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Dento-skeletal adaptation after bite-raising in growing rats with different masticatory muscle capacities

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SUMMARY The aim of this study was to analyse the effects of normal and hypofunctional masticatory muscles on dento-skeletal adaptation to posterior bite blocks in growing rats. Fifty-two young male rats were divided into two groups, fed a hard and soft diet, respectively, to develop different functional capacities in the masticatory muscles. Bone markers were inserted in the mandible on day 0. After two weeks, an appliance that raised the bite by 2 mm was inserted in half of each group. Lateral radiographs were taken on day 0, 14, 28, and 42 of the experiment. Images of the mandible were superimposed on the bone markers. Differences in cephalometric measurements were analysed by two-way ANOVA.

The reduced muscle capacity resulted in an upward growth of the snout and a shorter mandibular ramus with less bone apposition on its lower border. Bite blocks induced a more upward growth of the snout and a shorter mandibular ramus, and inhibited the eruption of the upper molars and intruded the lower molars. The rats with weaker masticatory muscles had less inhibitory effect of the posterior bite blocks on upper molar eruption and showed different bone apposition in the ramus, especially during the first two weeks. In conclusion, masticatory muscle capacity seems to influence the effect of the posterior bite blocks on both tooth eruption and skeletal adaptation. The results suggest that the characteristics of the masticatory muscles should be taken into account when predicting the efficiency of a functional appliance.

Introduction

Functional appliances are used for treatment of sagittal and vertical malocclusions in growing individuals. These appliances displace the mandible downwards (e.g. posterior bite blocks) and forwards (e.g. the Andresen activator and Herbst appliance), and cause stretching of the orofacial soft tissues and muscles. Treatment results with functional appliances are generally good, but show a large variation and may be unpredictable (Carels and van der Linden, 1987; Bishara and Ziaja, 1989; Hansen and Pancherz, 1992). This could be due to individual differences in the musculo-skeletal characteristics in the orofacial region. Some clinical studies have focused on the role of facial skeletal characteristics in the outcome of treatment with functional appliances (Pancherz, 1979; Malmgren and Ömblus, 1985; Ruf and Pancherz, 1997), but no study, to our knowledge, has analysed the influence of the characteristics of the masticatory muscles on the treatment effects of functional appliances. The dento-skeletal effects of various functional appliances have been analysed both clinically and in animal experimental studies (McNamara, 1977; Altuna and Woodside, 1985; Woodside *et al.*, 1987; Rowe and Carlson, 1990; Ferrari and Herring, 1995; Sugiyama *et al.*, 1999), but the characteristics of the masticatory muscles in the laboratory animals were quite homogeneous. It is therefore unclear whether the characteristics of the masticatory muscles would influence the results of treatment with functional appliances.

It is possible to induce experimentally reproducible changes in the functional characteristics of masticatory muscles by altering the consistency of the diet. This experimental model has been applied more commonly in the rat (Kiliaridis and Shyu, 1988; Kiliaridis *et al.*, 1988; Liu *et al.*, 1998). Based on such an experimental model, it was intended to test the hypothesis that differences in masticatory muscle capacity will lead to differences in treatment effects of functional appliances.

The aim of this study was to analyse the effects of normal and hypofunctional masticatory muscles on dento-skeletal adaptation to a bite block appliance (posterior bite blocks) in growing rats.

Material and methods

Fifty-two young male Sprague–Dawley rats, about three weeks old and weighing approximately 60 g, were obtained from Charles River (Uppsala, Sweden). Before the experiment, the animals were kept in quarantine for one week in accordance with the rules of the Laboratory Animal Department. The experimental design was approved by the Ethics Committee for Animal Research in Göteborg, Sweden.

Experimental design

The animals were divided into two groups of 26 rats each, fed either a hard or a soft diet to induce different functional masticatory muscles status. The hard diet consisted of food pellets (R34 Lactamin, Stockholm, Sweden). The soft diet was a mixture of food flour (R34 flour, Lactamin) and water at a ratio of 1:1, to obtain a porridge-like consistency. Fresh soft food was prepared daily and was delivered in ceramic bowls. Both hard and soft foods were delivered without limitation. The body weight gain was recorded weekly to monitor the growth and health of the animals.

Two weeks after the beginning of the experiment, the animals in each diet group were randomly assigned to two subgroups of 13 animals each: one subgroup received posterior bite blocks and the other served as controls. The groups were designated: hard diet bite block group, hard diet control group, soft diet bite block group, and soft diet control group (Figure 1). The experiment lasted 42 days.

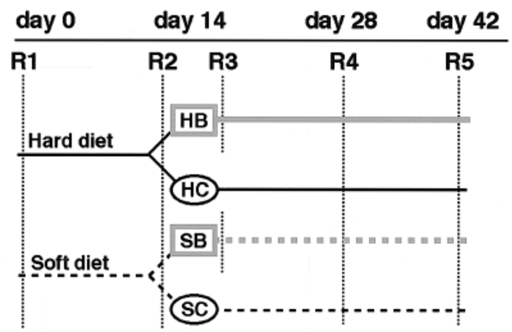

Figure 1 Experimental design. Full line, rats on a hard diet. Dotted line, rats on a soft diet. Grey, bite block groups. Black, control groups. HC, hard diet control group; SC, soft diet control group; HB, hard diet bite blocks group; SB, soft diet bite blocks group. Posterior bite blocks were inserted on day 14 (groups HB and SB). R1, R2, R3, R4, R5: radiographic recordings. R3: radiographic recording carried out immediately after the insertion of the bite blocks.

Bone markers were inserted at the beginning of the experiment to allow superimposition of the radiographic images. Soft tissue markers were inserted at day 14 of the experiment to monitor migration of the periosteum muscle insertion in the ramus region. Radiographs were taken at the beginning and on days 14, 28, and 42 of the experiment. Fibre striation and the length of the muscle belly and of the aponeurosis of the anterior deep masseter muscle were recorded. Details of muscle recordings have been published previously (Bresin *et al.*, 2000). Insertion of markers and of posterior bite blocks and all recordings were performed under anaesthesia, consisting of a mixture of ketamine 10 mg/kg b.w. (Ketalar® 50 mg/ml; Parke-Davis, Ann Arbor, MI, USA) and xylazine 5 mg/kg b.w. (Rompun vet® 20 mg/ml; Bayer, Leverkusen, Germany).

Posterior bite blocks

The posterior bite blocks were made of light-curing composite resin (Charisma®; Heraeus Kulzer, Hanau, Germany), after pressing it into a custom-made mould. The size of the appliance was 7 × 3 mm, and it was approximately 2 mm thick on the first molar and 1 mm thick on the third molar. The appliance was cemented on the two upper molar rows with Vitrebond™

cement (3M Dental Products Division; St Paul, MN, USA). The incisors could not come into contact after insertion of the appliance, thus possibly impairing biting ability and feeding. To avoid group differences because of this factor, a similar 'handicap' was created in the control animals by cutting the lower incisors once with a diamond disc (under anaesthesia) by approximately 2 mm. Because of the continuous eruption of the incisors, the incisal contact was re-established in all groups within one week.

Markers

At the beginning of the experiment two amalgam bone markers were placed in each right mandibular half. After dissecting the soft tissues, dental amalgam was plugged into holes drilled with a 0.6 mm diameter burr under cooling with saline solution. One marker was positioned just below the masseteric ridge by the first molar, and the other in the deepest point of the antegonial notch. The amalgam markers represented stable reference points, their location being unaffected by bone apposition, and thus allowed superimposition of the series of four radiographs for each rat. This permitted measurement of mandibular shape changes.

A soft tissue marker was inserted in the angular region of the right mandibular half on day 14 in all the animals. After perforating the mandible with a needle, a marker made of tantalum powder (Goodfellow Ltd, Cambridge, UK) and gelatine was inserted across the bone plate of the ramus through the needle. The marker was intended to allow measurement of movements of the periosteum and muscular insertion of the masseter during the rest of the experiment. The results will be reported in a separate study.

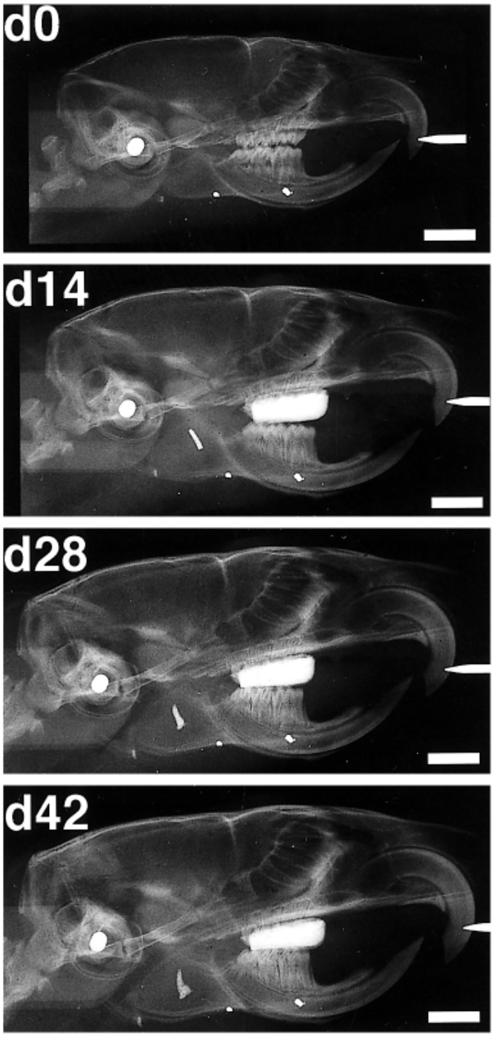

Figure 2 Lateral radiographs of a rat in the HB group, taken on day 0, 14, 28, and 42 of the experiment. The posterior bite blocks are positioned on the upper molars. Two amalgam markers are visible in the lower part of the mandible. A soft tissue marker was placed in the posterior part of the mandible on day 14. The round shade of the ear rod and the incisor pin of the cephalostat are visible on the left and right sides of the image, respectively. Reference line, 5 mm.

Radiographic recordings and cephalometric measurements

Radiographs of the skulls were taken in lateral projections on day 0, day 14 before and after insertion of the appliance, and days 28 and 42 of the experiment (Figure 2). The radiographic images were taken in a standardized way with a custom-made cephalostat on occlusal films, 57 × 76 mm (Ultraspeed DF50; Kodak, Rochester, NY, USA). The distance between the radiographic focus (D9; Ritter, Karlsruhe, Germany) and the film was 50 cm, and between the midsagittal plane and the film 2.5 cm, producing a magnification of 5.4 per cent. The exposure parameters were

50 kV, 10 mA, and 0.5 seconds. The radiographic films were processed in an X-ray film developing machine (Dürr XR24; Dürr Dental, Bietigheim-Bissingen, Germany).

The radiographic images were digitized with a flatbed scanner (Agfa Arcus II; Agfa-Gevaert, Leverkusen, Germany) and stored as 8-bit grey scale image files (PICT format). Identification of landmarks (Figure 3), registration of their co-ordinates, and superimposition of radiographs were performed with the image analysis program NIH Image version 1.61 PPC (available at http://rsb.info.nih.gov/nih-image; US National Institutes of Health, Bethesda, MD, USA) on an Apple computer.

Cephalometric measurements

In order to perform 'blind' measurements, the images were coded. The measurements were not adjusted for the geometric magnification produced by the radiographic projection.

Distances between selected landmarks, angles between reference lines (as listed in Table 1), plotting of group mean diagrams, and mandibular shape changes were calculated with a 'macro' protocol written in Microsoft Excel 5.0. Mandibular shape changes were described by the changes in position of selected mandibular landmarks after superimposing the series of radiographic images of each single mandible on the contour of the amalgam markers. The stability of the amalgam markers was checked by measuring the distance between the markers for each mandible on all four occasions.

Superimposition of radiographic images

Individual mandibular superimposition. The series of lateral radiographs of each rat was superimposed on the contours of the amalgam markers. This allowed measurement of bone apposition and tooth eruption in the mandible of each rat.

Average plot superimposition. Common 'fiducial points' for all mandibles were constructed in order to superimpose average mandibular plots. The two mandibular points Pg and Gn identified on day 0 images were selected for this purpose.

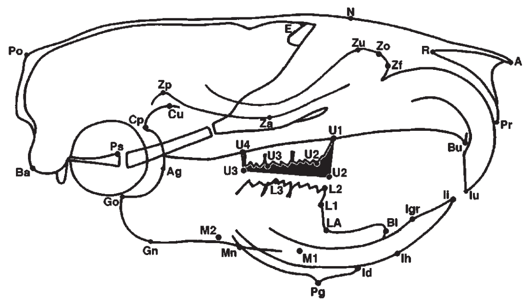

Figure 3 Landmarks used for cephalometric measurements. Ba, most posterior and inferior point of the occipital condyle; Po, most posterior point of the interparietal bone; E, intersection between the frontal bone and the posterior edge of the ethmoid bone; N, middle point on the nasofrontal suture; A, most anterior point of the nasal bone; R, intersection between the nasal bone plate and the premaxilla; Pr, anterior edge of the nasal floor; Iu, mean point of the incisal edges of the upper incisors; Bu, most prominent inferior point on the premaxilla, lingual to the upper incisors; U1, intersection between the maxillary bone and the mesial root of the upper first molar; U2, tip of the mesial cusp of the first molar; in HB and SB groups, the mean point of the most anterior and inferior edge of the bite blocks; U3, tip of the distal cusp of the second molar; in HB and SB groups, the mean point of the most posterior and inferior edge of the bite blocks; U4, intersection between the maxilla and the distal root of the upper third molar; Ps, most superior and posterior edge of the presphenoid synchondrosis; Zp, deepest point at the lower border of the zygomatic process of the temporal bone; Za, most prominent point on the zygomatic arch; Zu, deepest point on the posterior notch of the zygomatic process of the maxilla; Zo, most anterior and superior point on the zygomatic process of the maxilla; Zf, most anterior point on the zygomatic process of the maxilla; L3, tip of the distal cusp of the lower second molar; L2, tip of the mesial cusp of the lower first molar; L1, upper edge of the alveolar bone, mesial to the lower first molar; LA, deepest point between lower molar and incisor alveolar process; Bl, most anterior-superior point on the alveolar process of the lower incisor; Igr, posterior limit of the ground surface of the lower incisor; Ii, mean point of the incisal edges of the lower incisors; Ih, point of the labial surface of the lower incisor, opposite to point Bl; Id, most anterior-inferior point on the alveolar process of the lower incisor; Pg, most inferior point on the anterior part of the mandible; Mn, deepest point on the antegonial notch; Gn, most inferior point of the angular process of the mandible; Go, most posterior point of the angular process of the mandible; Ag, deepest point on the posterior border of the ramus; Cp, most posterior point on the condylar process; Cu, most superior point on the condylar process; M1, most superior-posterior point of the anterior amalgam marker; M2, most superior-posterior point of the posterior amalgam marker. The posterior bite blocks are represented by the black area.

Table 1 Mean and standard deviation (SD) of linear (mm) and angular (°) cephalometric measurements, and body weight (g) on days 0 and 14 of the experiment.

	Day 0		Day 14 Hard diet control		Day 14 Soft diet control		Day 14 Hard diet bite block		Day 14 Soft diet bite block		Day 14 Main effect diet
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	*P*
Skull											
Total skull length: Po–A	39.2	0.7	43.4	0.5	43.3	0.7	43.3	0.8	43.4	0.9	0.891
Height of											
Neurocranium: PS/Po–E	8.4	0.2	9.1	0.2	9.2	0.2	9.3	0.3	9.1	0.3	0.420
Viscerocranium: N–U1	9.4	0.2	10.7	0.3	10.5	0.2	10.6	0.3	10.4	0.3	**0.019**
Anterior viscerocranium: A–Pr	4.5	0.1	5.1	0.2	5.1	0.1	5.1	0.1	5.1	0.1	0.917
Zygomatic arch: Za/Zp–Zu	4.7	0.2	5.2	0.2	5.3	0.2	5.2	0.2	5.3	0.2	0.534
Upper alveolar process: E–U1	9.1	0.2	10.3	0.3	10.3	0.2	10.4	0.3	10.3	0.3	0.422
Upper crown/bite block: E–U2	10.7	0.2	12.1	0.4	12.1	0.2	13.7	0.3	13.6	0.3	0.288
Angles											
Premaxilla: E–Ps–Pr	27.6	0.7	29.4	0.8	29.2	0.9	29.4	0.9	28.7	0.6	**0.025**
Premaxilla: Ps–E/U1–Pr	25.4	1.1	27.7	1.1	27.2	1.1	27.9	1.3	26.7	1.1	**0.004**
Upper viscerocranium: Ps–E/N–A	133.0	1.2	130.7	1.3	131.8	1.3	130.6	1.6	132.1	1.2	**0.001**
Mandible											
Length of bony part: Go–Bl	20.7	0.4	23.7	0.6	23.8	0.3	23.6	0.5	23.4	0.6	0.716
Height of ramus region: Gn/Ps–Pr	6.2	0.3	7.5	0.3	7.3	0.3	7.8	0.4	7.6	0.4	**0.021**
Slope of the mandible: Ps–E/Gn–Pg	40.8	1.3	40.9	1.5	41.3	1.7	46.9	1.9	47.7	1.7	0.249
Total height of viscerocranium: N–Pg	18.1	0.3	20.7	0.3	20.4	0.2	22.7	0.4	22.5	0.4	**0.025**
Body weight	109	9	217	16	210	18	224	13	225	10	0.492

On day 14 the rats were divided into four groups of 13: hard diet bite block group; hard diet control group; soft diet bite block group; soft diet control group. Analysis of variance (ANOVA); *P*, probability value. *P* values are adjusted for body weight (as a covariate).

The two points were transferred to the images of the three following occasions by superimposition on the contours of the amalgam markers, for each individual rat. The two fiducial points were called 'Pg0' and 'Gn0'. The two mandibular points were chosen as they were easy to identify and because the shape of the mandibles was approximately identical for all the rats at the beginning of the experiment.

Mandibular shape changes were described as follows: molar eruption was represented by the movement of point L2 in relation to point Pg fiducial; apposition at the lower and posterior borders of the mandible was described by the changes in position of the points Pg, Gn, Go, and Cp from day 0 to day 42.

Statistical analysis

Group differences on days 14, 28, and 42, as well as the changes between days 14 and 28, and between days 14 and 42, were tested by two-way analysis of variance (ANOVA), including interactions between the experimental factors.

P values below 0.05 were required for the differences to be accepted as statistically significant. Since body weight showed a statistically significant relationship with some of the measurements, *P* values were adjusted using body weight as a covariate.

Distances between the amalgam markers, represented by points M1 and M2, were tested with a paired *t*-test between the four registrations.

Error of the method

The error of the methods of landmark identification was calculated according to the formula of Dahlberg (1949): $Se = \sqrt{\Sigma d^2/2n}$, where Σd^2 is the sum of the squared differences between pairs of recordings and n is the number of duplicate measurements. The coefficient of reliability of the measurements (Houston, 1983) was calculated using the formula $(1 - Se^2/St^2) \times 100$, where Se^2 is the variance of the error and St^2 is the total variance of the sample. Duplicate registration of landmark co-ordinates was performed in 20 rats with at least one month between the two sessions.

The error of the method varied between 0.04 and 0.08 mm for most linear measurements and exceeded 0.08 mm in the following measurements: Go–Bl (0.19 mm), Za/Zp–Zu (0.15 mm), Po–A (0.18 mm), and Ps/Po–E (0.10 mm). The error for the angular measurements varied between 0.3 degrees (E–Ps–Pr) and 0.5 degrees (Ps–E/Gn–Pg). The value of the coefficient of reliability was above 0.87 for most measurements except for Za/Zp–Zu (0.49), Ps/Po–E (0.73), A–Pr (0.76), and E–Ps–Pr (0.85). High values were found for N–Pg (1.0), E–U2 (0.99), Ps–E/Gn–Pg (0.98), N–U1 (0.97), and Gn/Ps–Pr (0.96).

Results

Body weight

The mean body weight on day 0 was 109 g (±8 g). Two weeks later, the mean body weights of the two groups receiving the bite blocks, although randomly selected, were slightly but statistically significantly larger compared with the control groups (Table 1). The body weight did not show statistically significant differences between the four groups later, either on day 28 or on day 42 (Table 2). Although the increase in body weight followed a similar trend in all groups (Figure 4), the rats that received the bite blocks showed less weight gain compared with the control rats (Table 3). The rats on a hard diet in both control and bite block groups grew less in the period between days 14 and 28.

Effect of the reduced masticatory function on dento-skeletal growth

Day 14. The rats on the soft diet had a shorter viscerocranium height (N–U1), as well as a more upward slope of the nasal bone and premaxilla, as indicated by the larger angle Ps–E/N–A and smaller angles E–Ps–Pr and Ps–E/U1–Pr (Table 1), compared with rats on the hard diet. The height of the mandibular ramus region (Gn/Ps–Pr) and the total height of the viscerocranium (N–Pg) were also shorter in the soft diet group. In the period day 0 to 14, bone growth on the lower (Gny) and posterior border (Go) of the mandibular angular region was less in the rats on the soft diet (Table 4).

Day 28. The same linear and angular measurement as for day 14 showed similar differences between the hard and soft diet groups on day 28 (Table 2). In addition, the slope of the lower border of the mandible (Ps–E/Gn–Pg) was steeper in animals on the soft diet.

Day 42. At the end of the experiment, both the heights of the viscerocranium (N–U1) and neurocranium (Ps/Po–E) were slightly shorter in the rats on the soft diet (Table 2). The slope of the nasal bones (Ps–E/N–A) had a more upward inclination in the rats on the soft diet. The height of the mandibular ramus measured up to the condylar head (Cu–Gn) was shorter in the rats on the soft diet (Figure 5) and the slope of the lower mandibular border (Ps–E/Gn–Pg) was steeper in this group. The total height of the viscerocranium (N–Pg) was slightly shorter in the control rats on the soft diet.

Days 14–28. A slightly smaller increase in height of the neurocranium (Ps/Po–E) was found in the rats on the soft diet during this period (Table 3). In the mandible, a smaller increase in height of the ramus region (Gn/Ps–Pr) was found in the animals on the soft diet. Bone apposition on the lower border of the angular process (Gny) tended to be less in the rats on the soft diet, while at the posterior border of the condyle (Cpx) it tended to be less in the rats on the hard diet, but neither of the differences reached a statistically significant level (Table 4).

Days 14–42. A smaller increase in height of the neurocranium (Ps/Po–E), and a slightly smaller increase in the height of the viscerocranium (N–U1) were found in the rats on the soft diet during this period (Table 3). The height of the ramus region (Gn/Ps–Pr) increased less in the rats on the soft diet and the inclination of the lower mandibular border (Ps–E/Gn–Pg) decreased less in this group.

Table 2 Mean and standard deviation (SD) of linear (mm) and angular (°) cephalometric measurements, and body weight (g) on days 28 and 42 of the experiment.

Day 28	Hard diet control		Soft diet control		Hard diet bite block		Soft diet bite block		Main effects		Interaction Diet–Appliance
									Diet	Appliance	
	mean	SD	mean	SD	mean	SD	mean	SD	*P*	*P*	*P*
Skull											
Total skull length: Po–A	46.2	0.4	46.3	0.7	46.0	0.8	46.1	0.9	0.761	0.398	0.777
Height of											
Neurocranium: PS/Po–E	9.6	0.2	9.6	0.2	9.8	0.3	9.6	0.2	0.064	0.138	0.368
Viscerocranium: N–U1	11.6	0.3	11.4	0.2	11.3	0.3	11.1	0.3	**0.011**	**0.0016**	0.970
Anterior viscerocranium: A–Pr	5.5	0.1	5.5	0.1	5.5	0.2	5.5	0.1	0.282	0.275	0.211
Zygomatic arch: Za/Zp–Zu	5.7	0.3	5.6	0.2	5.6	0.2	5.6	0.3	0.165	0.288	0.241
Upper alveolar process: E–U1	11.1	0.3	10.9	0.2	10.8	0.3	10.7	0.3	0.175	**0.010**	0.732
Upper crown/blocks: E–U2	13.0	0.3	12.9	0.2	14.2	0.4	14.1	0.3	0.291	**<0.0001**	0.427
Angles											
Premaxilla: E–Ps–Pr	29.9	0.5	29.1	0.8	29.3	0.7	29.0	0.7	**0.015**	**0.008**	0.095
Premaxilla: Ps–E/U1–Pr	28.3	1.0	27.1	1.1	28.2	1.1	27.5	1.1	**0.010**	0.954	0.233
Upper viscerocranium: Ps–E/N–A	130.3	1.0	132.2	1.3	131.1	1.2	132.3	1.3	**<0.0001**	0.072	0.167
Mandible											
Length of bony part: Go–Bl	25.3	0.3	25.3	0.4	24.8	0.5	24.6	0.3	0.278	**<0.0001**	0.365
Height of ramus region: Gn/Ps–Pr	8.5	0.3	8.0	0.4	8.4	0.3	8.0	0.4	**<0.0001**	0.841	0.740
Slope of the mandible: Ps–E/Gn–Pg	40.5	1.0	41.2	1.4	43.0	1.5	44.7	2.2	**0.001**	**<0.0001**	0.082
Total height of viscerocranium: N–Pg	22.6	0.3	22.1	0.3	23.2	0.4	23.0	0.4	**0.0008**	**<0.0001**	0.129
Body weight	307	22	306	21	289	23	304	20	0.211	0.084	0.180

Day 42	Hard diet control		Soft diet control		Hard diet bite block		Soft diet bite block		Main effects		Interaction Diet–Appliance
									Diet	Appliance	
	mean	SD	mean	SD	mean	SD	mean	SD	*P*	*P*	*P*
Skull											
Total skull length: Po–A	47.8	0.5	47.5	0.6	47.4	0.9	47.6	0.9	0.849	0.462	0.208
Height of											
Neurocranium: PS/Po–E	9.9	0.2	9.8	0.2	10.1	0.2	9.9	0.2	**0.003**	0.108	0.142
Viscerocranium: N–U1	12.2	0.3	11.9	0.2	11.8	0.3	11.5	0.3	**0.0001**	**<0.0001**	0.739
Anterior viscerocranium: A–Pr	5.7	0.1	5.8	0.3	5.7	0.2	5.6	0.1	0.841	0.144	0.060
Zygomatic arch: Za/Zp–Zu	6.0	0.2	5.8	0.3	5.8	0.4	5.9	0.3	0.310	0.619	0.172
Upper alveolar process: E–U1	11.7	0.3	11.5	0.3	11.5	0.4	11.4	0.3	0.168	0.080	0.687
Upper crown/blocks: E–U2	13.4	0.4	13.3	0.3	14.7	0.4	14.7	0.4	0.460	**<0.0001**	0.651
Angles											
Premaxilla: E–Ps–Pr	30.6	1.3	30.3	1.2	30.6	1.1	30.1	1.1	0.205	0.568	0.979
Premaxilla: Ps–E/U1–Pr	28.5	1.8	28.2	1.2	29.6	1.6	28.6	1.4	0.123	0.168	0.592
Upper viscerocranium: Ps–E/N–A	129.7	1.9	131.3	1.8	129.6	1.3	131.1	1.2	**0.0009**	0.981	0.701
Mandible											
Length of bony part: Go–Bl	26.1	0.4	26.0	0.5	25.8	0.6	25.5	0.6	0.324	**0.036**	0.358
Height of ramus: Cu–Gn	13.1	0.4	12.4	0.3	12.6	0.4	11.9	0.4	**<0.0001**	**0.0003**	0.790
Ramus region: Gn/Ps–Pr	9.0	0.3	8.4	0.4	8.7	0.4	8.2	0.5	**<0.0001**	0.098	0.746
Slope of the mandible: Ps–E/Gn–Pg	42.0	2.1	43.4	1.9	43.7	2.5	45.8	2.2	**0.003**	**0.007**	0.228
Total height of viscerocranium: N–Pg	23.8	0.5	23.3	0.5	23.9	0.4	23.9	0.5	**0.021**	**0.024**	**0.040**
Body weight	364	27	353	20	343	31	353	26	0.946	0.132	0.176

Thirteen rats in each group. Two-way analysis of variance (ANOVA); *P*, probability value. *P* values are adjusted for body weight (as a covariate).

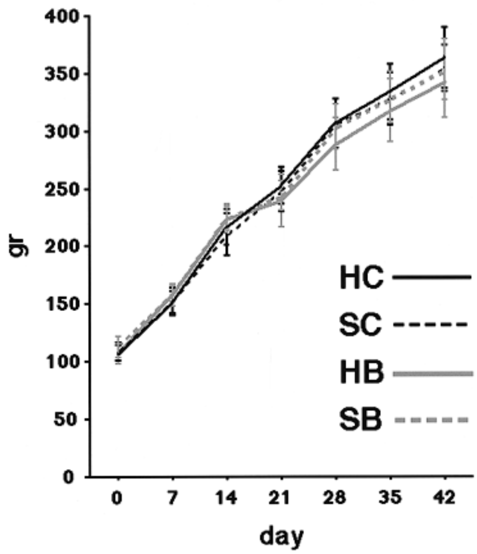

Figure 4 Linear plot of mean body weights; bars represent ±1 standard deviation. HC, hard diet control group; SC, soft diet control group; HB, hard diet bite block group; SB, soft diet bite block group.

Effect of the posterior bite blocks on dento-skeletal growth

The insertion of the posterior bite blocks on day 14 rotated the mandible downward and backward by 6 degrees (angle Ps–E/Gn–Pg, Table 1) and lowered the mandibular molars by 2.1 mm, as shown by the difference in the distance L2–U1 ($P < 0.001$). As a consequence, the height of the ramus region increased slightly (Gn/Ps–Pr, $P < 0.007$) and the total height of the viscerocranium increased by 2 mm (N–Pg, $P < 0.001$).

Day 28. Two weeks after insertion of the bite blocks, the height of the viscerocranium (N–U1) as well as the height of the upper alveolar process (E–U1) was shorter in rats wearing the bite blocks, while the height of the posterior bite blocks (E–U2) was still larger than that of the molar crowns of the control animals (Table 2). The premaxilla (E–Ps–Pr) was angulated slightly less downward in the rats wearing the bite blocks, and the total height of the viscerocranium (N–Pg) was still larger in this group compared with the control rats. The length of the mandible (Go–Bl) was shorter in the rats wearing the bite blocks, while its lower border (Ps–E/Gn–Pg) was angulated more downward in this group compared with the control rats.

Day 42. At the end of the experiment the height of the viscerocranium (N–U1) was still shorter in the rats wearing the bite blocks, and the height of the posterior bite blocks (E–U2) was still larger than that of the molar crowns of the control animals (Table 2). The total height of the viscerocranium (N–Pg) was still larger, although only slightly, in the rats wearing the bite blocks. The length of the mandible (Go–Bl) and the height of the ramus measured up to the condylar head (Cu-Gn) were shorter in the rats wearing the bite blocks compared with the control rats (Figure 5). The slope of the mandible (Ps–E/Gn–Pg) was still steeper in the rats wearing the bite blocks.

Days 14–28. The height of the viscerocranium (N–U1) increased less in the rats wearing the bite blocks while the height of the neurocranium (Ps/Po–E) increased slightly more in this group (Table 3).

The upper alveolar process (E–U1) increased less and the bite blocks (E–U2) erupted less than the molars in the control rats. Similarly, the total height of the viscerocranium (N–Pg) increased less in the rats wearing the bite blocks. In the mandible, the height of the ramus region (Gn/Ps–Pr) increased less in the rats wearing the bite blocks while the inclination of the mandible (Ps–E/Gn–Pg) decreased more in this group than in the control group. Bone apposition in the mandible did not show statistically significant differences between the bite blocks and control groups in this period (Table 4). The lower molars (Pg0–L2) were intruded in the rats wearing the bite blocks whereas they continued to erupt in the control rats.

Days 14–42. While the height of the viscerocranium (N–U1) increased less in the rats wearing the bite blocks until the end of the experiment, the height of the neurocranium (Ps/Po–E) increased slightly more in this group compared with the control rats (Table 3). The slope of the premaxilla (Ps–E/U1–Pr) became slightly steeper until the end of the experiment in the rats wearing the

Table 3 Mean and standard deviation (SD) of differences of linear (mm) and angular (°) cephalometric measurements, and body weight (g) between days 14 and 28 and between days 14 and 42 of the experiment.

Day 14–28	Hard diet control		Soft diet control		Hard diet bite block		Soft diet bite block		Main effects		Interaction Diet–Appliance
									Diet	Appliance	
	mean	SD	mean	SD	mean	SD	mean	SD	*P*	*P*	*P*
Skull											
Total skull length: Po–A	2.9	0.3	3.0	0.3	2.6	0.3	2.7	0.4	0.904	0.565	0.402
Height of											
Neurocranium: PS/Po–E	0.5	0.1	0.4	0.1	0.5	0.1	0.5	0.1	**0.034**	**0.007**	0.301
Viscerocranium: N–U1	0.9	0.1	0.8	0.1	0.7	0.1	0.7	0.2	0.202	**0.002**	0.891
Anterior viscerocranium: A–Pr	0.4	0.1	0.4	0.1	0.4	0.1	0.4	0.1	0.501	0.905	0.664
Zygomatic arch: Za/Zp–Zu	0.5	0.2	0.3	0.2	0.4	0.2	0.3	0.4	0.101	0.598	0.344
Upper alveolar process: E–U1	0.7	0.1	0.7	0.2	0.5	0.2	0.5	0.2	0.126	**0.020**	0.640
Upper crown/bite block: E–U2	0.9	0.2	0.8	0.1	0.4	0.2	0.6	0.2	0.740	**<0.0001**	**0.010**
Angles											
Premaxilla: E–Ps–Pr	0.5	0.6	0.0	0.7	–0.1	0.6	0.2	0.4	0.459	0.941	**0.020**
Premaxilla: Ps–E/U1–Pr	0.5	1.0	–0.1	0.6	0.3	0.8	0.8	0.5	0.517	0.051	**0.013**
Upper viscerocranium: Ps–E/N–A	–0.5	0.8	0.4	0.6	0.4	1.0	0.2	0.7	0.061	0.813	**0.024**
Mandible											
Length of bony part: Go–Bl	1.6	0.5	1.5	0.3	1.2	0.5	1.3	0.3	0.724	0.097	0.529
Height of ramus region: Gn/Ps–Pr	1.0	0.2	0.8	0.2	0.6	0.3	0.4	0.3	**0.003**	**0.0003**	0.588
Slope of the mandible: Ps–E/Gn–Pg	–0.4	1.5	–0.1	0.9	–3.8	1.7	–3.0	1.9	0.103	**<0.0001**	0.373
Total height of viscerocranium: N–Pg	1.8	0.2	1.7	0.2	0.6	0.2	0.5	0.4	0.093	**<0.0001**	0.611
Body weight increase	91	10	96	12	64	17	79	17	**0.014**	**<0.0001**	0.252

Day 14–42	Hard diet control		Soft diet control		Hard diet bite block		Soft diet bite block		Main effects		Interaction Diet–Appliance
									Diet	Appliance	
	mean	SD	mean	SD	mean	SD	mean	SD	*P*	*P*	*P*
Skull											
Total skull length: Po–A	4.4	0.3	4.2	0.4	4.0	0.5	4.2	0.4	0.552	0.512	0.213
Height of											
Neurocranium: PS/Po–E	0.8	0.2	0.7	0.1	0.9	0.2	0.7	0.2	**0.002**	**0.008**	0.689
Viscerocranium: N–U1	1.5	0.2	1.3	0.1	1.2	0.2	1.1	0.2	**0.0004**	**<0.0001**	0.697
Anterior viscerocranium: A–Pr	0.6	0.2	0.6	0.2	0.6	0.2	0.5	0.2	0.961	0.777	0.100
Zygomatic arch: Za/Zp–Zu	0.7	0.2	0.5	0.2	0.6	0.3	0.6	0.3	0.104	0.630	0.197
Upper alveolar process: E–U1	1.4	0.3	1.3	0.3	1.1	0.3	1.1	0.4	0.358	0.365	0.794
Upper crown/bite block: E–U2	1.3	0.3	1.2	0.2	1.0	0.3	1.1	0.3	0.876	0.236	0.336
Angles											
Premaxilla: E–Ps–Pr	1.2	1.2	1.1	1.1	1.2	1.1	1.4	1.3	0.954	0.283	0.883
Premaxilla: Ps–E/U1–Pr	0.8	1.5	1.0	1.1	1.7	1.4	1.9	1.5	0.681	**0.008**	0.821
Upper viscerocranium: Ps–E/N–A	–1.0	1.3	–0.5	1	–1.1	1.6	–1.1	1.6	0.437	0.249	0.652
Mandible											
Length of bony part: Go–Bl	2.4	0.6	2.3	0.6	2.2	0.7	2.1	0.4	0.583	0.859	0.955
Height of ramus region: Gn/Ps–Pr	1.5	0.3	1.2	0.3	0.9	0.4	0.6	0.3	**0.001**	**<0.0001**	0.680
Slope of the mandible: Ps–E/Gn–Pg	1.2	1.6	2.1	1.2	–3.2	2.9	–1.9	2.2	**0.040**	**<0.0001**	0.587
Total height of viscerocranium: N–Pg	3.1	0.3	2.9	0.3	1.3	0.5	1.3	0.4	0.563	**<0.0001**	0.200
Body weight increase	147	16	144	12	118	28	128	24	0.570	**0.0003**	0.253

Thirteen rats in each group. Two-way analysis of variance (ANOVA); *P*, probability value. *P* values are adjusted for increase in body weight (as a covariate).

bite blocks, and the total height of the viscerocranium (N–Pg) increased less in this group. The height of the mandibular ramus (Gn/Ps–Pr) increased less in the rats wearing the bite blocks, and the inclination of the mandible (Ps–E/Gn–Pg) decreased in this group whereas it increased in the control group. Similar to the period days 14–28, bone apposition in the mandible did not show statistically significant differences between the bite block and control groups over this longer period (Table 4). The lower molars (Pg0–L2) continued to be intruded in the rats wearing the bite blocks, although less than after the first two weeks with the appliance, whereas the lower molars erupted in the control rats.

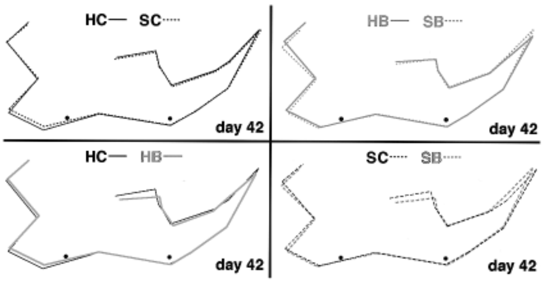

Figure 5 Mean diagrams of the mandibles on day 42 based on the cephalometric landmarks shown in Figure 2. The mandibles are superimposed on the two fiducial points Pg0 and Gn0 (black dots). HC, hard diet control group; SC, soft diet control group; HB, hard diet bite blocks group; SB, soft diet bite blocks group.

Interaction between function (diet) and posterior bite blocks and dento-skeletal growth

Days 14–28. Most of the statistical interactions between the two factors were found in the two weeks immediately following insertion of the bite blocks. The bite blocks (E–U2) erupted more in the rats on the soft diet compared with those on the hard diet, whereas the opposite

Table 4 Means and standard deviation (SD) of movements of landmarks Pg, Go, Gn, Cp, and L2 in the intervals indicated in the first column.

Landmarks and interval	Hard diet control		Soft diet control		Hard diet bite block		Soft diet bite block		Main effects		Interaction Diet–Appliance
									Diet	Appliance	
	mean	SD	mean	SD	mean	SD	mean	SD	*P*	*P*	*P*
Pg day 0–14 (26)	0.4	0.1	0.4	0.1					0.188		
Pg day 14–28 (13)	0.4	0.1	0.4	0.1	0.3	0.1	0.3	0.2	0.499	0.2432	0.736
Pg day 14–42 (13)	0.5	0.1	0.5	0.1	0.4	0.1	0.5	0.3	0.859	0.258	0.334
Gny day 0–14 (26)	0.8	0.2	0.5	0.2					**0.0013**		
Gny day 14–28 (13)	0.4	0.2	0.3	0.2	0.2	0.2	0.2	0.3	0.062	0.810	0.791
Gny day 14–42 (13)	0.7	0.3	0.5	0.3	0.2	0.2	0.4	0.4	0.944	0.114	**0.046**
Go day 0–14 (26)	2.8	0.3	2.5	0.4					**0.033**		
Go day 14–28 (13)	1.3	0.4	1.3	0.3	1.1	0.4	1.2	0.4	0.805	0.387	0.608
Go day 14–42 (13)	2.0	0.6	1.9	0.4	2.0	0.4	2.0	0.3	0.677	0.506	0.922
Cpx day 0–14 (26)	2.3	0.3	2.2	0.3					0.281		
Cpx day 14–28 (13)	1.5	0.2	1.7	0.2	1.4	0.5	1.6	0.4	0.232	0.697	0.891
Cpx day 14–42 (13)	2.4	0.4	2.6	0.2	2.3	0.6	2.6	0.3	0.055	0.336	0.811
Pg0–L2 day 0–14 (26)	0.8	0.2	0.7	0.2					0.695		
Pg0–L2 day 14–28 (13)	0.4	0.2	0.4	0.1	–0.2	0.1	–0.2	0.2	0.315	**<0.0001**	0.798
Pg0–L2 day 14–42 (13)	0.7	0.2	0.7	0.2	–0.1	0.2	–0.1	0.3	0.738	**<0.0001**	0.852

Gny, movement of Gn along the *y*-axis; Cpx, movement of Cp along the *x*-axis. The reference line drawn through the fiducial points Pg0–Gn0 represents the *x*-axis. The distances between the landmarks on different occasions describe bone apposition and tooth eruption in the mandible.
The number of rats in each group is given in parentheses. In the period day 0–14 the rats are pooled in the two main diet groups.
Two-way analysis of variance (ANOVA); *P*, probability value. *P* values are adjusted for increase in body weight (as a covariate).

effect was found for the molars between the two control groups (Table 3, Figure 6). Furthermore, while growth of the premaxilla (E–Ps–Pr, Ps–E/U1–Pr) and nasal bones (Ps–E/N–A) was directed slightly more downward in the rats on the hard diet among the control animals, the opposite was seen in the rats on the hard diet among the groups wearing the bite blocks.

Days 14–42. In the mandible, bone apposition at the lower border of the angular region (Gny) was less in the control animals on the soft diet, whereas the opposite occurred among the rats wearing the bite blocks, i.e. smaller bone apposition at this site was measured for the rats on the hard diet compared with those on the soft diet (Table 4, Figure 5).

On day 42, whereas the total height of the viscerocranium (N–Pg) was shorter in the control animals on a soft diet, no difference was detected among the two groups of rats wearing the bite blocks (Table 2).

Amalgam markers

The distance between the radiographic images of the amalgam markers decreased from day 0 to 14 by 0.01 mm on average (SD 0.05 mm, $P = 0.105$), from day 14 to 28 by 0.01 mm (SD 0.05 mm, $P = 0.03$), and from day 28 to 42 by 0.02 mm (SD 0.1 mm, $P = 0.002$). The total mean shortening of 0.04 mm of the intermarker distance represented 0.6 per cent of the initial mean intermarker distance of 7.09 mm (SD 0.84 mm).

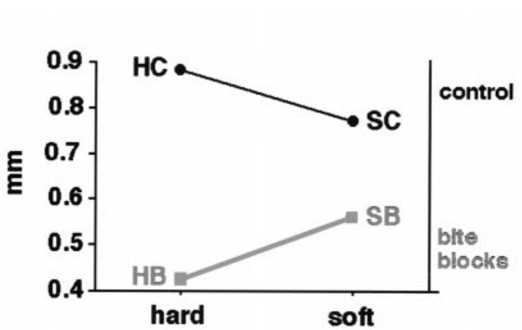

Figure 6 Interaction plot (ANOVA) of the eruption of the posterior bite blocks (E–U2) during the period day 14–28. The interaction is statistically significant ($P = 0.01$). HC, hard diet control group; SC, soft diet control group; HB, hard diet bite blocks group; SB, soft diet bite blocks group.

Discussion

This study demonstrated that both alteration of masticatory muscle function and the presence of posterior bite blocks *per se* led to an altered craniofacial growth pattern in rats. Differences in masticatory muscle capacity also affected the way that some craniofacial regions adapted to the presence of the bite blocks, particularly during the first two weeks after insertion of the bite blocks.

Effect of function on dento-skeletal growth

Differences in craniofacial morphology due to altered masticatory muscle function, such as the shorter vertical dimension of the ramus region, the more upward directed snout, and the shorter vertical dimension of the viscerocranium in the rats on the soft diet were consistent throughout the experiment and in line with previous studies based on this experimental model (Kiliaridis *et al.*, 1985; Ito *et al.*, 1988). Most of these differences could already be detected on day 14. Furthermore, a shorter height of the whole craniofacial complex and a backward, downward rotated mandible was found in the rats on the soft diet throughout the experiment. This could be the result of a reduced vertical sutural growth of the viscerocranium and of less bone growth at the lower border of the angular region, respectively.

The two amalgam markers in the mandible represented stable reference structures for the superimposition of the mandibular radiographic images. With this method, bone growth in the lower and posterior border of the ramus was demonstrated to be less in rats on the soft diet in the first two weeks of the experiment, and a similar tendency was found for the lower border of the ramus until the end of the experiment. This is in line with results from previous superimposition (Kiliaridis *et al.*, 1985; Kiliaridis, 1989) and histological studies (Kiliaridis, 1989; Yamada and Kimmel, 1991). The tendency to more posterior-directed growth at the condyle in rats on the soft diet, the shorter ramus, and the finding of a more posteriorly rotated mandible in this group suggest that muscle function may affect the growth pattern of the mandible.

Effect of the posterior bite blocks on dento-skeletal growth

Adaptation to the posterior bite blocks consisted mainly of a more upward-directed growth of the snout, whereas the height of the mandibular ramus was reduced. A dental effect of the bite blocks was the inhibited eruption of the upper molars bearing the bite blocks and the intrusion of the lower molars, especially during the first two weeks. Through inhibition of the eruption of the molars, forward rotation of the mandible was allowed, resulting in a decrease of the total height of the viscerocranium, which approached the size of the control group by the end of the experiment. The whole masticatory system thus demonstrated its capacity to neutralize the 'disturbing factor' represented by the posterior bite blocks during growth. The results concerning the growth direction of the snout are in line with those of previous animal studies, where the growth of the maxilla was also found to change to a more upward direction (McNamara, 1977; Altuna and Woodside, 1985; Ferrari and Herring, 1995).

In contrast, Sugiyama *et al.* (1999) found that the maxilla rotated downward in growing rats wearing bite blocks. This is possibly due to the fact that different landmarks were used for this measurement. The smaller viscerocranium height in the rats wearing the bite blocks in the present study can be explained by possible changes in direction of force upon the sutural complex, leading to reduced bone apposition in the area of the frontal-nasal suture (landmark N), and by inhibited development of the alveolar process of the upper molars (E–U1). The height of the neurocranium increased significantly more, although very slightly, in rats with normal masticatory function and in those wearing the bite blocks. It can be speculated that this may be due to a geometrical effect of the location of landmark Po, whose position may have been altered by different bone apposition in this area. This is an area of insertion of neck muscles whose activity may help to stabilize the head during incision of food pellets in rats on the hard diet, or which would allow an altered head posture during feeding due to the presence of the appliance.

In the mandible, the finding of a reduced ramus height induced by the bite blocks is in line with the results of previous studies on growing rats (Sugiyama *et al.*, 1999) and on growing monkeys wearing bite blocks (McNamara, 1977). The length of the mandible was negatively affected by the presence of the appliance. It can be speculated that the relocation of the angular region in a lower and more posterior position altered the relationship of the bone structure with the neighbouring connective tissues and resulted in altered posterior periosteal bone apposition. Superimposition on the amalgam markers revealed a clear intrusion of the lower molars during the first two weeks after insertion of the bite blocks. This is also in line with previous studies with bite blocks where either inhibited eruption (McNamara, 1977; Sugiyama *et al.*, 1999) or intrusion of the molars (Ferrari and Herring, 1995) was found.

Influence of masticatory muscle capacity on the effect of the posterior bite blocks on dento-skeletal growth

Masticatory muscle function influenced the eruption of the upper molars bearing the bite blocks, so that reduced masticatory function resulted in less inhibition of their eruption. It can be speculated that lower muscular contractile forces and less occlusal loads in the bite block group on the soft diet may have restrained eruption of the upper molars less. No difference was found in the magnitude of intrusion of the lower molars in relation to muscle function. The different effect on the upper and lower molars may be due to possible differences in response to occlusal loads between the upper and lower molars.

In the skull, the rats wearing the bite blocks with higher functional demands showed a more upward directed growth of the snout compared with the rats with lower functional demand, opposite to the differences between the two control groups. It can be speculated that a different direction of loads on the upper incisors due to lowering of the mandible after the insertion of the bite blocks may have altered sutural growth in the maxilla–premaxilla in rats on the hard diet.

In the mandible, bone apposition at the lower border of the mandible was least in the rats wearing the bite blocks with higher functional demands and greatest in control rats with higher functional demands. It can be speculated that the cumulative effect of larger contractile forces and continuous passive stretching of the deep masseter may have inhibited periosteal bone apposition at the lower border of the mandible. However, a larger increase in total height of the ramus region was found in this same group compared with rats on the soft diet. This may have been due to compensatory vertical growth at the condyle in rats wearing the bite blocks on the hard diet. The reduced growth of this area in the hard diet group may also be related to less somatic growth as indicated by the smaller weight gain of this experimental group.

Posterior bite blocks and body weight increase

Although the rats were randomly assigned to the four groups on day 14, the rats that received the bite blocks weighed more than the control rats. In the following two weeks, the rats wearing the bite blocks, especially the ones on the hard diet, grew less than the control rats. The bite blocks possibly disturbed the ability of the rats on the hard diet to gnaw food pellets, since the incisors did not have occlusal contact in the first week, thus impairing feeding. Similarly, the control rats on the hard diet grew less compared with those on the soft diet, possibly because their gnawing capacity was impaired after shortening the incisors on day 14. Still, all groups showed a similar trend, with an increase in body weight. Since body weight or increases in body weight significantly affected some of the cephalometric measurements, the influence of body weight was compensated for by adjusting all P values after an analysis of covariance, using body weight as a covariate.

Advantages and limitations of the experimental model

The use of different dietary consistencies has previously proved effective in inducing different functional demands on the masticatory muscles (Kiliaridis *et al.*, 1988; Liu *et al.*, 1998) and made it possible to obtain two groups with a ‘controlled’ variation of muscular characteristics.

Whereas differences in the functional condition of the masticatory muscles existed at the insertion of the posterior bite blocks, the effect of the dietary consistency on the muscles may have been affected by the presence of the appliance. Thus it is unclear whether muscular differences between the two diet groups wearing the posterior bite blocks were maintained until the end of the experiment.

The advantage of the posterior bite blocks, compared with an appliance that would have kept the mandible in a protruded position, was that they allowed free antero-posterior and jaw-opening movements. However, the presence of the appliance seemed to disturb the rats’ feeding, especially in the group on the hard diet. The appliance worked in a similar way to a functional appliance, i.e. it stretched some of the masticatory muscles, among them the deep masseter muscle (Bresin *et al.*, 2000). The limitation of the appliance design is that it was mostly intended to influence molar tooth eruption and not, as some clinical functional appliances do, to produce sagittal tooth movements.

The distance between the images of the amalgam bone markers used for radiographic superimposition showed a slight shortening during the experiment. The two mandibular halves form a V-shape in the horizontal plane, meeting in the symphysis, and the opening of the ‘V’ seems to increase during growth (unpublished data), with the symphysis as the fulcrum of rotation. The distance between the marker images in the lateral projection therefore became shorter. The use of amalgam markers allowed an anatomically more correct superimposition procedure compared with other reference structures such as the occlusal plane.

Vertical growth at the condyle could unfortunately not be measured with this superimposition method, since the radiographic image of the condyle could not be distinguished before day 42.

Clinical implications of the findings

A large variation of the characteristics of the masticatory muscles, such as the magnitude of bite force (Proffit and Fields, 1983) and the thickness of the masseter muscle among young individuals (Raadsheer *et al.*, 1996), has been reported in the literature. In this study, it could be demonstrated that masticatory muscle function is able to influence the effect of a functional appliance on both tooth eruption and growth of the jaws. Although the results of this investigation cannot be applied directly to clinical orthodontics, it is tempting to speculate on the basis of the present finding that the effects of functional appliances, both on tooth eruption and on growth of the jaws, may be different in subjects with different muscular characteristics. The results of the present study suggest, for example, that the intrusive effect of a functional appliance may be enhanced in subjects with stronger masticatory muscles.

In the future, the analysis of muscular characteristics such as muscle thickness with non-invasive methods or the registration of bite forces may enable the clinician to individualize the choice of the orthodontic appliance. In subjects with weaker muscles, the clinician may, for example, foresee a lower efficiency of the appliance and may therefore consider a longer treatment time. Clinical investigations are required to corroborate the findings of this study.

Conclusions

This study demonstrated that both reduced masticatory muscle capacity and the presence of posterior bite blocks *per se* affected the craniofacial growth pattern and tooth eruption in rats. Differences in masticatory muscle capacity also influenced the way that some craniofacial regions and upper molar eruption were affected by the posterior bite blocks, particularly during the first two weeks after their insertion.

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