The Influence of an Experimentally-Induced Malocclusion On Vertebral Alignment in Rats: A Controlled Pilot Study

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ABSTRACT: There is a growing interest in the relationship between occlusion and posture because of a greater incidence of neck and trunk pain in patients with occlusal dysfunction. The study was designed to verify whether an alteration of the spinal column alignment may be experimentally induced in rats as a consequence of altering dental occlusion and also to investigate whether the spinal column underwent any further changes when normal occlusion was then restored. Thirty rats were divided into two groups. Fifteen (15) rats (test group) wore an occlusal bite pad made of composite resin on the maxillary right first molar for a week (T1). The same rats wore a second composite bite pad for another week on the left first molar in order to rebalance dental occlusion (T2). Fifteen rats were included in an untreated control group. All the rats underwent total body radiographs at T0 (before the occlusal pad was placed), at T1 (one week after application of a resin occlusal bite pad on the maxillary left first molar) and at T2 (one week after application of a second resin occlusal bite pad on the maxillary right first molar). A scoliotic curve developed in all the test rats at T1. There were no alterations of spinal position observed in any of the control rats. Additionally, the spinal column returned to normal condition in 83% of the test rats when the balance in occlusal function was restored. The alignment of the spinal column seemed to be influenced by the dental occlusion.

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Investigators have sought to produce scoliotic curves in several animal species using a variety of techniques.[1-15] These experiments on various animal models suggest possible anatomic or functional influence for each of these elements (spinal, neuromuscular, metabolic, endocrine) in the etiology of idiopathic scoliosis. In dentistry, the study of the relationship between occlusal problems and the spine are of increasing interest. This is the result of a greater incidence of pain in the muscles of the neck, trunk, the upper and lower limbs, and in the temporomandibular joints (TMJ) of patients with occlusal dysfunction.[16]

There are several conditions that impede normal trunk alignment in the frontal plane, and it should be interesting to investigate whether such conditions also affect dental occlusion.

Previously, Muller-Wachendorff[17] investigated 420 children with various postural disorders. Among the 164 children diagnosed as scoliotic, 60 (37%) were observed to have crossbites. Later, a noticeable prevalence of unilateral crossbite (11-15%) was shown in a group of Swedish children.[18-19] Finally, Huggare, et al.[20] investigated 22 young adults who had been previously treated for scoliosis and were using a Boston-brace and were diagnosed or had a history of treatment for lateral cross-bite in 55% of the subjects, compared with 18% in the control group.

Since these studies were based on a cross-sectional study method, no conclusion could be drawn with regard to the mechanism used or "what caused what". For that reason, the purpose of this study was to evaluate any changes occurring in the position of the spinal column on the frontal plane in a group of rats, both when the occlusion was experimentally altered and then following rehabilitation of the condition. Previous studies[17-21] have underlined a notable prevalence of cross-bite malocclusion in patients diagnosed with, or recently treated for, scoliosis. In the current study, the occlusion was altered in such a way as to induce the rat into a crossbite occlusal relationship.

Material and Methods

Thirty female Sprangue Dawley rats weighing 350 g (average age, 309 days) were used in the study: 15 in the study group and 15 in an untreated control group. All the rats in the two groups underwent spinal radiographs at baseline (T0) before any change in occlusion had occurred. In the rats in the study group, an occlusal bite pad made of composite and measuring 0.5 mm in height, was applied to the upper right molar, (**Figure 1**).

To apply the bite pad, the following procedures were used. The rats were anesthetized in a bell-shaped glass chamber, which contained ether-soaked cotton balls. The rats were also injected intra-peritoneally with benzodiazepine (5-10 mg/Kg body weight) and ketamine (20-40 mg/Kg body weight). The occlusal pad was made from a controlled measure of composite resin (**Figure 1**). A hollow plastic cylinder, 0.5 mm in height, was used to measure the composite which was then spread over the whole occlusal surface of the molar. The unilateral pad created a dysfunction in the bite position as an induced premature occlusal contact. The occlusion on a single premature contact was not stable so the rats adjusted their bite to a more stable position. They deviated the way they closed their jaw, either to the right or the left side. This deviation in closing movement caused a crossbite occlusion on the side to which the mandible deviated. The altered bite position is evident when looking at the rat's occlusal midline which deviated to the left side (**Figure 2**).

The rats in the study group wore the occlusal pad for one week (T1) then underwent another total body radiograph of the spinal column to evaluate any changes as a result of the experimental premature contact. At this time (T1), the rats in the control group also underwent another total body radiograph. After the radiographs were taken, a second occlusal pad was applied to the left upper molar in order to rebalance the occlusion. The new contact on the opposite side induced the rats to straighten their closing movement. The rats in the study group wore the second occlusal pad for one week (T2), then both groups underwent the last total body radiographs of the spinal column.

Radiographs

At T0, T1, and T2, each rat had undergone two total body radiographs, the first one on the frontal plane and the second on the sagittal plane. In order to perform

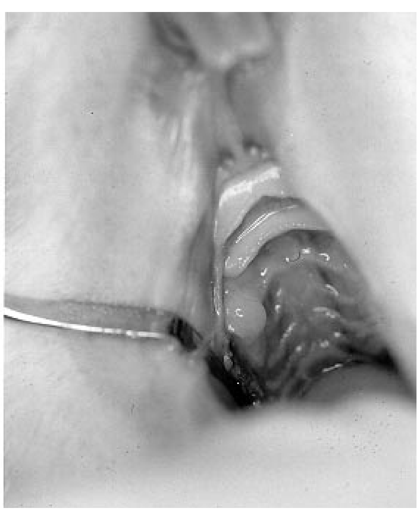

Figure 1
In the rats in the study/therapy group, a composite bite pad (less than 0.5 mm in height) was applied to the upper right molar. One week later, the same was applied to the opposite side to balance the occlusion.

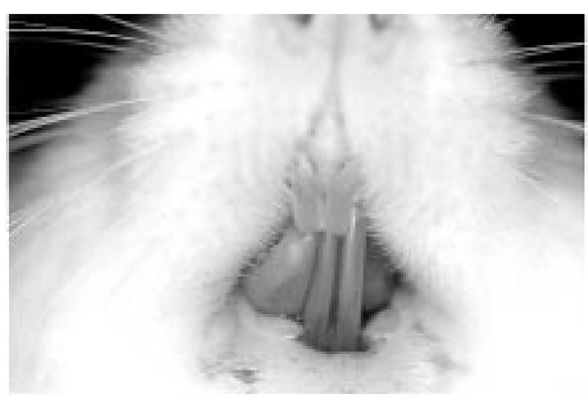

Figure 2
The lower incisal midline deviated to the left side after the first occlusal bite pad was applied to the maxillary right first molar.

spinal radiographs in a reproducible and standardized way and according to the canons of veterinary medicine, the rats were placed on the table as follows: on their backs with the spinal column resting on the surface; and in muscle traction via their fore- and hind-limbs.

Radiographs were taken using ALEM 100KW, 100ma. Radiographic specifications were 40Kw and 50mA for 0.2 seconds. The distance between the focus and the film was fixed to 90 cm. Three-M (Minnesota Mining and Manufacturing Co., St. Paul, MN) photographic films (24x30) were used. The method used for the procedure was as follows:

1. A bottomless and lidless metal box (**Figure 3A**) was built to an appropriate size to be placed on the radiographic table. The box was equipped with two runners with two metal rods each, parallel to each other on the horizontal and sagittal planes (**Figure 3B**). These rods were used to anchor the fore- and hind-limbs of the rats and were long enough to nearly touch the table in order to guarantee traction of the animals as close as possible to the table.
2. For each rat, the four limbs was anchored in the metal box by using four bandage-type adhesive strips of the same length, one for each limb. Before each of the four limbs were anchored, two reference lines were drawn on every adhesive strip, each one perpendicular to the long axis of the strip and a distance of two cm from each contour of the strips (**Figure 4**). These reference lines were necessary to allow for wrapping the strip around the rods (on the metal base) and around the limbs the same number of millimeters. Additionally, the adhesive strips were always attached by the same operator and at the same points on the wrists and ankles of the rats and near the head of the metal rods (**Figure 5**).

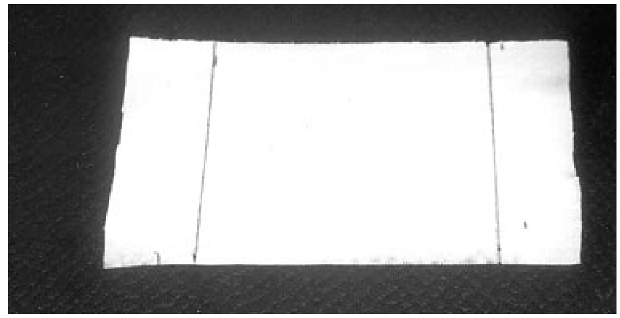

Figure 4
Measured adhesive strips applied two cm from each end of the box.

3. A series of lines, perpendicular to the long axis of the radiographic table, were drawn with a glass marking pencil by the operator. These lines were important to allow reroducible radiographs each time. In fact, for every radiograph, the joints of the fore- and hind-limbs were positioned using the same lines (**Figure 6**). Reference points were thus constant and unchangeable.
4. Each rat was anesthetized and affixed to the rods of the runners on the metal box after carefully checking the alignment of the four limbs using the reference lines (**Figure 6**). After the rat was anchored to the runners, it was then put in traction (**Figure 7**). The force was measured using a force gauge and the measurement noted on the chart for each rat (**Figure 8**). During successive radiographs (at T1 and T2), the same force as in the first examination was applied. The first radiograph (on the frontal plane) was taken with the rat on its back with the spinal column resting on the surface. The second radiograph (the lateral body radiograph) was taken soon after the radiograph on the frontal plane. The rats were positioned in profile while keeping the four legs affixed to the rods of the runners on the metal box and checking the alignment of all four limbs carefully with regard to the reference lines.

Variables considered on radiographs: Rats normally

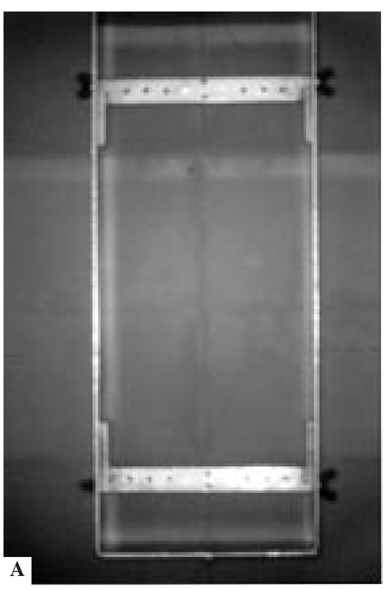

Figure 3 (**A** and **B**)
Bottomless, lidless metal box (**A**) used to position rats to be radiographed. The box has two runners and two metal rods parallel to each other on the horizontal and saggital planes (**B**).

Figure 5
The rat is anchored into the box for radiographing using the rods and adhesive strips.

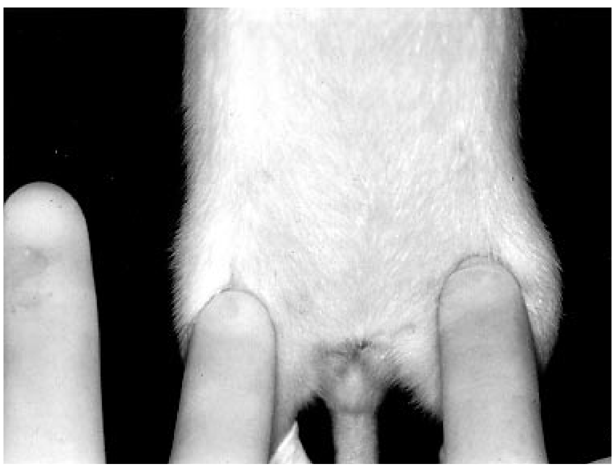

Figure 6
Using perpendicular lines drawn in the box, the rat's joints are kept in alignment.

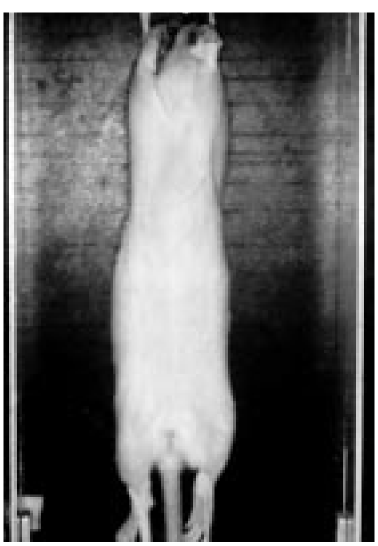

Figure 7
The rat shown in traction in preparation for radiographing.

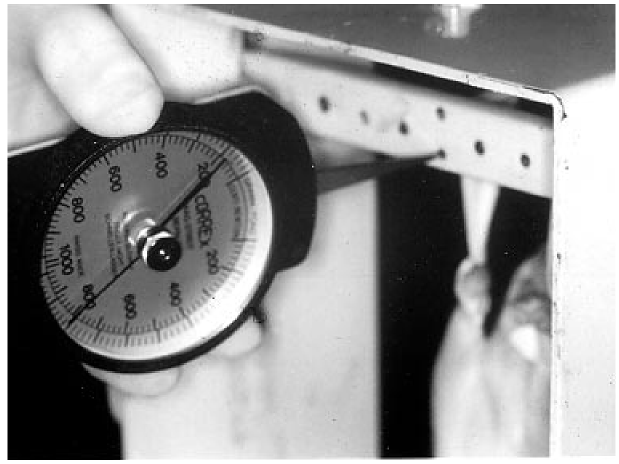

Figure 8
The force used to suspend the rat is measured using a force gauge.

have 30 vertebrae: seven cervical, twelve thoracic, seven lumbosacral and four caudal spine vertebrae. Spontaneous fusion often occurs in the lumbosacral block.

Variables on the frontal radiographs: The C4, T1, T6, T10, and L4 vertebrae were chosen as reference vertebrae for the evaluation of the alteration of column posture. A true vertical was traced on the radiographs parallel to the margin of the radiograph and intersecting at the center of the body of the fifth vertebra (which corresponds to the center of the pelvis) (**Figure 9**). The centers of C4, T1, T6, T10, and L4 vertebrae were marked on the radiographs by the same operator (blinded as to the group identity) and used as reference points. Five variables were evaluated, respectively: distance (measured in mm) between the reference points (the centers of the C4, T1,

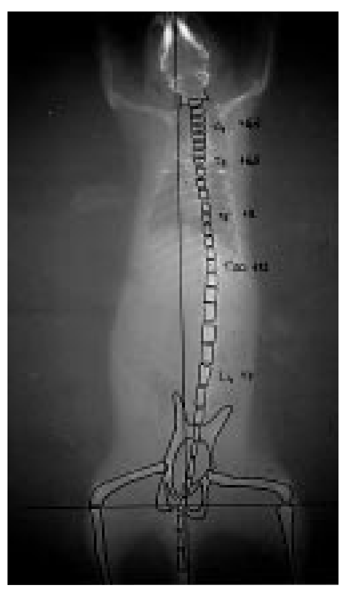

Figure 9
Reference points and lines: **C4** is the center of the fourth cervical vertebra; **T1** is the center of the first thoracic vertebra; **T6** is the center of the sixth thoracic vertebra; **T10** is the center of the tenth thoracic vertebra; **L4** is the center of the fourth lumbosacral vertebra. The line representing true vertical was traced parallel to the margin of the radiograph and was centered on a point that was the same for all radiographs: the center of the last fifth vertebra.

T6, T10, and L4 vertebrae) and the true vertical. The five radiographic variables indicated the alignment of the spinal column, since the greater the distance, the more inclined that segment of spinal column. The distance was considered positive if the body of the vertebra was positioned on the right of the true vertical. Otherwise, it was considered negative. The overlap between a reference point and true vertical was considered as 0 value. All measurements were taken by the same operator, blinded to the group identity.

Variables on lateral radiographs: The outline of all vertebrae was traced onto the radiographs by the same operator (blinded to the group identify) and used as reference points. The alignment of the spinal column was calculated by using a geometrical construction. The angle between the tangent line to the upper contour of the upper vertebra involved in the curvature and the tangent line to the lower contour of the lower vertebra involved in the curvature was traced. Perpendicular lines to those just described were then traced. The downward opening angle between these two lines defined the angle of the curvature. The greater the angle, the more curved that segment of the spinal column.

Method Error

In order to evaluate the method error of the radiographic procedure, two radiographs of four animals at T0 were made after loosening and repositioning the rat. The measurements of the first and the second radiographs were taken and the method error was calculated using Dahlberg's formula[22]:

$$\delta = \sqrt{\Sigma d^2/2N}$$

where d is the difference between the first and the second measurement and N is the number of double measurements. In order to evaluate the error inherent to the landmark identification on the radiographs, the same measurements on 15 radiographs were taken once again by the same operator a week later and the method error calculated by using Dahlberg's formula.

Data

The Statistical Package for Social Sciences program (SPSS, Inc., Chicago, IL) was used to analyze the data. Descriptive statistics included means and standard deviations (SD) for each variable considered at T0, T1, and T2. Since the data did not show a normal distribution, nonparametric statistics were computed to test significance.

The difference between the test and control groups at each experimental session (T0, T1, T2) was tested using the Mann-Whitney "U" test, and Friedman's two-way analysis of variance (ANOVA) was used to test the significant differences among the distances (mm) between the reference points (the centers of the C4, T1, T6, T10, and L4 vertebrae) and the true vertical from T0 to T2. The Bonferroni corrected Wilcoxon's signed rank test was used as a post-hoc test to verify the significance of the differences between T0-T1, T1-T2, and T0-T2, respectively. Differences were considered significant at $p<0.05$.

Results

During the weeks from T0 to T1 and to T2, the rats were observed carefully. During the hours soon after the first occlusal pad was applied to the upper right molar, the rats in the study group spent nearly all of their time opening and closing their mouths. However, the presence of the unilateral occlusal pad did not appear to impair their activities. After the first occlusal pad was applied, they were compelled to deviate their jaw-closing movements to the right or the left side, in order to experience a more stable occlusal condition. No difficulties in drinking, feeding, or defending themselves were observed. When the second occlusal pad was applied to the left upper molar in order to rebalance the occlusion, the rats in the study group experienced a new occlusal balance and after a few tries, were able to open and close their mouths normally without deviation.

The observation of radiographs [**Figure 10** (**A-C**) and **Figure 11** (**A-C**)] revealed that no change in the alignment of the spinal column occurred in any of the rats that did not receive an occlusal pad. However, a change in the alignment of the spinal column was observed in all of the rats that received a unilateral occlusal pad. The deformities were similar to those found in human idiopathic scoliosis (**Figure 10B** and **Figure 11B**). The convexity of the curve was directed to either side, with no consistent preference. No rat developed limb paralysis or showed difficulty in walking or running.

At T2, when the second occlusal pad was applied to the left upper molar to rebalance the occlusion, radiographs revealed the straightening of the spinal columns of all the rats in the study group.

Error in landmark localization and in the radiographic procedure was less than 5% of the total variance in the whole sample for all the variables. The analysis of the tracings on total body radiographs revealed no significant differences in each variable between the study and the control group at T0 and no significant changes in the alignment of vertebrae from T0 to T1 and T2 in the control group (**Table 1**). On the contrary, significant changes in the alignment of T6 vertebra ($p<0.01$) and T10 vertebra ($p<0.05$) were observed in the study group at T1, compared with the results at T0 (**Table 1**).

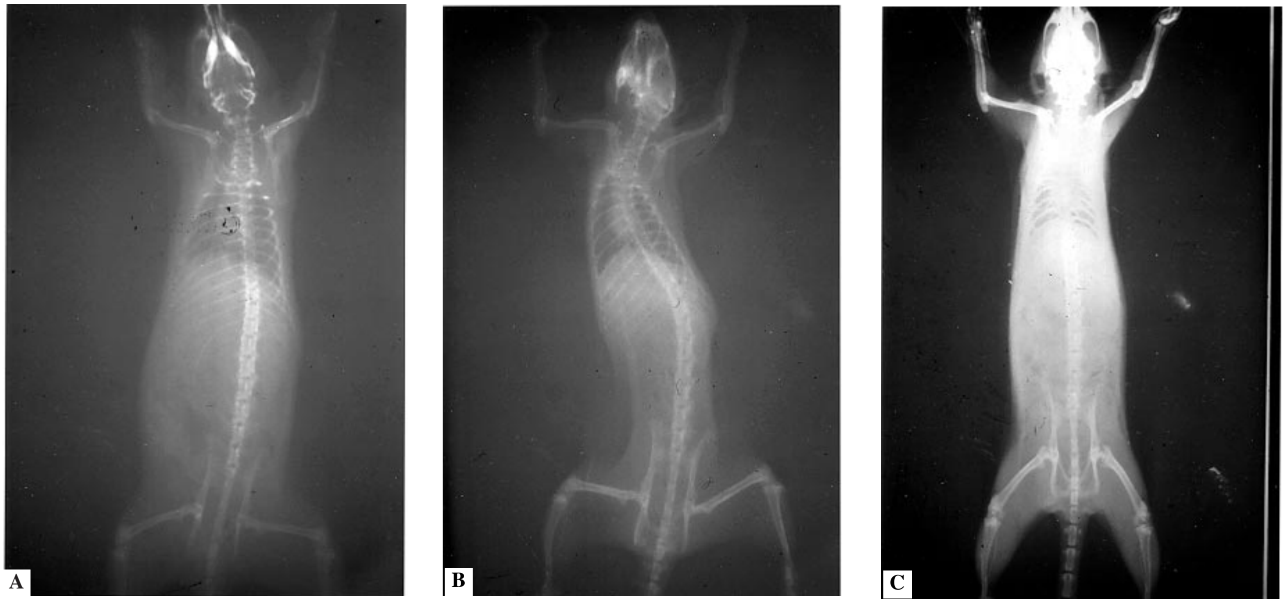

Figure 10 (**A**-**C**)
Radiograph of one rat in the study group at T0 (**A**) before application of the composite; at T1 (**B**) one week after application of the composite; and at T2 (**C**) one week after composite application on the balancing side. Note the convexity of curvature at T1, especially in the thoracic area.

Finally, no significant changes in the alignment of the spinal column were observed in the study group from T0 to T2, but significant changes in T6 vertebra and T10 vertebra were observed from T1 to T2 (respectively, $p<0.05$ and $p<0.02$) (**Table 1**). At T1, rats in the study group showed a significantly different alignment of all vertebrae when compared with rats in the control group (**Table 1**). The observation of radiographs revealed that no significant change in the alignment of the spinal column occurred in any of the rats on the lateral plane (**Table 2**).

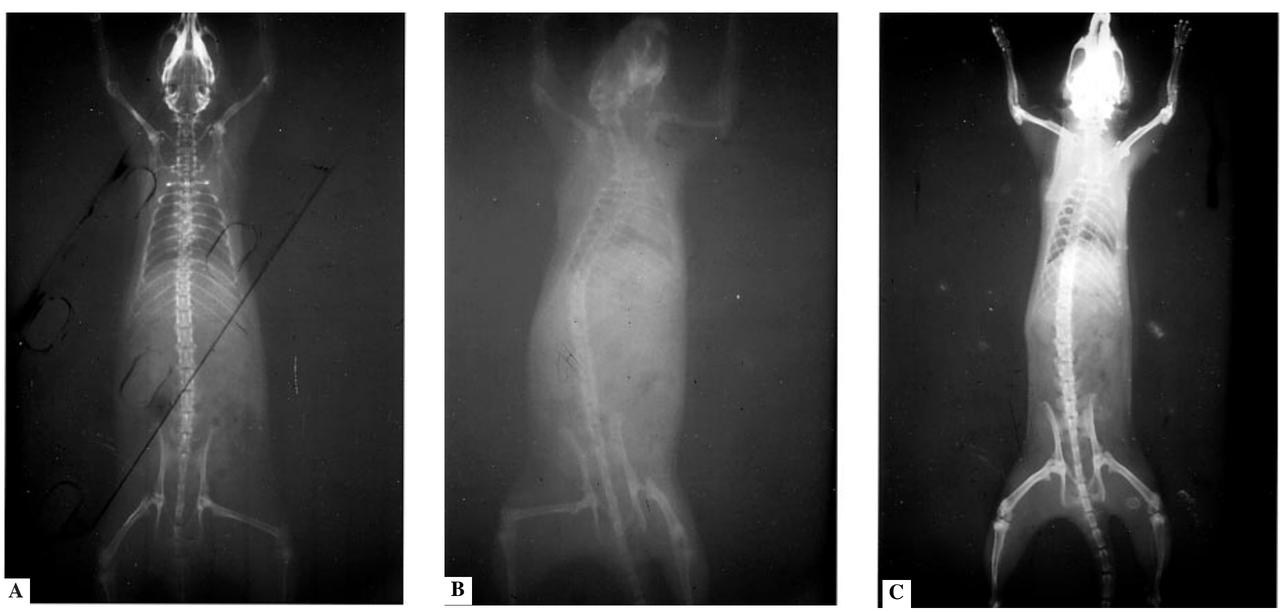

Figure 11 (**A**-**C**)
Radiograph of one rat in the study group at T0 (**A**) before application of the composite; at T1 (**B**) one week after application of the composite; and at T2 (**C**) one week after composite application on the balancing side. Note the convexity of curvature at T1, especially in the thoracic area. This rat deviated to the opposite side from the rat in **Figure 10**.

Table 1

Spinal Column Misalignment (Mean and Standard Deviation Calculated as Distance Between the Center of Vertebral Bodies and the Vertical Line) in the Both the Study and the Control Groups

	T0			T1			T2			Difference	
Vertebra	Test	Difference	Control	Test	Difference	Control	Test	Difference	Control	Test	Control
C4	2.90±3.96	NS	3.20±3.99	-0.53±4.56	p<0.05	3.10±3.85	1.30±1.30	NS	2.87±3.96	NS	NS
T1	2.60±4.51	NS	2.90±2.98	-1.63±5.37	p<0.01	2.83±3.80	1.37±1.37	NS	2.90±2.87	NS	NS
T6	1.40±5.08*	NS	1.73±4.77	-4.87±5.45‡	p<0.01	1.93±4.11	1.17±1.17	NS	1.87±4.84	p<0.05	NS
T10	0.67±4.94†	NS	1.30±4.95	-6.07±10.35§	p<0.05	1.53±5.13	2.60±2.60	NS	1.30±5.03	p<0.01	NS
L4	0.57±3.36	NS	1.80±6.55	-4.10±7.05	p<0.05	0.67±2.70	-0.23±4.10	NS	0.67±3.70	NS	NS

*Significantly different from T1 at p<0.01 level
†Significantly different from T1 at p<0.05 level
‡Significantly different from T2 at p<0.05 level
§Significantly different from T2 at p<0.02 level

Discussion

In the current study, the change in the occlusion of the rats was brought about by using an occlusal bite pad made of composite that was less than 0.5 mm in height. In order to assure that the occlusal pad was made in the same shape and size for the whole study sample, a quantitative method was followed. The same hollow plastic cylinder of 0.5 mm in height was used to measure the amount of composite to ensure the same amount was used for all rats (**Figure l**). The same operator then spread the composite resin over the whole occlusal surface of the molar tooth for all the rats. Finally, particular care was taken to make the second occlusal pad the same height as the previous pad in the same animal, since this important procedure allowed the restoration of normal occlusion. The method employed for taking radiographs was mainly conceived to assure the standardization and reproducibility of the radiographic procedure. In fact, the procedure described in the *Materials and Methods* (points 1 and 2) section allowed the researcher to employ the same positioning procedure for all the rats. The procedure described in point 3 allowed for the exact repositioning of the rat in the metal box as it was positioned for the first radiograph. The aim of the positioning procedure was to show the spinal curvature without error. The extremely flexible nature of the rat's spine and the normal, extensive thoracolumbar kyphosis necessitated very careful positioning of each rat, because false-lateral curves could have easily been generated, especially if the normal thoracolumbar kyphosis was misaligned. We realized that any deviation of the kyphosis from the sagittal plane could easily be misinterpreted as a long lateral curve in the radiographs. Care was taken to eliminate false curves by keeping the limbs symmetrical, keeping pelvic and shoulder girdles parallel to each other, and ensuring that the kyphosis was aligned in the sagittal plane as much as possible. Since the evaluation of method error showed no significant differences between a set of two radiographs taken twice using this procedure and confirmed the validity of the method, the authors strongly recommend the use of this procedure in future investigations of this type.

On the total body radiographs, the alignment of the spinal column was defined as the distance (mm) between the centers of C4, T1, T6, T10, and L4 vertebrae marked on the radiographs and true vertical—a line traced on the radiographs parallel to the lateral margins and intersecting the center of the pelvis (**Figure 9**). The greater the distance, the more inclined that segment of the spinal column was considered.

This method is unusual for the evaluation of scoliosis angle, since Cobb's angle is more frequently employed.

Table 2
Spinal Column Curvature (Mean and Standard Deviation) Calculated as the Downward Opening Angle on Lateral Radiographs in Both the Study and the Control Groups

	T0			T1			T2		
	Mean	SD	T0vs.T1	Mean	SD	T1vs.T2	Mean	SD	T0vs.T2
Control group	6.67	4.68	NS	6.53	4.36	NS	6.60	4.60	NS
Study group	6.73	4.25	NS	5.40	2.99	NS	8.00	4.09	NS

Cobb's angle is defined as the upper angle between the perpendicular line to the tangent line to the superior contour line of the first upper vertebra included in the pathological curvature and the perpendicular line to the tangent line to the inferior contour line of the first lower vertebra included in the pathological curvature. The choice of variables was made by the authors, because the variables employed in our study appear to better show the level of regional balance, instability, and alignment of the spinal segments. Cobb's angle, however, better clarifies the shape of the whole scoliotic curvature. In order to evaluate the level of regional changes rather than the degrees of the whole curvature, the authors preferred to employ this particular method. Among all the vertebrae, C4, T1, T6, T10, and L4 were selected as indicators of spinal column alignment; firstly, because they represent all the segments in the spinal column of the rats (except for the caudal spine segment); and secondly, because the distances between C4 and T1, T1 and T6, T6 and T10, T10 and L4 may be nearly considered the same. Rats, in fact, normally have 30 vertebrae: seven cervical, twelve thoracic, seven lumbosacral and four caudal spine vertebrae.

In the current study, the radiographs at T1 were taken one week after the positioning of the first occlusal pad, and those at T2 one week after the second occlusal pad was applied. The choice of a week as the period for wearing the unilateral occlusal pad and, then, the second occlusal pad, was made by considering the average lifespan of a man at 70 years and of a rat at three years and the average time necessary in humans for the appearance of the first symptoms of temporomandibular joint pathology after an alteration of occlusion, which was calculated (Dr. Farrar to Dr. D'Attilio in a personal communication, 1984) to be six months.

Expressing the month and years in days and laying out the following proportion:

25550 days: 180 days = 1085 days : x days,
the result of x=7.64 days is obtained.

After a week from the application of the second occlusal bite pad, all the rats in the study as well as in the control group underwent another radiograph to evaluate the effects induced on the spinal column by rehabilitation of the occlusal plane.

There are a number of theories on the etiology and pathogenesis of idiopathic scoliosis. These theories include genetic,[23] musculoskeletal,[24-25] metabolic, and chemical factors,[26] as well as abnormalities of the central nervous system.[27] Based upon these theories, many investigators have sought to produce scoliotic curves in several species using a variety of techniques.[1-15]

The assumption on which this study was based is that there is an anatomical and functional relationship between the stomatognathic apparatus and the spinal column. This relationship is hypothesized by several authors based upon various observations[16,28-31]:

1. Neurophysiological principles of convergence and sensitization: A constant input, such as a nociceptive input, on second-order neurons may increase the sensitivity of these neurons. Then, non-nociceptive neural impulses from other areas within the same segment, which converge onto these neurons, may give rise to altered sensations from these areas. For the craniocervical region, for example, a constant nociceptive input from, the upper part of the trapezius muscle can lead to an increased sensitivity of the spinal trigeminal nucleus and, consequently, non-nociceptive stimuli from the masticatory system would then lead to painful sensations from the trigeminal region.[28] This occurs as the different input converges onto the nucleus caudal portion of the trigeminal spinal tract nucleus.[29] As a consequence, in a recent study a significantly higher prevalence of cervical spinal pain was observed in a group of patients with craniomandibular pain than in a matched control group without craniomandibular pain. The prevalence of cervical spine pain was higher in patients with craniomandibular arthrogenous pain (64%) than in patients with craniomandibular myogenous pain (58%) or with both myogenous and arthrogenous pain (53%);[16]

2. Anatomical details: There is an anatomical relationship between the mandible and the cervical column, since the cranium and mandible have muscular and ligament attachments to the cervical area. The function of the head, neck, and jaws is closely interrelated, forming a combined functional system.[28] Festa, et al.[30] observed a significant correlation between mandibular length and cervical lordosis angle on lateral skull radiographs (in natural head position) in Caucasian adult women with a skeletal class II malocclusion. The longer the mandibular body was, the straighter the cervical column appeared to be.[30] In a group of 50 Caucasian adult women with internal derangement, compared with a control group of 50 Caucasian women without internal derangement, cephalometric tracings on lateral skull radiographs in natural head position showed a significantly lower cervical lordosis angle ($p<0.05$).[31]

Based on these observations, which underline an anatomical and a neurophysiological interrelationship between the spinal column and the stomatognathic apparatus, the purpose of this study was to evaluate any changes occurring in the positioning of the spinal column on the frontal plane in a group of rats, both when the occlusion was experimentally altered and following rehabilitation of the condition. Many transversal studies reported a history or the presence of cross-bite in young patients with idiopathic scoliosis.[17,20] In the current study, the alteration to the occlusion was performed in such a way as to induce the rat into a cross-bite occlusal relationship.

The most important findings of this study were that the functional cross-bite induced by a unilateral occlusal pad, caused a significant change in the alignment of the T6 vertebra ($p<0.01$, from T0 to T1) and T10 vertebra ($p<0.05$, from T0 to T1) in the group of treated rats and, what is more important, this change disappeared at T2, when the second occlusal pad was applied to the other side. In fact, no significant difference was observed between T0 and T2. Between group analysis revealed significant differences in the alignment of all the vertebrae considered at T1 (**Table 1**). This evidence leads to the postulate that an occlusal functional cross-bite might contribute to experimental scoliosis in rats. Interestingly, scoliosis curvature observed in the rats of the study group involved a long thoracic curve, and the spinal deformities were similar to scoliosis observed after lesions induced in the hypothalamus, produced by Yamada, et al.[32] and to scoliosis induced in a group of bipedal rats using pinealectomy by Machida, et al.[14]

However, the associations between the occlusal features and lesions in the hypothalamus or melatonin deficiency secondary to pinealectomy are unknown. The occurrence of scoliosis in our sample was probably associated with the fact that a normal spine requires a precise and delicate mechanical balance of equilibrium and postural tone. Disturbances in the primary structures, support structures, position of the spine, and related neural or muscular components could possibly result in development of scoliosis. The result obtained may be considered a movement of adaptation that occurs in relationship to the constraints imposed by morphology and degrees of freedom of each joint, as well as the elasticity and architecture of the surrounding soft tissue. These adaptations may be specific to the constraints imposed by muscle tissue in reference to a new occlusal equilibrium and, in consequence, affect the overall geometry of the spinal column. These adaptations may reflect a strategy used by the neuromuscular system to move the center of gravity over the sacral base and within the base of support, providing a horizontal vestibular and visual frame of reference. At the time of the initial evaluation, the rats had no discrepancy in their occlusion, but after the cross-bite was induced, they all showed a curvature of the spine.

We noted that the convexity of the observed curve was directed to either side, with no consistent preference, suggesting that the side of the curve cannot be predicted, or more probably, that the sample in this investigation was too small to study the probability of spinal inclination to one side or the other. Further investigations, using larger samples, could possibly clarify the real mechanism of these findings and forecast the direction of spinal column inclination.

The mechanism may be related to the consequential tilt of the first cervical vertebra (Cl) that affects the tilt of adjacent vertebra, destabilizing the vertical alignment of the spine, changing the functionality of each muscle, and in the end, an asymmetrical distribution could then affect the orientation of the vertebrae, contributing to the functional deformity of the spine.[21-22]

Although from our observations it could be assumed that the current study mechanism induced vertebral misalignment, our sample was possibly too small to predict the side of vertebral inclination.

The relationship between the cervical part of the spinal column and the stomatognathic apparatus has been previously shown by several authors. For example, in a cephalometric study of adult Caucasian females in skeletal class II, Festa, et al.[30] showed a significant correlation between mandibular length and cervical lordosis angle. D'Attilio, et al.[31] also showed a significantly lower cervical lordosis angle in patients with internal derangement when compared to a control group of patients without internal derangement. Moya, et al.[33] investigated the effect of an occlusal stabilization splint on craniocervical relationships in 15 patients with muscle spasms in the sternocleidomastoid and trapezius muscles. Their patients underwent two lateral skull radiographs, with and without a splint inserted in the mouth. They found that soon after the insertion of the splint, the device caused a significant extension of the head on the cervical spine and a significant decrease in cervical spine lordosis. Hellsing, et al.[34] studied 125 children, aged 8, 11, and 15 years, for the development of cervical lordosis (measured on lateral skull radiographs), thoracic kyphosis, and lumbar lordosis (measured using a kyphometer) and showed a highly significant correlation between the thoracic and lumbar curvatures and a negative correlation between the inclination of the lower part of the cervical spine (from the fourth to the sixth vertebra) to a true vertical and the thoracic curvature.

In a follow-up investigation, Hellsing, et al.[35] showed an association between thoracic kyphosis and some craniofacial morphological variables. The curvature of the thoracic spine increased with facial prognathism and the anteroposterior dimension of the mandible. This may suggest that the curvature of the thoracic spine has a compensatory mechanism in maintaining body balance. Although these results concerned humans and may be not appropriate for describing postural adaptations in rats, they might suggest a close relationship between the stomatognathic apparatus and the spinal column which could be valid for all vertebrate animals. Based on the findings, the scoliotic curvature observed in our sample was probably related to the consequential tilt of the first cervical vertebra (C1) which affects the tilt of adjacent vertebra, destabilizing the vertical alignment of the spine.

Limitations of the Study

This study must be considered a pilot investigation because of the small sample, and because the results did not make clear any long-term effects of the occlusal pad on spinal alignment, since the period for wearing the first or the second occlusal pad was only one week. No conclusions about the possible application of the results to a human model were possible, since the results of the current study were based upon a quadruped model (rat) which could influence the development of particular mechanisms of adaptation. Further research in this area will clarify the relationship between occlusion and the functional aspects of body posture and the spinal curvature in bipedal vertebrate animals and in humans.

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