2.89E–02	3.47	0.032	mm
LMD	10	1.00E–03	56	1.37E–02	7.28	0.032	mm
LLI	10	2.40E–04	56	3.63E–03	6.60	0.015	mm
MDI	10	4.50E–05	56	8.77E–04	5.13	0.007	mm
BLM1	10	1.75E–04	56	7.12E–04	24.57	0.013	mm
BLM3	10	5.30E–04	56	1.35E–03	39.39	0.023	mm
BONE HISTOMORPHOMETRY							
OSWpp	10	7.44E–01	56	1.68E+01	4.44	0.863	μm
CBWpp	10	2.78E+00	56	1.75E+04	0.02	1.666	μm
BARpp	10	6.44E–02	56	4.14E+00	1.56	0.254	μm/day
BALpp	10	1.63E–02	56	4.39E+00	0.37	0.128	day
OSWhp	10	4.71E–01	56	2.19E+00	21.50	0.686	μm
CBWhp	10	8.38E–01	56	2.74E+03	0.03	0.916	μm
BARhp	10	4.26E–02	55	2.00E+00	2.13	0.206	μm/day
BALhp	10	9.13E–03	55	7.41E–02	12.32	0.096	day
SLims	10	4.90E+00	45	3.20E+04	0.02	2.214	μm
ORSims	10	1.46E–01	56	4.11E+00	3.55	0.382	μm/day
DBims	10	8.56E–01	45	1.21E+03	0.07	0.925	μm
SLips	10	7.62E+00	45	5.30E+03	0.14	2.761	μm
ORSips	10	2.78E–02	56	3.55E+00	0.78	0.167	μm/day
DBips	10	4.28E+00	45	1.32E+03	0.32	2.069	μm
SPpms	10	2.77E+03	31	7.97E+06	0.03	52.599	μm
SGRpms	10	6.51E+00	31	1.11E+02	5.85	2.552	μm/day
DBpms	10	4.43E–01	31	3.54E+01	1.25	0.666	μm
SPtps	10	2.91E+02	56	3.99E+05	0.07	17.069	μm
DBtps	10	5.17E–01	56	3.83E+01	1.35	0.719	μm

n, number of double determinations; Se^2, variance of the error of method for the measurements on two different occasions, $Se^2 = \Sigma d^2/2n$ (Dahlberg, 1948); Se, error of the method; *d*, difference between two measurements; *N*, number of samples in the total material; SD_{tot}^2, variance in the total material.

variables were considered to present no problems in analysis. For the variables BLM1 and BLM3 in dental arch measurements and OSWhp and BALhp in histomorphometrical measurements, the percentages of error variance to total variance were large; however, as the measurement errors were all small, the large percentages of error variance to total variance were considered to be due to small total variances.

Body weight and body length measurements

Results of the body weight and body length measurements for the control and test groups are given in Figure 5. Although body weight gain in the test group was higher than that in the control group in the middle of the experimental period ($P < 0.001$–0.05), no significant differences were found between these two groups at the end of the experimental period. No significant differences in body length were found between the two groups throughout the experimental period.

Dental arch measurements

Results of the dental arch measurements are shown in Table 3. The maxillary widths between the first molars (IMWB1, IMWL1) and between the third molars (IMWB3, IMWL3) were smaller in the test group than in the control

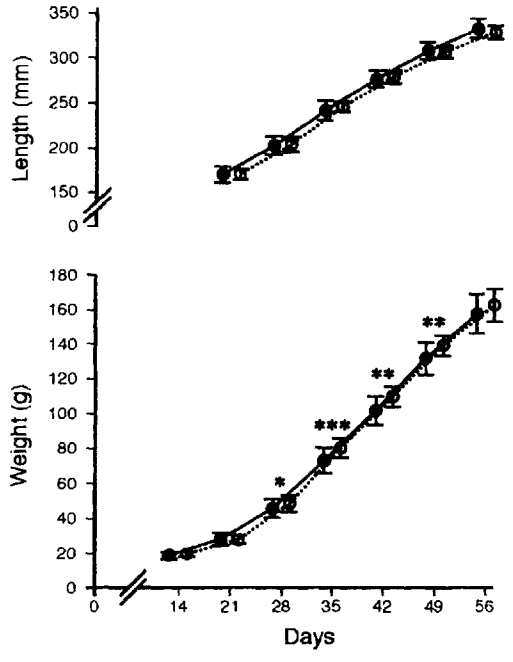

Figure 5 Age-related changes in body weight and body length (mean value ± 1SD) in the control group (●—●) and the test group (○·····○). Significance of mean differences: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

group. Since no significant differences were found in the buccolingual distance of the molars (BLM1, BLM3), the sizes of the molars were not responsible for these differences in arch width. To confirm the morphological change in the molar dentition, the ratio of the maxillary width between the third molars (IMWB3) to the maxillary width between the first molars (IMWB1) was calculated. In addition, the differences between the groups were tested for significance with the non-parametric Mann–Whitney *U*-test. This analysis demonstrated a significantly smaller value in the test group (1.076 ± 0.013) than in the control group (1.094 ± 0.012) ($P < 0.001$), indicating that the molar dentition in the test group had a more parallel form than that in the control group, and the inhibition of lateral growth of the maxilla at the third molar area was more marked than that in the first molar area in the test group.

The distance between the incisor and first molar (DIM1) in the test group was significantly larger than that in the control group ($P < 0.01$). However, the width of the incisor (LLI, MDI) in the test group was smaller than that in the control group ($P < 0.001$). To exclude the effect of the morphological difference of the incisor on the incisor to first molar distance, the distance between the first molar and the labiolingual centre of the incisor was calculated from the formula: distance between first molar and labiolingual centre of the incisor = distance between incisor and first molar (DIM1) – 1/2 labiolingual distance of incisor (LLI), and the significance of difference was again analysed by the unpaired *t*-test. This analysis also demonstrated a significantly larger value in the test group (9.892 ± 0.121) than in the control group (9.702 ± 0.177) ($P < 0.001$), confirming a more protrusive growth of the lower part of the maxilla and premaxilla in the test group even after the morphological difference of the incisors was corrected.

Bone histomorphometry

The results of the bone histomorphometrical measurements are given in Table 4. Some sections that could not be measured accurately due to poor preparations were excluded.

The bone appositional rate (BAR), which indicates the velocity of drift of the palate, in the first molar area (area of the palatal process of the maxilla) was significantly lower in the test group than in the control group ($P < 0.001$). On the contrary, the BAR in the third molar area (area of the horizontal plate of the palatine bone) was significantly higher in the test group than in the control group ($P < 0.05$). Although the bone appositional lag time (BAL) at the occlusal surface of the palate in the first molar area was not different between the test and control groups, a significantly shorter BAL was found in the third molar area in the test group compared with the control group ($P < 0.001$). However, calcified bone widths in both the palatal process of the maxilla (CBWpp) and the horizontal plate of palatine bone (CBWhp) in the test group were significantly smaller than in the control group ($P < 0.001$). These results suggested that, at the third molar area, bone resorption of the nasal surface of the palate was accelerated in spite of an accelerated bone

Table 3 Mean values and standard deviation (SD) for the dental arch measurements ($n = 28$)

Variables (mm)	Control group		Test group		Significance of difference	Ratio (test/control)
	Mean	SD	Mean	SD		
IMWB1	7.144	0.091	6.949	0.102	***	0.973
IMWB3	7.816	0.108	7.476	0.080	***	0.956
IMWL1	3.794	0.083	3.593	0.076	***	0.947
IMWL3	5.375	0.093	5.051	0.089	***	0.940
DIM1	10.790	0.182	10.931	0.124	**	1.013
LMD	6.568	0.143	6.525	0.081	NS	
LLI	2.175	0.038	2.078	0.032	***	0.955
MDI	1.125	0.026	1.089	0.020	***	0.968
BLM1	1.963	0.025	1.976	0.027	NS	
BLM3	1.469	0.040	1.481	0.032	NS	

Values in mm as they were measured with slide calipers. Differences between the groups were tested for significance with the unpaired t-test. Ratios of the mean values in the test group to the mean values in the control group were calculated.
$^{**}P < 0.01$; $^{***}P < 0.001$; NS, not significant.

Table 4 Mean values and standard deviation (SD) for bone histomorphometry

Variable	Control group			Test group			Significance of difference	Ratio (test/control)
	n	Mean	SD	n	Mean	SD		
CORTICAL BONE								
OSWpp (μm)	28	12.9	3.3	28	7.9	3.2	***	0.616
CBWpp (μm)	28	586.8	56.0	28	339.7	31.0	***	0.579
BARpp (μm/day)	28	9.9	2.1	28	8.0	1.5	***	0.816
BALpp (day)	28	1.8	2.9	28	1.0	0.3	NS	
OSWhp (μm)	28	5.2	1.5	28	4.3	1.3	*	0.824
CBWhp (μm)	28	184.3	35.9	28	101.3	26.9	***	0.550
BARhp (μm/day)	28	6.7	1.6	27	7.5	1.1	*	1.116
BALhp (day)	28	0.8	0.3	27	0.6	0.2	**	0.728
SYNCHONDROSIS								
SLims (μm)	28	832.0	62.2	17	482.4	26.1	***	0.580
ORSims (μm/day)	28	7.7	1.5	28	4.8	1.2	***	0.618
DBims (μm)	28	171.3	20.2	17	112.3	18.3	***	0.655
SLips (μm)	28	332.6	43.3	17	216.7	50.2	***	0.651
ORSips (μm/day)	28	4.6	1.0	28	1.3	0.8	***	0.290
DBips (μm)	28	113.9	27.9	17	60.8	21.5	***	0.534
SUTURE								
SPpms (μm)	16	7936.6	2959.3	15	5480.9	2089.4	*	0.691
SGRpms (μm/day)	16	58.7	9.9	15	54.3	11.1	NS	
DBpms (μm)	16	49.6	5.7	15	45.5	5.6	*	0.916
SPtps (μm)	28	2370.4	613.4	28	1726.1	469.6	***	0.728
DBtps (μm)	28	47.1	4.6	28	38.2	4.1	***	0.813

Differences between the groups have been tested for significance with the unpaired t-test. Ratios of the mean values in the test group to the mean values in the control group were calculated.
$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; NS, not significant; n, sample size.

apposition on the oral surface of the palate in the test group. Furthermore, the velocity of drift of the palate was slower in the first molar area, and faster in the third molar area in the test group than in the control group, suggesting an anterior rotational growth of the palate in the test group.

The distance between bones (DBims, DBips) and ossification rate in the synchondrosis (ORSims, ORSips) of the midpalatal suture was significantly smaller in the test group than in the control group in both the first and third molar areas ($P < 0.001$). To compare the ossification rate in the synchondrosis of the midpalatal suture between the first and third molar area, the ratio of the ossification rate in the synchondrosis of the interpalatine suture (ORSips) to the ossification rate in the synchondrosis of the intermaxillary suture (ORSims) was calculated. In addition, the differences between the groups were tested for significance with the non-parametric Mann-Whitney *U*-test. This analysis demonstrated a significantly smaller value in the test group (0.289 ± 0.204) than in the control group (0.617 ± 0.174) ($P < 0.001$), indicating greater inhibition of lateral growth of the maxilla in the distal area. This agreed with the results of dental arch measurements. Histological examination of the midpalatal suture revealed a cartilaginous joint in the first molar area and a transition of a cartilaginous joint, to joint by collagen fibre bundles in the third molar area. The test group had a smaller sutural perimeter in the transverse palatine suture (SPtps) ($P < 0.001$) and also a shorter distance between bones in the transverse palatine suture (DBtps) ($P < 0.001$) than the control group. Although bone apposition was observed in the transverse palatine sutures in both groups, the sutural growth rate (SGRtps) was not measured in this area because of a highly complicated interdigitation.

Compared with the control group, the sutural perimeter in the premaxillary–maxillary suture (SPpms) in the test group was significantly shorter ($P < 0.05$), and the distance between bones in the premaxillary–maxillary suture (DBpms) was also shorter ($P < 0.05$), but no significant differences in sutural growth rate in the premaxillary–maxillary suture (SGRpms) were found.

Discussion

Food consistency and jaw movement

Previous studies which investigated the effect of food consistency on the growth of the craniofacial complex employed a kneaded diet (Beecher and Corruccini, 1981; Bouvier and Zimny, 1987), powder diet (Sukekawa and Ito, 1990) and liquid diet (Hinton, 1988; Kuroe, 1991) as the soft diet model, and solid pellet diet as the hard diet model. Although not mentioned explicitly, these studies assumed that the mode of masticatory movement, e.g. the jaw-closing muscle activity and the pattern of movement, changed linearly with the increase in food consistency. In fact, trigeminal motor output during mastication is known to be modified by periodontal sensory afferents, and thus by the food consistency (Inoue *et al.*, 1989).

To examine the effect of occlusal loading on the growth of the jaw bone, however, it is more logical to compare the two cases of food consistency, with and without the presence of occlusal loading. Among aforementioned soft diet models, it is reported that liquid diet yields 'lapping' and leads to preservation of the free-way space and interference of the molar contact (Hiiemäe and Ardran, 1968). Thus it is assumed that occlusal loading does not occur during the intake of a liquid food. Thexton *et al.* (1980) confirmed that 'lapping' is controlled by a neural mechanism completely different from that which controls the 'chewing' movement that takes place during solid food intake. As the activation of masticatory movement is primarily dependent on the presence of occlusal loading in the mouth, the food consistency itself exerts only a secondary effect (Morimoto *et al.*, 1991). This means that the comparison between the effect of solid food intake and that of liquid food intake may be expressed as the comparison between the effect of 'mastication' and that of 'lapping'. The aim of this study was to investigate possible effects of occlusal loading on the growth of the maxillary complex. Accordingly a liquid diet, which involves no molar contact and where zero occlusal loading to the maxillary complex is assumed, and a solid diet were used as the test foods.

The effect of food consistency on constitutional growth

The two groups showed a difference in body weight gain during the middle of the experimental period. The test group was significantly heavier than the control group, agreeing with the results of Kim (1990) and Kuroe (1991), but contrary to those of Kiliaridis *et al.* (1985) and Katsaros *et al.* (1994), who found that rats fed a hard diet were heavier than rats fed a soft diet, and also to the results of Watt and Williams (1951), Moore (1965), Beecher and Corruccini (1981) and Kiliaridis (1986), who did not find any difference in body weight of animals fed hard and soft diets. These differences among various studies may be due to the physical consistency of the soft diet model, the time of changing from a hard diet to a soft diet, and adaptation of eating practice to the altered food consistency. In this study, the rats in the test group were provided a liquid diet before weaning, so that they were considered well adapted to taking the liquid diet. Additional factors that may account for the difference in body weight between the groups found in this study include a 4-fold greater water intake in the test group than in the control group and the easily digestible nature of the ground food (Kim, 1990; Kuroe, 1991). However, the two groups showed no differences in body length during the experimental period, in accordance with the results of previous studies (Kim, 1990; Kuroe, 1991), indicating that morphological difference of the viscerocranium might be independent of the difference in constitutional growth.

Food consistency and bone appositional pattern on the palatal region of the maxillary complex

The most interesting finding in the present study is the difference in vertical growth of the palate between the groups and a more anteriorly directed growth rotation of the palate in the test group. This difference in growth pattern agreed with that described by Kiliaridis *et al.* (1985) who reported that the craniofacial morphology and the growth pattern of rats fed a soft diet was changed to a more orthocranial direction than rats fed a hard diet. Studies which examined the mechanism underlying this change have reported a marked decrease in bone apposition of local bone in areas of muscle insertion in the anterior part of the upper viscerocranium (Engström *et al.*, 1986), and changes in fibre size and composition (Kiliaridis *et al.*, 1988) and in contractive capacity of the masticatory muscles (Kiliaridis and Shyu, 1988). Moreover, a recent study has reported decreases in sutural width and length in the areas of muscle insertion in the anterior part of the upper viscerocranium (Katsaros *et al.*, 1994). However, the region examined in the present study is not an area of muscle insertion, and therefore the difference in growth pattern of the palate demonstrated in this study cannot be caused directly by effects such as muscle contraction.

The differences in calcified bone width in the palatal process of the maxilla and in the horizontal plate of the palatine bone between the two groups confirmed that occlusal loading was transmitted not only to the teeth and alveolar bones but also to the basal bone of the maxilla and the palate, and affected bone apposition in the basal bone. In the test group, a decrease in bone appositional rate at the occlusal surface of the palate (BARpp) correlated with a decrease in calcified bone width (CBWpp), but in the third molar area the bone appositional rate in the occlusal surface of the palate (BARhp) was higher than in the control group, even though the calcified bone width (CBWhp) was smaller than in the control group. This apparently contradictory finding may indicate an increase in bone resorption on the nasal surface of the palate. This might have occurred in a condition of zero occlusal loading, under which the palate was under a relatively strong influence from growth of elements other than the craniofacial structure, such as the neurocranium and rhinopharynx. The posterior part of the palate, due to its location, may be affected by growth of the neurocranium more than the anterior part of the palate. A correlation between growth of the neurocranium and that of the viscerocranium was also reported in a study that examined cranial suture closure (Babler, 1988); it was found that growth of the upper viscerocranium was affected by positional changes in the cranial base due to brain growth. The difference in growth pattern of the palate demonstrated in this study may be caused by the effect of growth of

structures such as the neurocranium on the growth of the palate under different occlusal loading conditions. The different growth pattern of the upper viscerocranium induced by different food consistency may be caused not only by a difference in mechanical force generated by the masticatory muscles acting on the bones in the muscle insertion area, but also to a different degree by the effect of growth of structures such as the neurocranium on growth of the upper viscerocranium under different occlusal loading conditions.

The lateral growth of the maxilla in the test group was inhibited compared with that of the control group, in accordance with the results of Watt and Williams (1951), Moore (1965) and Beecher and Corruccini (1981). In the present study, the results of the synchondrosis reaction in the midpalatal suture agreed with those of Hinton (1988). Together with the finding that the mechanical force generated during occlusion or mastication acts to separate the intermaxillary suture (Ishida, 1972), it is possible that occlusal loading during mastication has a direct effect on the lateral growth of the maxillary molar region.

Moreover, the extent of the inhibition of lateral growth in the liquid diet group was different in the first and third molar areas, and the lateral growth of the maxilla of the liquid diet group was inhibited more markedly in the distal area. Histological examination of the midpalatal suture revealed a cartilaginous joint in the first molar area, and a transition of the cartilaginous joint to a fibrous joint in the third molar area. This difference in histological structure of the midpalatal suture had also been confirmed in mice, and was considered to be an adaptation to mastication in the molar area, and to the function of the muscles running from the posterior part of palatine bone to the soft palate and pharynx in the more distal part of the molars (Sukekawa *et al.*, 1988). The difference in the amount of lateral growth in the first and third molar areas may be due to a sutural response between the synchondrosis and the fibrous suture of different degrees of functional stimulation. The suture in the first molar area, which is a synchondrosis, may have an autonomous growth ability besides the growth activation due to functional stimulation, and the suture in the distal part of the third molar area, which is a fibrous suture, may have no autonomous growth ability.

The tongue movement in liquid diet ingestion differs from that in solid diet ingestion (Thexton and McGarrick, 1988, 1989, 1994). If there is a difference in the tongue pressure to the palate, the contact pattern of the tongue to the palate, and the muscle movement and activity of the soft palate between liquid diet ingestion and solid diet ingestion, the difference in the bone growth of the palate observed in this study between the two groups may be attributable to the tongue and muscle of the soft palate.

An unexpected finding of a difference in the anteroposterior distance of the maxilla and premaxilla in the liquid and solid diet groups, contradictory to previous studies, was obtained. No significant difference in bone apposition in the premaxillary–maxillary suture was found between the groups, suggesting that difference in food consistency may not affect the forward growth of the maxilla in rats. However, the perimeter and the distance between bones in the premaxillary–maxillary suture were significantly shorter in the test group than in the control group. Similar results were reported in a study which observed the morphology of the premaxillary–maxillary suture while the incisors of rats were shortened periodically so as to effect no incisor contact, suggesting that the premaxillary–maxillary suture is the place where occlusal loading of gnawing is relieved (Sukekawa and Ito, 1990). In the present study, feeding with a liquid diet reduced the occlusal loading on the incisor to zero, or at least to a level lower than in the solid diet group. Under this condition, incisor eruption may be different to a normal feeding condition. In fact, a previous study (Kiliaridis, 1986) confirmed that the amount of incisor eruption in rats fed a soft diet was smaller than that in rats fed a hard diet. There is a possibility that the morphology of the incisors, such as the curvature of the incisor root, may also be different, and the discrepancy in growth pattern observed in this region may be induced by the continuously erupting incisors during the lifetime of the rat.

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