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The effect of dental occlusal disturbances on the curvature of the vertebral spine in rats

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Abstract

Aim: To determine how either a unilateral increase or decrease of the bite affects the curvatures of the vertebral spine. Also, to determine associations between the side of the bite alteration and the side of the displacement at the vertebral spine.

Introduction: There is still controversy if alterations on the bite could affect the curvatures of the vertebral spine.

Methods: 25 male mature Sprague Dawley rats were included in the study. Five animals in the control group received no alteration on their bite. Bite was increased on five animals on the right upper molars and five had that on the left molars. Molar teeth were extracted on the right side on other five animals and other five had that on the left side. Radiographs were taken on days 0, 7, 14 and 21. Distances from C4, T1, T6, T10 and L4 to a true vertical line were recorded on frontal radiographs and, from C4, T6 and L3 on lateral radiographs. Measurements were contrasted with repeated measures analysis. Significance was determined at 95 per-cent level of confidence.

Results: The repeated measured analysis revealed statistically significant differences between the amount of the curvature at C4, T1 and T6 over time on the frontal radiographs ($p<0.05$), as well as at C4, T6 and L3 on the lateral radiographs ($p<0.0001$).

Conclusion: Alterations in the dental occlusion affects the normal curvatures of the vertebral spine in rats. No associations were found between the side of alteration and the side of displacement of the vertebral spine.

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Introduction

An increase in the physiological curvature at the cervical region of the vertebral spine has been associated with alterations of the dental occlusion. (1-5) Nevertheless, a direct causation from disturbances in the dental occlusion on the alteration of the curvatures at the vertebral spine has not been established. (6)

Although there are studies reporting that an alteration or pathology of the temporomandibular joint (TMJ) mechanics would lead to perturbations of the normal force acting on the head and neck, (7) only one animal study in the literature has reported that dental occlusal disturbances directly affect the curvature of the vertebral spine. (8) Therefore, more investigation is required to determine if alterations in the dental occlusion can directly have an effect on the curvatures of the vertebral spine, as well as to understand the pathophysiology of such a phenomenon.

In order to understand the dynamics of the vertebral spine and its associations with the head and the dental occlusion, it is important to understand the anatomy, physiology and dynamics of the vertebral spine in humans. Also, it is necessary to appraise the various animal models which have been validated to investigate pathological conditions in the vertebral spine. So, the following sections refer to the normal anatomy of the vertebral spine in humans and its dynamics, as well as its associations with the head and dental occlusion. The current knowledge on the associations between the dental occlusion and the curvature of the vertebral spine is also assessed.

The anatomy and physiology of the vertebral spine in humans

In the vertebrate species, the vertebral spine is the support of the head, as well as the container of the neural tissue innervating and sensing the body. The support of the head is provided by the cranio-vertebral joints at the superior end of the vertebral spine. In humans, the vertebral spine is divided by regions: the cervical region composed of seven vertebrae; the thoracic region with twelve vertebrae; the lumbar region with five vertebrae and the sacral and coccygeal regions with five and four vertebrae respectively. The vertebrae forming those two last regions are fused forming the sacrum and coccyx.

Each region presents a physiological curvature (Figure 1), which increases under pathological conditions.

The vertebrae at each region of the vertebral spine present some particular differences. In general, a vertebra is composed by the body at the anterior portion and the vertebral arch located at the posterior part of the vertebrae. They produce a space in the internal part of the vertebrae, where the spinal cord's tissues run. The vertebral arch is where the inter-vertebrae joints are and it is composed by seven processes: the spinous process is located and pointing posteriorly; two transverse processes are located at the posterior sides of the vertebrae; and, four articular processes are positioned vertically, with two at the superior part and the other two at the inferior part. Thus, the two inferior articular processes join the two superior articular processes of the following vertebra, forming the inter-vertebral joint. (9)

At the superior end of the vertebral spine there are some variations in the first two vertebrae's joints. The superior articular processes of the first cervical vertebra, known as atlas, articulate with the occipital condyles forming the atlanto-occipital joint. The second vertebra, known as axis, has an odontoid process which enters into the atlas forming the atlanto-axial joint. (9) Those cranio-vertebral joints play an important role, as they facilitate the translation and rotation of the head.

Physiological curvatures of the vertebral spine

The vertebral spine in humans presents some physiological curvatures. Although in a horizontal view it is normally aligned, sagittally it presents some slight curvatures from the top to the bottom (Figure 1). Thus, on the cervical portion and at the lumbar region an anterior slight curvature, named lordosis, is present. In that context, those regions are characterized by a convexity on the anterior aspect and a concavity at the posterior aspect. Conversely, on the thoracic region there normally is a posterior slight curvature, named kyphosis. A normal curvature at the vertebral spine is between 20 to 40 degrees. (10) When those curvatures exceed forty degrees they are considered pathological being known as hyper-lordosis (Figure 2) and hunchback respectively.

On the other hand, a lateral deviation of the vertebral spine on the coronal plane may occur as a pathological entity and it is known as scoliosis (Figure 3). All those deviations from the physiological curvatures of the vertebral spine may be the results of compensatory adaptations. (11, 12)

The co-activation pattern in humans

Human bipedal posture requires a co-activation pattern at different regions of the musculo-skeletal system, maintaining a well-balanced activity between the muscles of the neck, trunk and extremities. Any disturbance of that equilibrium means one group of muscles may be overwhelming or more active than the others. It may be interpreted that such imbalances are only involving the muscles, but that is not the case. The muscles are driven by the nervous system, which at the end is the one responsible for the co-activation pattern. (13) If there is a dysfunction at any level, the co-activation of a group of muscles is altered together with the joints and the soft tissues, enhancing and perpetuating the dysfunction. (14)

Thus, the imbalance in a group of muscles can result in an unfavorable position (eg. excessive flexion or extension of the head). Such a disturbance of the co-activation pattern may be expressed as a painful lesion in the form of trigger points, (13) which are reproducible hyperirritable sites in taut bands of muscle which can express pain in a more extensive area and may or may not include the muscle containing the trigger points. (15, 16) For example, an excessive extension of the head creates an imbalance, which wrongly co-activates the sternocleidomastoid and short extensor muscles in the neck, restricting the movement at the atlanto-occipital joint, and then, expressed as a trigger point in the trapezius muscle. (13)

Control of the vertebral spine’s orientation

The vertebral spine is an unstable structure and is supported by the muscles, fascia and ligaments attached to it. Simultaneously, those tissues supporting the vertebral spine are governed by the Central Nervous System (CNS). The CNS has to continuously interpret the internal and external force and loads delivered on the vertebral spine and rapidly produce the required adjustments to overcome those challenges. (17, 18) That process involves processing the information received from the peripheral mechanoreceptors, the vestibular and visual systems; and then, contrasting that input against the internal body dynamics in order to produce a coordinated response at the muscles attached to the vertebral spine. In that context, the CNS has to control and adjust the vertebral spine's orientation and the translation and rotation of the vertebrae. (19)

Today, it is known that the physiological alignment of the vertebral spine is maintained by the muscles of the neck, trunk and abdomen. Under the control of the CNS, these muscles adjust their activity based on the feedback from the environment and the body dynamics. (17) For example, an increase in the muscular activity of the transverse abdominus, obliquus internus and obliquus externus muscles at the abdominal region is counteracted by the co-contraction of the antagonist muscles to offset unwanted mechanical side effects on the supporting structures. (20) Thus, discrete combinations of a muscle's activity by the CNS determine the position of the structures composing the vertebral spine at the three spatial planes, (21) determining the physiological or pathological curvature of the vertebral spine. However, further investigation is required to identify potential challenges from the environment, such as mandibular posture and/or dental occlusion, which could produce an adaptive response in the CNS and modify the muscles' activities at the head, neck, trunk or abdomen, which may alter the physiological curvature of the vertebral spine.

Dynamics of the head

Through the cranio-vertebral joints described previously, the head can be displaced in several directions. It can be displaced forward (flexion), backward (extension) and laterally, as well as rotate. The combination of all those movements is known as circumduction. Flexion and extension of the head are shown in Figure 4.

The atlanto-occipital joint allows flexion and extension, whereas the atlanto-axial joint allows the rotation of the head. Flexion is produced by the longus capitis, rectus capitis anterior, anterior fibers of the sternocleidomastoid and the supra and infrahyoid muscles. Extension is produced by the rectus capitis posterior, obliquus capitis superior, splenius capitis, longissimus capitis and the trapezius muscles. Lateral flexion is produced by the unilateral action of the splenius capitis, obliquus capitis superior, rectus capitis lateralis, longissimus capitis and sternocleidomastoid muscles. Finally, rotation is produced by the ipsilateral action of the obliquus capitis inferior, rectus capitis posterior, longissimus capitis and splenius capitis muscles, with the contralateral action of the sternocleidomastoid and semispinalis capitis muscles. (22) All these muscles are innervated by the suboccipital, greater occipital, lesser occipital nerves, as well as the posterior rami of the C3-C7 nerves.

The movements of the head described above are produced by the mentioned muscles, but limited by the ligaments. At the atlanto-occipital joints, two ligaments are found: the anterior atlanto-occipital connecting the anterior arch of the atlas to the anterior margin of the foramen magnum; and, the posterior atlanto-occipital connecting the posterior arch of the atlas to the posterior margin of the foramen magnum. At the atlanto- axial joint, there are four ligaments controlling that articulation: the apical, running from the apex of the odontoid process to the anterior margin of the foramen magnum; the alar, joining the odontoid process to the medial sides of the occipital condyles; the membrane tectoria, which attaches at the occipital bone and covers the posterior surface of the odontoid process and the other three ligaments; and, the cruciate, which consists of two portions: the transverse part attached to the lateral mass of the atlas on each side binding the odontoid process to the anterior part of the atlas; and, the vertical part, running from the posterior surface of the body of the axis to the anterior margin of the foramen magnum. (9) Thus, from reviewing the anatomy of the vertebral spine it is evident that there is a close functional relationship between the neck, the cranium and the mandible, which suggests that any variation in the dynamics of either one of those structures may affect the dynamics of the others. (23)

Associations between craniofacial structures and vertebral spine

Some associations between the position of the head and the mandible with the curvature and morphology of the vertebral spine have been extensively reported in the literature. (1-5) Also, a correlation between the morphology of the vertebral spine with the morphology of the craniofacial structures has been established. (24, 25) This section refers to the up-to-date knowledge in that field and some studies reporting on associations between temporomandibular joint disturbances (TMDs) and body posture.

The position of the head atop the flexible neck has been described as an inverted pendulum model. (26, 27) In that context, the head has an intrinsically unstable point of equilibrium achieved by the application of restoring forces and torques. (7) Among the factors determining the balance of the head atop the cervical spine is eye dominance. (28) The visual and sensory-motor systems are able to effect changes in the determination of the horizontal relationship of the jaws, (29) as well as to be associated with significant changes in the electrical activity of some head and neck muscles. (30)

Another factor associated with the stability of the head on the cervical spine is the position of the mandible. There are reports demonstrating that changes in mandibular position affect body posture, (31) and modifications in the contraction of masticatory muscles may affect the whole body. (32) That latter statement is further supported by studies showing that surgical variation of the position of the mandible affects the structures of the neck. Marsan and colleagues reported that mandibular setback in patients with Class III malocclusions produces a posterior and inferior movement of the hyoid bone and a significant flexion of the neck. (33) Although head and body positions have little effect on two- and three dimensional (3D) airway dimensions in patients with jaw protrusion, (34) mandibular advancement itself increases the volume of the oropharynx, (35) and reduces upper airway airflow resistance. (36) Such effect on the structures of the neck produced by changes in the mandibular posture can result from changes in the function of the supra- and infra-hyoid muscles, (37) as well as from co-activation of the sternocleidomastoid muscles by changes in the masticatory muscles. (38)

Together with the posture of the mandible, the craniofacial morphology and the state of the dental occlusion may affect the physiological curvatures of the vertebral spine, and consequently, the position of the head atop the flexible neck. An association between the skeletal facial patterns and the cervical vertebral morphology has been found. (24, 25) A study reported that children with skeletal Class III malocclusion showed a significantly lower cervical lordosis than children with skeletal Class I, whereas children with skeletal Class II malocclusion show higher extension of the head upon the vertebral spine, suggesting that the posture of the neck seems to be strongly associated with the sagittal structure of the face. (39) Another study reported that children with open bite, a vertical alteration of the craniofacial structure associated with oral breathing, show greater extension of the head related to the cervical spine and reduced cervical lordosis when compared with physiological breathing subjects. (40) Thus, compensatory muscle function caused by extension or flexion of the head may play an important role in determining craniofacial morphology. (41)

Regarding malocclusions, a high correlation between abnormal positions of the craniocervical region and the malposition of teeth have also been found. (42, 43) Extended head posture is associated to lower dental arch crowding. (44) That suggests that an imbalance of the occlusal support could modify the muscular activity of some muscles in the neck, such as the sternocleidomastoid. (45) Furthermore, a lateral inclination of the occlusal plane could create an imbalance between the right and left masticatory muscles, which act antagonistically on the displacement of the cervical spine. (46)

Finally, an association of head and body posture with temporomandibular disorders (TMDs) has also been reported. (47, 48) That was initially observed in an animal study where chronic alteration of the mandibular posture in a vertical direction produced progressive remodeling of the mandibular condyle in young adult monkeys. (49) More recently, a study in humans observed posterior rotations of the pelvis and reduction in the physiological thoracic curvature in patients with TMDs. (48) Also, an improvement in the condyle-fossa relationship was correlated to a decrease in the forward head posture. (47) In that context, an alteration or pathology of the temporomandibular mechanics would lead to perturbations of the normal force acting on the head and neck, (7) and poor posture causes muscle imbalance which may act as a developmental factor for TMDs, among others. (50)

Therefore, it appears that alterations in the craniofacial structures, the temporo-mandibular joint or the dental occlusion may produce changes in the position of the head atop the cervical spine, and so, affecting the physiological curvatures of the whole vertebral spine. On the other hand, there are reports supporting the idea that the physiological curvatures of the vertebral spine alter due to molecular/genetic alterations. (51-53) The following section reports on the current knowledge on environmental and genetic influences on the vertebral spine's curvatures.

Pathological curvatures of the vertebral spine and their associations

Dental occlusion

Idiopathic scoliosis, a deformation of the vertebral spine, is defined as a spinal curve or curves on the horizontal plane of more than 10 degrees. (54) That spinal deformation has no known etiology. In humans, it may present at an early age, being more noticeable during the adolescence. (55) Idiopathic scoliosis has a higher prevalence in men when it presents during infancy, whereas it is more common in woman when appears during the adolescence. (55) A multifactorial cause has been reported in the literature. Factors such as genetics, congenital defects, faulty segmentation of the vertebrae, environmental influences and muscular imbalance have been associated with it. (1, 54, 56-58)

The effect of the environment on the appearance of scoliosis has been observed by means of the Milwaukee brace therapy. The Milwaukee brace is a device designed to treat scoliosis in young patients and it has been reported to negatively impact craniofacial growth and development by reducing the vertical dimension in the mouth. (59) For that reason today is recommend using it concomitantly with a dental removable splint to avoid that effect on the dental vertical dimension. (60)

More recently particular attention has focussed on the potential association between occlusal disturbances on the teeth and the curvatures of the vertebral spine. Reports from the last century presented clinical cases where a relationship between dental occlusion and scoliosis was observed because increased kyphosis and postural defects. (1, 2) During the last decade, more studies on this association have been reported in the literature. In a cohort study of 98 subjects with scoliosis and 705 controls, a higher prevalence of unilateral Angle's Class II malocclusion was evident among patients with scoliosis compared with the control group (21.9% vs. 8.5%). It was mainly associated with a deviation of the midline both in the upper and lower dental arches. (5) Similar observations were reported by Lippold and Korbamacher in their studies. (3, 4) In a more recent study, 213 children aged 7 to 15 years old with a Angle's Class II malocclusion and horizontal maxillary overjet were evaluated to determine the association between the skeletal and dental occlusion with the curvature of the spine. Twenty-eight per cent of children with skeletal malocclusions presented deviations in the cervical vertebral column, whereas 17% in the group with tooth crowding with non-skeletal malocclusion showed deviations. The authors concluded that there are associations between cervical column morphology, craniofacial morphology, and head posture in children with horizontal maxillary overjet. (61)

The current scientific literature reports several malocclusions that could be associated with deformities or increased curvatures on the vertebral spine. Among them, unilateral Class II malocclusion, increased overjet, decreased overbite and unilateral crossbite are the most common ones associated with scoliosis, increased lordosis of the cervical spine and reduced kyphosis of the thoracic spine. (3-5, 62, 63) An animal study reported that unilaterally raising the bite in rats causes a deviation of the vertebral spine, mainly at the thoracic region, after one week. (8) The same study showed that the vertebral spine tends to align back when the occlusal plane is even. Nevertheless, that study did not report if there was an association between the side of bite increase and the direction the vertebral spine deviates towards. In that context, that animal study supports the idea of discrepancies in the height of the occlusal plane between the right and the left side affecting the alignment of the vertebral spine. That is further supported by case reports in the literature saying that the position of the mandible seems to affect the body posture, (62) and that occlusal interferences causes functional abnormalities in the upper cervical spine and the sacroiliac joint. (64) Other studies also stated that the posture of the neck seems to be strongly associated with the sagittal and vertical structure of the face, (39) and the vertical relationship between the jaws has been associated with cervical spine body fusion and cervical spine deviations. (65, 66) However, there is no enough evidence to support a correlation between dental occlusion disturbances and the spinal curvature. (6)

As mentioned above, the animal study by D' Attilio (8) showed that there may be a direct causation from the dental occlusion status affecting the curvatures of the vertebral spine. Concomitantly, a mathematical three-dimensional finite elements study reported that a lateral inclination of the dental occlusal plane produces a displacement of the cervical spine, and therefore, mandibular lateral displacement may play a compensatory role in posture control.(67) However, those studies did not produce data to understand why and how that effect could occur. Similarly, human studies have found an association between dental occlusion status and the curvatures of the vertebral spine. The latter are cross-sectional studies which are able to report an association, but they did not produce information to determine if progressive changes in the dental occlusion can affect the physiological curvatures of the vertebral spine, and so, be able to determine a direct causation. In that context, the current literature does not reveal potential pathways linking dental oclusal changes with modifications in the vertebral spine curvatures.

Molecular/Genetic influences

The vertebral spine derives from the mesodermal embryological tissue surrounding the neural tube. Several genes express at that stage to precisely pattern the morphology of the spine in humans. (68) A disruption in that mechanism produces segmentation defects, which may affect the skeletal structures and muscles of the vertebral spine and result in curvatures such as scoliosis, kyphosis or lordosis. (68) Although those spinal disorders have been associated with abnormal muscular function, connective and bone tissues malfunctions and neuroendocrine entities, genetics is considered an important factor when evaluating their etiology. (51)

Several studies support the involvement of genetics in the appearance of pathological curvatures in the vertebral spine. It definitively plays an important role in the formation of the lumbosacral curvature during the intra-uterine period. (69) A recent study mapped some regions on chromosomes 9 and 16, where some genes can associate with familial idiopathic scoliosis. (70) Another report involves a melatonin signaling dysfunction hypothesizing that some chemical factors act as regulators of the deviations in growth, bone formation, mineralization and/or resorption occurring at the vertebral spine when a disorder appears. (71) Therefore, genetics and molecular expressions should be considered as one of the potential factors altering the physiological curvatures in the vertebral spine. However, the major difficulty to understand the mechanisms involved in those disorders is their phenotypic and genetic heterogeneity. (53)

A connection between the TMJ, the masticatory muscles, the suprahyoid muscles and the cranio-vertebral joints have been reported in the literature, (72, 73) which affect the short extensors muscles of the neck and the sternocleidomastoid muscles, as well as the cervico-thoracic junction and the diaphragm. (73) There are also several reports showing an association between the state of dental occlusion and the horizontal alignment of the vertebral spine. (3-5) Nevertheless, there is no enough evidence to support a correlation between dental occlusion disturbances and the spinal curvature. (6) Although human studies have reported an association between dental occlusion and the curvature of the vertebral spine, only one animal study has demonstrated that compensatory adaptations may occur in the vertebral spine when the dental occlusion is modified. (8) Accordingly to that study, disturbances of dental occlusion in rats causes mainly an increase of the vertebral spine’s curvature in the horizontal plane (scoliosis) with a minimal effect on the vertebral curvatures in the sagittal plane (lordosis and kyphosis). Such an effect is further supported by clinical reports, where scoliosis has been associated with disturbances in the dental occlusion.

Further investigation is required to elucidate how and to what extent the dental occlusion may be modified to affect the biomechanics of the vertebral spine. The difficulties to perform longitudinal studies in humans point out the necessity of more animal studies to elucidate if changes in the dental occlusion directly affect the curvatures of the vertebral spine. Thus, animal studies may produce insights on the pathophysiology of such an effect and may produce information for the dental community on how alterations of the dental occlusion may affect other areas of the body, such as the vertebral spine.

The animal model

Animals are used for studies where permanent functional or structural modifications are intended to be performed in order to understand the effect of those variables on the biology of the body. However, choosing an animal model to infer results and make them applicable to humans require using a validated animal model.

Several animals have been validated as suitable models of the human vertical column. Among them, the sheep and deer resemble the human's vertebral spine morphology. (74, 75) Even more, some species such as rabbits, guinea pigs, cats and monkeys have been used as models for human's vertebral spine's kinematics. (76) Recent studies have demonstrated that the rat is a valid model to study the responses to a range of circumstances that may affect the CNS, as well as biomechanical events that may affect the vertebral spine. (77, 78) Thus, the rat was chosen as the animal model for this study based on the suitability of that animal model compared with other species and the support found in the literature validating the rat as a model when studying the vertebral spine in mammals.

The rat's vertebral spine

The vertebral spine of the rat (Figure 5) is composed by seven cervical, thirteen thoracic, six lumbar and four sacral vertebrae, with the last sacral vertebra connecting with the tail which is composed of approximately thirty vertebrae. (79) The cervical vertebral spine of the rat is oriented vertically and not horizontal or obliquely as suggested by the macroscopic appearance of the neck. (80) That vertical orientation allows horizontal rotations of the skull around the odontoid process of the axis at the atlanto-occipital joint, as it occurs in humans. (80)

Specific Aims and Hypothesis

Based on the review of literature, we hypothesize that: Occlusal disturbances affecting the vertical dimension of the dental occlusal plane increases the horizontal and sagittal curvatures of the vertebral spine. Additionally, we hypothesize that the deformation of the vertebral spine on the horizontal plane leads toward the opposite side of the dental occlusal plane disturbance.

To test the proposed hypotheses, an animal study has been designed using the rat as animal model. This study aimed to determine first, how both a unilateral increase and a unilateral decrease in the dental occlusal plane affects the normal curvature of the vertebral spine in rats; and second, to observe if there is an association between the side of the occlusal alteration and the side of the deformation in the vertebral spine.

Research Design and Methods

This study was approved by the Research and Ethics Compliance Committee at the University of Manitoba, Canada under the reference number 12-038, July 9, 2012.

Animals

Twenty-five sexually mature male Sprague Dawley rats (20 – 22 weeks old) were used in the study. They were maintained under a 12 hours light/darkness cycle and fed with regular rats' pellets and water *ad libitum*. The animals were randomly assigned to one control group and four experimental groups. Those animals in the control group received no alteration on their bite and served as controls for the others in the experimental groups. Ten animals were in the experimental group 1 (Exp-1). In that group, the dental occlusal plane was unilaterally increased by means of adding a flat horizontal 1 mm thick bite pad (dental composite) on the whole occlusal surface of the upper molars (Figure 6). Five animals received the bite pad on the right upper molars (Exp-1 R) and the other five received the bite pad on the left molars (Exp-1 L). The bite pad induced a premature contact at mouth closing increasing the dental vertical dimension.

The other ten animals composed the second experimental group (Exp-2). The dental occlusion in five animals was unilaterally affected by means of extracting the upper molars on the right side (Exp-2 R), whereas, the other five animals had extracted the molars on the left side (Exp-2 L). (Figure 7)

Surgery and alteration of the dental occlusion

Bite increase

To apply the bite pad to the animals in the Exp-1 group the following procedure was followed: the rats were anaesthetized with a mixture of Ketamine (75-100 mg/Kg) and Xylazine (5-10 mg/Kg). A prefabricated flat horizontal 1 mm thick bite pad was glued to the occlusal surface of the upper molars by means of liquid composite. As explained above, the occlusal pad was glued on the right molars in five animals, whereas the other five received it on the left upper molars. The animals wore the bite pad for 21 days.

Bite decrease

The animals in the Exp-2 group were similarly anaesthetized as described previously for the bite increase procedure. Under general anaesthesia, the upper molars were unilaterally extracted. Again, five animals had molars extracted on the right side, while on the other five animals molars were extracted at the left side.

Control group

The five animals in the control group were similarly anaesthetized as the other animals, but no procedure was performed on those. Sham-surgery was performed to eliminate any bias that the general anesthesia might produce on the neural system potentially affecting the vertebral spine during the recovery time after the surgical procedure.

Radiographs

At experimental day 0, immediately after anesthesia (D0), experimental day 7 (D7), experimental day 14 (D14) and at the end of the experimental period, day 21 (D21) after the initial procedure, two total body radiographs, one on the frontal plane and one on the sagittal plane, were taken. A portable x-ray machine (Image-X70 Plus, Dent-X, Elmsford, New York, USA) was used. The machine was set at 0.72 seconds of exposure with an exposure coefficient of 0.988 per-cent. A standard 4 sensitivity film was used. The x-ray machine was positioned at 1.7 meters from the collimator to the cassette holding the film. The machine's tube-head was placed parallel to the floor with the cone targeting the geometrical center of the cassette (Figure 8).

For the x-ray at D0 the animals were anaesthetized as described above for the surgical procedure. For the x-rays at D7, D14, D21, the animals were slightly anaesthetized as described above, but using the minimal dose for the anesthetics; ketamine and xylazine.

In order to perform spinal radiographs in a reproducible manner and standardized way and according to the cannons of veterinary medicine, the rats were placed on a table under muscle traction via their fore- and hind-limbs as described by D'Atillio. (8) Animals were placed on their backs with the spinal column resting on the surface for the frontal view (Figures 9 A & B), whereas they were hanging by means of muscle traction close to the table facing to the left side for the sagittal x-ray projection. For the later x-ray projection, the alignment of the four limbs was carefully maintained to the reference lines. Also, during successive radiographs the same force was applied to the limbs when holding the animal in position by means of measuring that force with a force gauge. The experimental time line is shown in the Appendix A, Figure 10.

Measurements were made on the radiographs. For that, the seven cervical, 13 thoracic and 6 lumbar vertebrae were identified on each radiograph. On an antero-posterior view, the rat's vertebral spine present a slight lordosis (anterior curvature) at the cervical and lumbar spines, whereas a kyphosis (posterior curvature) is present on the thoracic spine, as it is in all mammals (Figures 9 A & B). As in humans, no misalignment in the horizontal plane is observed in healthy rats.

Data collection

The curvature of the vertebral spine was determined following the methods proposed by D'Attilio. (8) A true vertical line (TVL) was traced on the radiographs parallel to the margin of the radiograph and intersecting at the center of the body of the fifth lumbar vertebra, which corresponds to the center of the pelvis. Then, the centers of the 4th cervical (C4), the 1st thoracic (T1), the 6th thoracic (T6), the 10th thoracic (T10), and the 4th lumbar (L4) vertebras were marked and used as reference points. So, five distances in millimeters were obtained on each frontal radiograph by measuring horizontally from the five vertebras reference point to the TVL (Figure 8A). Those distances indicated the alignment of the vertebral spine at each segment, and so, the more inclined a segment is, the higher the distance was. If the reference point deviated to the right, the distance was recorded as a positive number; otherwise it was recorded as negative value. An overlap between the vertebral reference point and the TVL was computed as zero value. TVL and the reference points for all measurements at the various vertebrae referred above were draw on acetate tracing paper.

On the lateral radiographs, a similar protocol was followed for the measurements. However, only three reference points were considered: C4, T6 and L3. The TVL was traced maintaining the same parameters expressed above, parallel to the border of the film and intersecting at the center of the fifth lumbar vertebra. Then the distances from the reference points at C4, T6 and L3 to the TVL were used to determine the curvature in a sagittal perspective of the upper (cervical), middle (thoracic) and lower (lumbar) vertebral spine curvatures. Again, a positive value was recorded when the curvature was towards frontal, while a negative value was given when the curvature towards dorsally.

In order to avoid identification of the experimental group by the operator, the radiology technician maintained identifying the animals at the three groups determined for the study. She delivered the radiographs to the researcher with the head portion on the radiograph covered with masking tape. In that way, the researcher was blinded on the experimental group the animal's radiograph belongs to. The curvature of the vertebral spine in the animals was measured as previously described on both experimental groups and the controls at all the experimental periods.

Statistics

The primary outcome for this study was the amount of increase in the spinal curvature during the experimental period. Measurements were made at eight pre-determined locations on the spine. Comparisons were made within the groups who had a unilateral increase in dental occlusion and the group who had a unilateral decrease in dental occlusion in order to determine if there was an association between the side of the alteration and the displacement of the curvature. Then, the results from both experimental groups were individually contrasted against the controls in order to determine differences in the amount of increase of the vertebral curvature during the experimental period.

Based on results from previous studies, it was anticipated a 4 mm difference in curvature between the two groups. Assuming a common standard deviation of 2 mm, it was given a 98% power to detect this difference between the groups, having 10 subjects per group (5 with the modification to the right side and 5 with the modification to the left side). The statistical package nQuery Advisor, 7.0 (Statistical Solutions, Saugus, MA, USA) was used to perform the power analysis.

Three measurements were made by the researcher on each radiograph and the median of those three measurements used as final result. To determine the reliability of the measurements, ten radiographs were measured three times each, and the results analyzed by calculating the inter-rater reliability by means of determining the Intraclass correlation.

As measurements were taken at eight locations on each subject, the final results were analyzed first with repeated measures analysis using the statistical package for Social Sciences program (SPSS, Inc. Chicago, IL) to determine differences of the mean between the two experimental groups and the controls. The stage at the experimental period when a significant difference existed between the groups was determined through the repeated measures analysis. Then, statistical differences between the groups at that stage of the experimental period were determined through one-way analysis of variance using the SPSS software.

Results

During the experimental period, at day 7, one of the animals composing the bite increase on the right side group died during the delivering of the anesthesia. Post-mortem exam revealed no systemic problem or a cause associated with the procedure. The report from the veterinary was death could be caused by a high level of stress during the anesthesia, which caused a heart attack. Therefore, that experimental group remained with four animals for the rest of the experimental period.

Animals weight

All animals recorded a weight gain during the 21-days experimental period. No Statistical significance was reached when the weight of the animals were statistically contrasted. The controls reported a weight gain higher than the others submitted to a surgical procedure (154.4 g on average). Between the experimental animals, those in the extraction group gained less weight comparing with those animals where the bite was increased (98.4 & 109.0 g vs 109.2 & 137.3 g on average). Although there was a lesser gain in those animals receiving a surgical procedure, they maintained healthy and with normal behavior during the experimental period. The recorded data of rats' weight during the experimental period in all animals is presented in the appendix C.

Reliability

All measurements were repeated by the same investigator three times with an interval of one week, between them. Inter-rater reliability was computed by determining the intraclass correlation (ICC). On the frontal radiographs, the ICC was 0.78 at C4; -0.36 at T1; 0.34 at T6; 0.63 at T10; and, 0.54 at L4. On the lateral radiographs, the ICC was 0.89 at C4; 0.87 at T6; and 0.78 at L3. The mean of the three measurements was used as final data for statistical analysis.

Experimental results

When data from D0 at each of the chosen points on the vertebral spine, for both frontal and lateral radiographs, were contrasted against those from D7, D14 and D21, the repeated measurement analysis computed some significant variations over time between the distances recorded from the TVL to the centre of the landmark vertebrae on both perspectives, frontal and sagittal. ANOVA also computed significant differences between the groups of alterations and controls when the data at a significant stage of the experimental period was contrasted, as explained below.

Horizontal plane (frontal radiographs)

The repeated measures analysis for C4 revealed no statistically significant differences between the treatment groups (p=0.2849), but did find that the amount of curvature varied over time (p=0.0192). As compared to baseline, at D14, the mean (SE) displacement was 1.60 (0.67) mm to the left (p = 0.0204).

At the thoracic spine, the repeated measures analysis for T1 revealed no statistically significant differences between the treatment groups (p=0.3081), but did find that the amount of curvature varied over time (p=0.0121). As compared to baseline, at D14, the mean (SE) displacement was 1.69 (0.63) mm to the left (p=0.0095). At T6, the repeated measures analysis revealed no statistically significant differences between the treatment groups (p=0.35170), but did find that the amount of the curvature varied over time (p=0.0060). As compared to baseline, at D14, the mean (SE) displacement was 1.41 (0.58) mm to the left (p=0.0175).

The repeated measures analysis for T10 and L4 revealed no statistically significant differences either between the treatment groups (p=0.6689 & 0.3453 respectively) or the amount of the curvature over time (p=0.0853 & 0.1385 respectively). The means and SEM for all measurements at C4, T1, T6, T10 and L4 are presented on Table 1, appendix A.

The repeated measures analysis reported that there was a statistical significant variation at C4, T1 and T6 on D14 when contrasting the measurements from the frontal radiographs.

Then, ANOVA computed a statistical significant difference between the Exp-2 group and controls at C4 (p=0.0007); between the Exp-1 group and controls, as well as between the Exp-2 group and controls at T1 (p= 0.002 and p=0.007 respectively); and, between the Exp-2 group and controls at T6 (p=0.0027).

Sagittal plane (lateral radiographs)

The repeated measures analysis for C4 revealed a statistically significant differences between the treatment groups (p=0.0207), as well as for the variation in the amount of curvature over time (p<0.0001). As compared to baseline, at D7, the mean (SE) displacement was 3.07 (0.60) mm forward (p < 0.0001)

At T6, the repeated measures analysis revealed statistically significant differences between the treatment groups (p=0.0132), as well as for the variation in the amount of curvature over time (p<0.0001). As compared to baseline, at D7, the mean (SE) displacement was 2.52 (0.39) mm forward (p<0.0001).

The repeated measures analysis for L3 revealed no statistically significant differences between the treatment groups (p=0.2177), but did find that the amount of curvature varied over time (p<0.0001). As compared to baseline, at D14 and D21, the mean (SE) displacement was 0.35 (0.16) mm backward (p=0.0296) and 0.49 (0.16) mm forward respectively.

Thus, the repeated measures analysis reported that there was a statistical significant variation between the groups over time at C4 and T6 on D7, when contrasting the measurements from the lateral radiographs. However, ANOVA did not compute a statistical significant difference between the two experimental groups and controls at that experimental period.

The mean and SEM for the measurements performed at C4, T6 and L3 are shown in Table 2. The results of the repeated measures statistical analysis for both, horizontal and sagittal perspectives are presented in appendixes A.

Discussion

The results from this study did report significant differences on the curvatures of the vertebral spine when the dental occlusion was altered. The repeated measures analysis showed that there was a significant effect on D14 at the horizontal perspective, whereas at D7 a significant effect was observed on the lateral perspective. Then, one-way ANOVA demonstrated that at T1 both, decreasing and increasing the vertical dimension, affects the thoracic curvature on the horizontal perspective, by significantly reducing the distance between the landmarks and the TVL. On the other hand, at C4 and T6 the cervical and thoracic spines were also affected, but only when the bite was decreased. At those landmarks, the distance between the landmarks and the TVL was also significantly reduced. The results also showed that on the lateral perspective, the distances at C4 and T6 significantly increased, which means that the curvature of the cervical and thoracic spines displaced forward.

The cervical spine displaced towards the right side after 14 days of the bite alteration. Similarly, the thoracic spine displaced to the same side also after 14 days of the bite alteration. However, that displacement at the thoracic spine occurred at the superior (T1) and middle (T6) portions. No deviations at the lower part of the thoracic spine (T10) or at the lumbar spine were observed. Comparing with the results reported by the other animal study, (8) they found an effect of altering the bite on the vertebral spine after 7 days of the alteration. Considering that the effect appeared later in the current study, the present results agree with the statement that there is an association between the state of the dental occlusion and the curvatures of the vertebral spine, and furthermore, they demonstrated that such an effect can be produced by both, increasing or decreasing the height of the dental occlusion, particularly at the thoracic spine. However, it looks like that effect can appear between 7 to 14 days in the rats. Even more, a decrease of the bite appears to affect more significantly the cervical spine, as it concomitantly affects the cervical, as well as the superior and middle parts of the thoracic spines.

The current results also computed a significant change in the curvatures of the vertebral spine from a lateral perspective. The results showed that the lordosis observed on the cervical portion of the vertebral spine significantly increased on those animals where the bite was increased, regardless the side of alteration, after 7 days. As mentioned previously, decreasing the bite on the right side produced a displacement of the cervical spine forward, whereas a reduction on the left side displaced the cervical spine backward.

Those effects on the reduced bite group were also observed after 7 days of the extractions. Interestingly, the thoracic spine displaced forward after 7 days in the animals where the bite was increased, whereas it displaced backward in those animals where the bite was decreased. Thus, the results from this study demonstrated that an alteration of the dental occlusion can also affect the curvatures of the vertebral spine on a sagittal perspective. In that context, the current results disagree with the previous animal study, where non effect was observed when the lateral radiographs were measured. (8) The difference could be due to the methodology used by both studies when measuring on the lateral radiographs. In that study, D' Atilio and coworkers measured the angles formed by 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. In the current study, the distance from the geometrical center of C4, T6 and L3, the vertebrae at the center of the curvature, was directly measured to the TVL. The measurement used here appears to be simpler and any deviation of that point at the center of the vertebra located at the middle of the curvature infers any displacement of that portion of the vertebral spine. Therefore, the current results support the idea that an alteration of the height of the dental occlusion can affect the curvatures of the cervical and thoracic spines on the lateral plane, as it does on the horizontal plane.

This study followed the same methodology reported in a previous animal study. (8) However, some differences were accounted. In that animal study the bite was increased in rats only on the right side. In the current study, the bite was increased in five animals on the right side, but it was increased in other five animals on the opposite side. This study went further including a decreased bite in ten animals by means of extracting the molar teeth. Therefore this study looked at a wider scope, analyzing the effect of both increased and decreased vertical dimension of the dental occlusion in order to elucidate if they could differently affect the curvature of the vertebral spine. To the knowledge of this author, the present study is the second time that an animal study with rats has been designed in order to elucidate such an association. For that reason, the results from the current study is being mainly discussed against that study by DÁtilio and coworkers. (8)

Another important point to discuss here is the fact that significant differences were computed between the measurements on both, horizontal and lateral perspectives over the time, even though the rats' body was stretched by gravity when taking the radiographs. To avoid any side deviation, the animals were hanging with a similar stretching force on each side/pawns. In that context, it could be said that any difference computed between the groups and at any of the stages of the experimental period infers a real morphological deviation of the vertebral spine in the animals.

The review of the literature showed that although there are many clinical publications proposing an association between the state of the dental occlusion and the curvatures of the vertebral spine, (1-5, 39) the above mentioned animal study was the only one reporting an association between them. (8) Nevertheless, a 3D-finite elements study suggested that an increase in the height of the dental occlusion modifies the stress distribution in the cervical spine, which could displace the vertebral spine due to the effect on the masticatory muscles. (67) Such an action on the masticatory muscles would act antagonistically displacing the cervical spine. In other words, the cervical spine would displace towards the opposite side of the bite increase when observing the vertebral spine in a horizontal perspective. (67) That study would also support an association between the status of the bite and the curvature of the cervical spine.

This study reported changes on the sagittal curvature of the cervical spine which displaced forward at C4 and T6 when the bite was altered. That could suggest that the vertebral spine compensated displacing forward whenever an alteration of the bite was produced in the animals. That phenomenon was observed when the sides of alteration were compared on the lateral radiographs, but no similar associations were observed either on the horizontal plane or at the other sites on the sagittal plane. The association reported in a previous 3D-finite elements study at the cervical spine was on the horizontal plane, in a simulated case where the bite was increased. (67) The association observed in the current study was also at the cervical spine, but on the sagittal plane only. In that context, the results from this study could not fully prove the hypothesis that an association between the side of occlusal alteration and that direction the vertebral spine displaces towards exists, and so, that hypothesis remained unresolved.

The current results agree with those from the previous animal study, stating that an increase in the height of the dental occlusion affects the physiological curvatures of the cervical and thoracic spines in rats. However, the results observed in that previous study were more dramatic than those observed here. An alteration of the dental occlusion can produce a shift of the mandible at closing, which can produce a deviation of the mandible either to the side or to the front. The differences in the results from both studies could be due to the inclination given to the pad used to increase the bite. In the previous study, D'Atilio and coworkers expressed that the alteration of the occlusion was performed in such a way to induce the rats into a cross-bite occlusal relationship. (8) So, they created an inclined plane forcing the shift of the mandible to the side. In this study, the pad cemented on the molar teeth was parallel to the floor with no inclination to the side. Thus, in the study by D'Atilio and coworkers the pad cemented on the right molars could shift the mandible towards one of the sides, and therefore, the action of the muscles and ligaments attaching from the mandible to the vertebral spine could affect the vertebral spine higher towards the side. So, their results presented a significant deviation on the vertebral spine on the frontal plane rather than on the lateral plane. Contrary, the pad cemented on the molar teeth on the current study with no inclination could produce a displacement of the mandible forward. In such a way, the muscles and ligaments attached from the mandible to the vertebral spine could pull the mandible forward resulting in a totally different situation, where the vertebral spine was affected on both directions, horizontally and sagittally. Future research should consider the inclination of the pad used to increase the bite in the animals, as it looks like it could affect the results, particularly when considering the directional effect that such increase can have on the curvature of the cervical and thoracic spines.

Another difference at the present study was that the alteration in the dental occlusion was maintained during the 21 days of the experiment, rather than stabilizing the bite by adding composite on the opposite side after 7 days, as performed by D'Atilo and coworkers. In that way, the results from the present study were able to show that the effect on the vertebral spine can appear later. Furthermore, the current study also demonstrated that after 14 days of maintaining an increase or decrease of the height of the bite, the cervical and thoracic spines tend to realign. That could be a result of adaptation. In that context, the results observed by D'Atilio and coworkers where the vertebral spine tended to realign after stabilizing the dental occlusion, appears to occur even though the bite alteration is maintained for an extended period.

Supporting the idea that could be an adaptive process occurring when the bite alteration is maintained over an extended period of time, is what happened at the lumbar spine in this study. By maintaining the bite alteration for a longer period of time, the current results permitted to observe an event not previously reported. After 14 days of changing the height of the dental occlusion, the lumbar curvature displaced forward, while the cervical and thoracic curvatures realigned. Changes in the status of the dental occlusion produce modifications in the relationship between the maxillaries, as well as in the activity of the muscles attached to the mandible. (81) Similarly, compensations and adaptations to the new situations at the occlusal dental plane could occur in animals due to changes in the mastication pattern. (82) In the rats, several muscles are attached to the mandible and the cervical spine. Thus, changes in the height of the dental occlusion, can produce changes in the activity of those muscles simultaneously attaching to the mandible and the vertebral spine, so affecting the curvatures of the vertebral spine at the cervical and thoracic portions. As the body tends to adapt to that new situation, the cervical and thoracic spines tend to realign, having an effect on the curvature of the lumbar spine, which tended to increase its lordosis as the superior portion of the vertebral spine realigns. That proposed effect on the lumbar spine observed during the course of this study in the animals is supported by some studies in humans showing that restoring the thoracic kyphosis produces changes in the lordosis of the lumbar spine later. (83) Even more, compensatory mechanisms have been reported in the vertebral spine occurring in patients with degenerative spine disorders. (84) Based on the results from this study, it can be proposed that changes in the height of the dental occlusion affect the lordosis of the cervical spine, as well as the kyphosis of the thoracic spine. If such alteration is maintained, the curvatures at the cervical and thoracic spines tend to realign, but affecting the lordosis at the lumbar spine, which occurs later.

Genetics is also involved in the appearance of pathological curvatures in the vertebral spine. (69-71) Although the rat strain used for this study was the same to that used in a previous report, the source where the animals were acquired from, as well as the timing for both studies are different. Furthermore, neither this study nor the previous one included a genetic map of the animals involved in order to determine the potential presence of genes associated with curvatures of the vertebral spine. (70) So, it could happen that genetics between the animals in both studies could be slightly different and that could also account for some of the different results observed between both studies.

The present study supports the hypothesis that the physiological curvatures of the vertebral spine are affected when the height of the dental occlusion is either increased or reduced. An explanation for that phenomenon observed in the animals in this study can be that an alteration in the bite forced the mandible to close at different levels on each side. A situation like that can modify the inputs from the mechanoreceptors at the temporomandibular joint and periodontal ligament, which affects the activity of some muscles of the neck, such as the splenius muscle, so affecting the morphology of the vertebral spine and the posture of the head in the rats. (85, 86)

Even though this is an animal study and there are limitations of performing this type of studies in humans, these results insights further scientific evidence for those case-reports suggesting an association between the state of the dental occlusion and the alignment of the vertebral spine. (1-5) Before translating these results to humans, it is important to understand the animal model used here. Rats maintain a horizontal orientation of the cervical spine permitting similar rotations of the head as it occurs in humans. (80) However, rats functions as a quadruped animal, which does not fully resemble that occurring on a bipedal vertebrate. Therefore, a different animal model such as monkeys, which function most of the time in a bipedal position, should be consider in the future when designing an animal study intending to fully understand the effect of altering the dental occlusion on the physiological curvatures of the vertebral spine.

Conclusions

The present study supports the hypothesis that occlusal disturbances affecting the height of the dental occlusion increases the horizontal and sagittal physiological curvatures of the vertebral spine. However, decreasing the bite appears to have a higher impact on the cervical and thoracic spines.

The results from this study also showed that those alterations occurring in the cervical and thoracic spine tend to reverse if the change in the dental occlusion is maintained, producing modifications in the curvature of the lumbar spine later.

This study showed that the vertebral spine displaced forward on the sagittal aspect when the bite is altered, regardless the side of alteration. In that context, the current results did not produce evidence supporting an association between the side of alteration and the side the vertebral spine displace towards on the horizontal and sagittal planes.

Further studies with bipedal animal models are suggested in order to fully understand the pathways how an alteration in the dental occlusion can affect the physiological curvatures of the vertebral spine in humans.

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Appendix A: Tables

		Vertebra C4				Vertebra T1				Vertebra T6				Vertebra T10				Vertebra L4			
	Group / Day																				
		1	7	14	21	1	7	14	21	1	7	14	21	1	7	14	21	1	7	14	21
Controls	**Mean**	**1.6**	**1.3**	**-3.8**	**-0.4**	**1.8**	**1.1**	**-3.7**	**-0.6**	**1.8**	**0.9**	**-3.3**	**-0.1**	**1.2**	**0.9**	**-2.1**	**0.2**	**-0.1**	**-0.1**	**-0.5**	**0.0**
	SE	**1.1**	**1.0**	**2.7**	**0.3**	**0.9**	**1.1**	**2.7**	**0.4**	**0.5**	**1.1**	**2.6**	**0.2**	**0.4**	**0.8**	**2.3**	**0.2**	**0.1**	**0.0**	**0.4**	**0.0**
Increase R	**Mean**	**-1.1**	**1.5**	**-2.6**	**1.3**	**-0.2**	**1.6**	**-2.3**	**1.2**	**-0.4**	**1.6**	**-2.4**	**0.9**	**-0.5**	**1.1**	**-0.9**	**0.9**	**0.0**	**0.1**	**-0.3**	**0.1**
	SE	**1.5**	**0.7**	**0.5**	**1.1**	**0.7**	**0.5**	**0.6**	**1.1**	**0.6**	**0.6**	**0.7**	**0.9**	**0.7**	**0.6**	**1.2**	**0.7**	**0.1**	**0.1**	**0.1**	**0.0**
Increase L	**Mean**	**0.5**	**1.7**	**1.9**	**0.0**	**0.3**	**1.9**	**1.7**	**-0.2**	**-0.1**	**2.2**	**1.4**	**-0.1**	**-0.1**	**1.8**	**1.2**	**-0.1**	**0.0**	**0.3**	**0.0**	**-0.1**
	SE	**0.4**	**1.2**	**0.8**	**1.0**	**0.4**	**1.2**	**0.6**	**0.9**	**0.3**	**0.9**	**0.8**	**0.6**	**0.3**	**0.9**	**0.5**	**0.5**	**0.0**	**0.3**	**0.1**	**0.0**
Decrease R	**Mean**	**1.5**	**0.7**	**0.2**	**1.1**	**1.4**	**0.7**	**0.4**	**0.7**	**0.8**	**0.7**	**0.4**	**0.4**	**0.5**	**0.5**	**0.4**	**0.2**	**0.0**	**0.0**	**0.1**	**0.1**
	SE	**0.9**	**0.7**	**0.7**	**0.4**	**0.6**	**0.7**	**0.7**	**0.4**	**0.3**	**0.7**	**0.7**	**0.4**	**0.3**	**0.6**	**0.7**	**0.2**	**0.0**	**0.1**	**0.1**	**0.1**
Decrease L	**Mean**	**0.5**	**0.5**	**-0.7**	**0.5**	**0.3**	**0.5**	**-0.9**	**0.3**	**-0.1**	**0.2**	**-1.3**	**-0.1**	**0.2**	**0.0**	**-0.8**	**0.0**	**0.0**	**0.0**	**-0.1**	**0.0**
	SE	**0.7**	**0.6**	**0.6**	**0.6**	**0.7**	**0.4**	**0.5**	**0.5**	**0.6**	**0.3**	**0.7**	**0.4**	**0.5**	**0.2**	**0.8**	**0.2**	**0.1**	**0.1**	**0.3**	**0.0**

Table 1. Mean and Standard Error of the mean computed for the measurements recorded on the frontal radiographs at the five chosen vertebrae. C4: Fourth Cervical; T1: First Thoracic; T6: Sixth Thoracic; T10: Tenth Thoracic; L4: Fourth Lumbar

		Vertebra C4				Vertebra T6				Vertebra L3			
	Group / Day												
		1	7	14	21	1	7	14	21	1	7	14	21
Controls	**Mean**	**3.1**	**8.8**	**7.3**	**6.8**	**3.7**	**7.4**	**6.6**	**5.1**	**-0.7**	**-0.8**	**-1.0**	**0.8**
	SE	**0.7**	**0.6**	**1.5**	**1.0**	**0.6**	**0.7**	**1.0**	**0.5**	**0.2**	**0.2**	**0.2**	**0.6**
Increase R	**Mean**	**7.7**	**8.9**	**7.8**	**7.1**	**6.1**	**7.3**	**5.9**	**4.5**	**-0.1**	**-0.1**	**-0.4**	**0.2**
	SE	**1.8**	**1.0**	**0.9**	**0.7**	**0.9**	**0.7**	**0.8**	**0.6**	**0.1**	**0.1**	**0.1**	**0.1**
Increase L	**Mean**	**8.8**	**12.2**	**7.8**	**6.7**	**6.0**	**9.4**	**5.4**	**5.6**	**0.1**	**-0.6**	**0.0**	**0.3**
	SE	**0.6**	**0.7**	**0.9**	**0.6**	**0.5**	**0.4**	**0.4**	**0.5**	**0.1**	**0.3**	**0.2**	**0.2**
Decrease R	**Mean**	**6.9**	**9.0**	**6.2**	**7.7**	**5.5**	**7.1**	**4.2**	**5.2**	**0.1**	**-0.2**	**-0.5**	**0.5**
	SE	**1.4**	**0.8**	**1.0**	**0.6**	**0.6**	**0.5**	**0.8**	**0.3**	**0.2**	**0.3**	**0.3**	**0.1**
Decrease L	**Mean**	**5.9**	**8.4**	**5.8**	**5.6**	**4.1**	**6.6**	**4.5**	**3.6**	**0.0**	**-0.1**	**-0.4**	**0.1**
	SE	**1.2**	**0.9**	**0.7**	**0.8**	**0.3**	**0.6**	**0.6**	**0.5**	**0.1**	**0.2**	**0.2**	**0.1**

Table 2. Mean and Standard Error of the mean computed for the measurements recorded on the lateral radiographs at the three chosen vertebrae. C4: Fourth Cervical; T6: Sixth Thoracic; L3: Third Lumbar

Solution for Fixed Effects

Effect	group	day	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept			-0.01999	0.6723	19	-0.03	0.9766
Group	decrease L		0.5541	0.7513	19	0.74	0.4698
Group	decrease R		1.2401	0.7513	19	1.65	0.1153
Group	increase L		1.3834	0.7513	19	1.84	0.0812
Group	increase R		0.1229	0.7969	19	0.15	0.8791
Group	control		0	.	.	.	.
Day		21	-0.1958	0.6760	69	-0.29	0.7729
Day		14	-1.6042	0.6759	69	-2.37	0.0204
Day		7	0.4667	0.6695	69	0.70	0.4881
Day		1	0	.	.	.	.

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
group	4	19	1.36	0.2849
day	3	69	3.53	0.0192

Table 3. Statistical results computed with the Repeated Measures Analysis for C4 on the frontal radiographs.

Solution for Fixed Effects

Effect	group	day	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept			0.1002	0.6206	19	0.16	0.8734
Group	decrease L		0.4115	0.6796	19	0.61	0.5520
Group	decrease R		1.1519	0.6796	19	1.69	0.1064
Group	increase L		1.3102	0.6796	19	1.93	0.0690
Group	increase R		0.4567	0.7209	19	0.63	0.5339
Group	control		0	.	.	.	.
Day		21	-0.5500	0.6361	69	-0.86	0.3902
Day		14	-1.6958	0.6358	69	-2.67	0.0095
Day		7	0.3542	0.6465	69	0.55	0.5856
Day		1	0	.	.	.	.

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
group	4	19	1.29	0.3081
day	3	69	3.92	0.0121

Table 4. Statistical results computed with the Repeated Measures Analysis for T1 on the frontal radiographs.

Solution for Fixed Effects

Effect	Group	day	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept			0.08349	0.5801	19	0.14	0.8871
Group	decrease L		-0.1301	0.6496	19	-0.20	0.8434
Group	decrease R		0.7422	0.6496	19	1.14	0.2674
Group	increase L		1.0104	0.6496	19	1.56	0.1363
Group	increase R		0.09591	0.6890	19	0.14	0.8908
Group	Control		0	.	.	.	.
Day		21	-0.2500	0.5821	69	-0.43	0.6689
Day		14	-1.4167	0.5820	69	-2.43	0.0175
Day		7	0.6583	0.5749	69	1.15	0.2561
Day		1	0	.	.	.	.

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
group	4	19	1.18	0.3517
day	3	69	4.51	0.0060

Table 5. Statistical results computed with the Repeated Measures Analysis for T6 on the frontal radiographs.

Solution for Fixed Effects

Effect	Group	day	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept			0.1196	0.5135	19	0.23	0.8183
Group	decrease L		-0.2120	0.5864	19	-0.36	0.7217
Group	decrease R		0.3257	0.5864	19	0.56	0.5851
Group	increase L		0.6206	0.5864	19	1.06	0.3031
Group	increase R		0.06431	0.6220	19	0.10	0.9187
Group	Control		0	.	.	.	.
Day		21	-0.07083	0.5051	69	-0.14	0.8889
Day		14	-0.7167	0.5036	69	-1.42	0.1592
Day		7	0.5542	0.4849	69	1.14	0.2571
Day		1	0	.	.	.	.

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
group	4	19	0.60	0.6689
day	3	69	2.30	0.0853

Table 6. Statistical results computed with the Repeated Measures Analysis for T10 on the frontal radiographs.

Solution for Fixed Effects

Effect	Group	day	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept			-0.1440	0.09184	19	-1.57	0.1335
Group	decrease L		0.1286	0.1034	19	1.24	0.2290
Group	decrease R		0.1826	0.1034	19	1.77	0.0935
Group	increase L		0.2061	0.1034	19	1.99	0.0609
Group	increase R		0.1171	0.1097	19	1.07	0.2990
Group	Control		0	.	.	.	.
Day		21	0.02917	0.09165	69	0.32	0.7513
Day		14	-0.1333	0.09157	69	-1.46	0.1499
Day		7	0.06667	0.08979	69	0.74	0.4603
Day		1	0	.	.	.	.

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
group	4	19	1.19	0.3453
day	3	69	1.90	0.1385

Table 7. Statistical results computed with the Repeated Measures Analysis for L4 on the frontal radiographs.

Solution for Fixed Effects

Effect	Group	day	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept			5.4587	0.6508	19	8.39	<.0001
Group	decrease L		-0.03767	0.7491	19	-0.05	0.9604
Group	decrease R		1.0396	0.7491	19	1.39	0.1812
Group	increase L		2.4114	0.7491	19	3.22	0.0045
Group	increase R		1.4312	0.7945	19	1.80	0.0875
Group	Control		0	.	.	.	.
Day		21	0.3583	0.6345	69	0.56	0.5740
Day		14	0.5500	0.6315	69	0.87	0.3868
Day		7	3.0792	0.6017	69	5.12	<.0001
Day		1	0	.	.	.	.

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
group	4	19	3.75	0.0207
day	3	69	10.92	<.0001

Table 8. Statistical results computed with the Repeated Measures Analysis for C4 on the lateral radiographs.

Solution for Fixed Effects

Effect	Group	day	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept			5.0167	0.4182	19	12.00	<.0001
Group	decrease L		-0.9779	0.4767	19	-2.05	0.0543
Group	decrease R		-0.1303	0.4767	19	-0.27	0.7876
Group	increase L		0.9396	0.4767	19	1.97	0.0635
Group	increase R		0.2604	0.5056	19	0.52	0.6124
Group	Control		0	.	.	.	.
Day		21	-0.1917	0.4120	69	-0.47	0.6432
Day		14	0.2750	0.4109	69	0.67	0.5056
Day		7	2.5250	0.3965	69	6.37	<.0001
Day		1	0	.	.	.	.

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
group	4	19	4.20	0.0132
day	3	69	19.90	<.0001

Table 9. Statistical results computed with the Repeated Measures Analysis for T6 on the lateral radiographs.

Solution for Fixed Effects							
Effect	Group	day	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept			-0.4050	0.1642	19	-2.47	0.0234
Group	decrease L		0.3372	0.1873	19	1.80	0.0877
Group	decrease R		0.4086	0.1873	19	2.18	0.0419
Group	increase L		0.3859	0.1873	19	2.06	0.0533
Group	increase R		0.3402	0.1986	19	1.71	0.1031
Group	Control		0	.	.	.	.
Day		21	0.4958	0.1618	69	3.07	0.0031
Day		14	-0.3583	0.1613	69	-2.22	0.0296
Day		7	-0.2458	0.1556	69	-1.58	0.1188
Day		1	0	.	.	.	.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
group	4	19	1.59	0.2177
day	3	69	11.48	<.0001

Table 10. Statistical results computed with the Repeated Measures Analysis for L3 on the lateral radiographs.

Appendix B: Figures

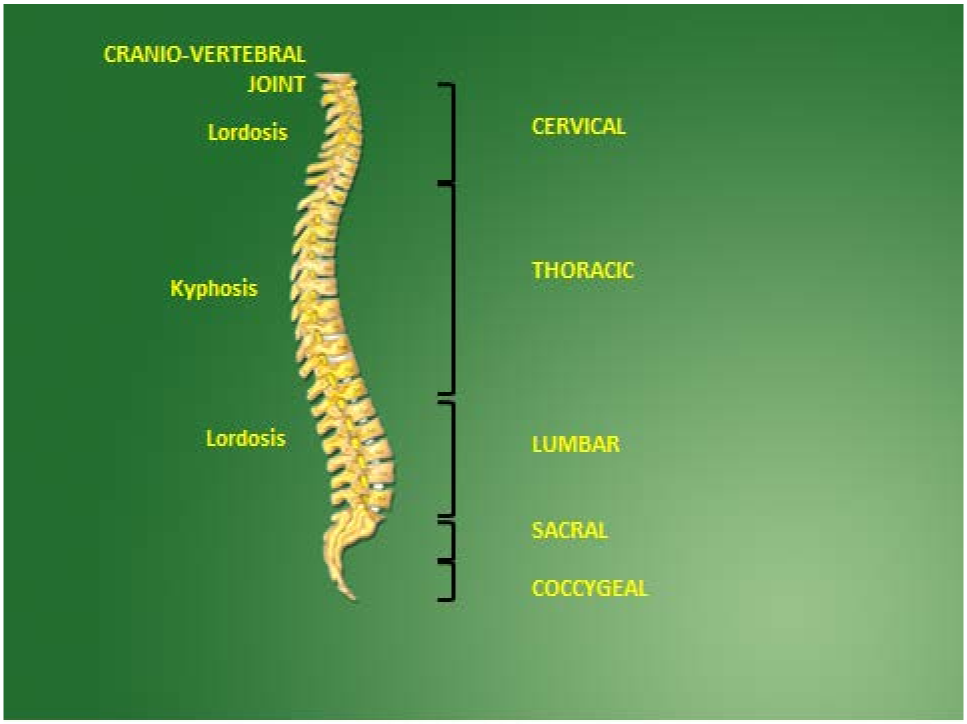


Figure 1. Diagram showing the various sections of the vertebral spine in humans with their associated curvatures.

Figure 2. Radiograph showing an increased curvature at the cervical region of the vertebral spine (Hyperlordosis).

Figure 3. Radiograph showing a lateral deviation of the vertebral spine towards the right side (Scoliosis).

Figure 4. Radiographs showing an extension (left) and flexion (right) of the head, affecting the curvature of the vertebral spine.

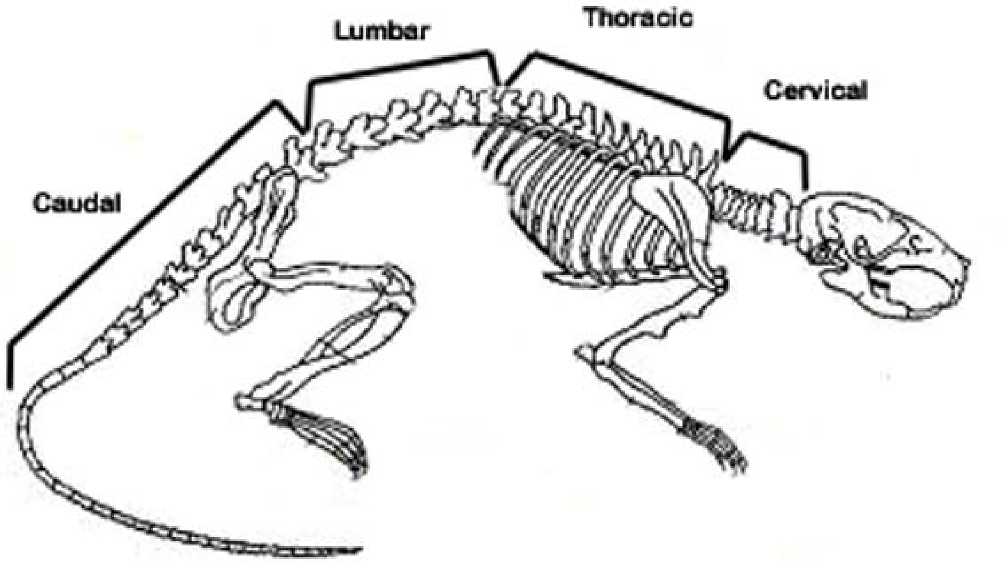


Figure 5. Diagram showing the various sections of the vertebral spine of rats.

Figure 6. Photograph showing the increase pad cemented on the maxillary molars of the rats in the Exp-1 group involved in this study.

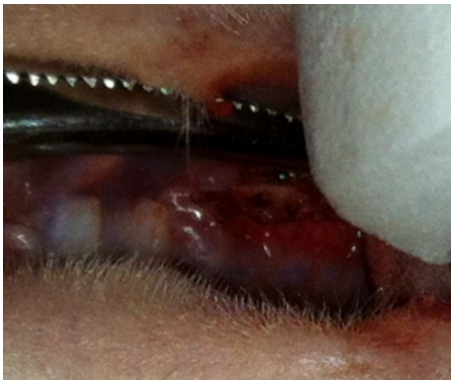


Figure 7. Photograph showing the site of extraction of the maxillary molars of the rats in the Exp-2 group involved in this study.

Figure 8. Photograph showing the set for the x-ray equipment used for taking the radiographs during the experimental period in this study.

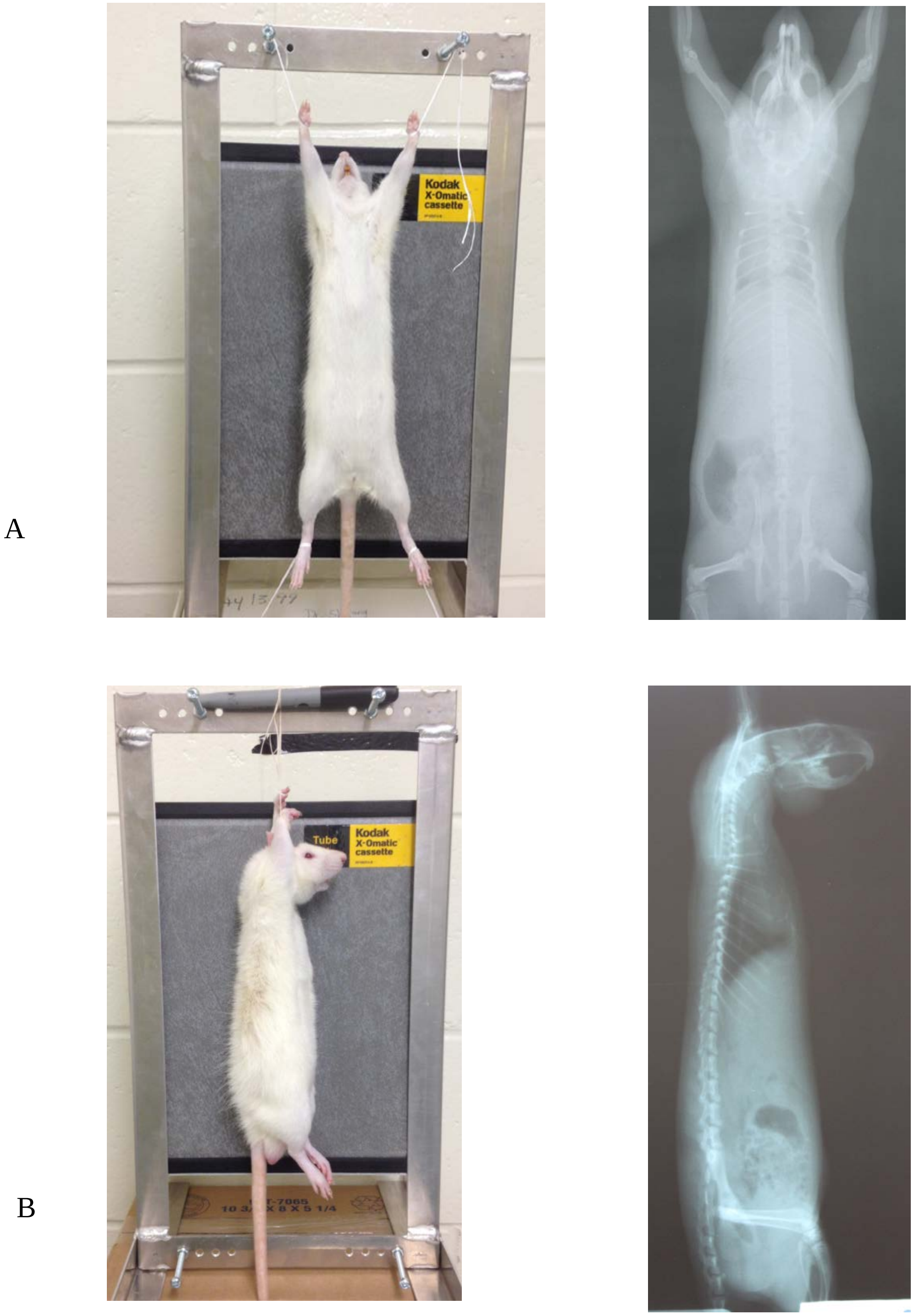


Figure 9. Photographs showing the position of the animals when taking the frontal (A) and lateral (B) radiographs during the experimental period in this study.

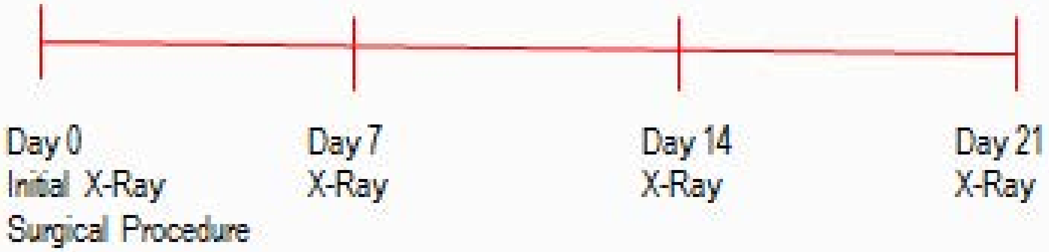
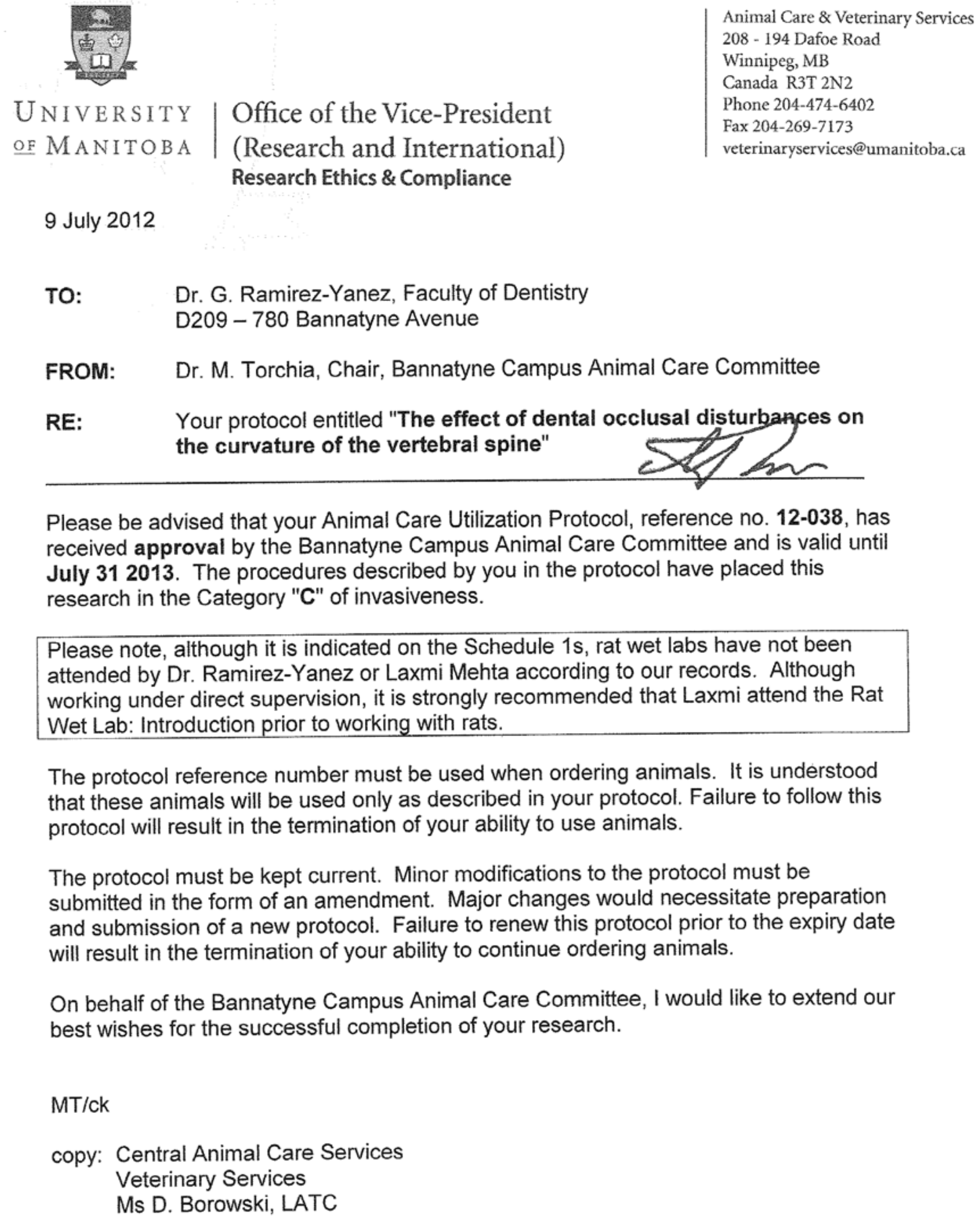


Figure 10. Diagram showing the designed timeline for this study, when the x-rays where taken for all the animals involved in the study.

Appendix C: Copy of survey instrument



UNIVERSITY OF MANITOBA | Office of the Vice-President (Research and International)
Research Ethics & Compliance

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9 July 2012

TO: Dr. G. Ramirez-Yanez, Faculty of Dentistry
D209 – 780 Bannatyne Avenue

FROM: Dr. M. Torchia, Chair, Bannatyne Campus Animal Care Committee

RE: Your protocol entitled "**The effect of dental occlusal disturbances on the curvature of the vertebral spine**"

Please be advised that your Animal Care Utilization Protocol, reference no. **12-038**, has received **approval** by the Bannatyne Campus Animal Care Committee and is valid until **July 31 2013**. The procedures described by you in the protocol have placed this research in the Category "**C**" of invasiveness.

Please note, although it is indicated on the Schedule 1s, rat wet labs have not been attended by Dr. Ramirez-Yanez or Laxmi Mehta according to our records. Although working under direct supervision, it is strongly recommended that Laxmi attend the Rat Wet Lab: Introduction prior to working with rats.

The protocol reference number must be used when ordering animals. It is understood that these animals will be used only as described in your protocol. Failure to follow this protocol will result in the termination of your ability to use animals.

The protocol must be kept current. Minor modifications to the protocol must be submitted in the form of an amendment. Major changes would necessitate preparation and submission of a new protocol. Failure to renew this protocol prior to the expiry date will result in the termination of your ability to continue ordering animals.

On behalf of the Bannatyne Campus Animal Care Committee, I would like to extend our best wishes for the successful completion of your research.

MT/ck

copy: Central Animal Care Services
Veterinary Services
Ms D. Borowski, LATC