Bone response to orthodontic forces in diabetic Wistar rats

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**Introduction:** Patients with type 1 diabetes have shown decreased bone mineral density (BMD) values. The need for orthodontic treatment in diabetic patients is usually associated with occlusal problems and the occurrence of abnormalities in the development of the jaws. The aim of this study was to analyze bone response of insulin-treated and untreated diabetic rats after applying orthodontic forces. **Methods:** Wistar rats were divided into 3 groups: experimental orthodontics, experimental diabetes and orthodontics, and experimental diabetes treated with insulin and experimental orthodontics. Orthodontic forces were applied the first day of the seventh week. Forty-eight hours after placement, all the animals were killed, and the maxillae were excised and processed using routine histologic techniques. **Results:** Bone activity in the periodontal cortex of the dental alveolus showed a significant decrease in bone formation and erosive areas in diabetic animals as compared with controls. A recovery of these parameters could be observed in the group with experimental diabetes treated with insulin and experimental orthodontics. Bone volume in the interradicular bone showed no significant differences among groups. **Conclusions:** People with diabetes should not receive orthodontic treatment until their metabolic status normalizes. Bone response to orthodontic forces in insulin-treated diabetic subjects does not differ significantly from that observed in healthy subjects. (Am J Orthod Dentofacial Orthop 2011;139:S76-82)

Type 1 diabetes, previously known as insulin-dependent, type I or juvenile diabetes, accounts for 5% to 10% of diabetic patients and has a peak incidence at 10 to 14 years of age. It is caused by an absolute deficiency in insulin secretion, which results from an autoimmune destruction of pancreatic β cells. Type 2 diabetes, formerly referred to as insulin-independent, type II or adult-onset diabetes, accounts for 90% to 95% of diabetic patients, including many who are insulin resistant and often have relative insulin deficiency.

Long-term complications of diabetes include retinopathy, nephropathy, peripheral neuropathy, and autonomic neuropathy, causing gastrointestinal, genitourinary, and cardiovascular symptoms, as well as sexual dysfunction.

Alteration in bone metabolism is another common observation among long-term complications found in diabetic patients, even in patients treated with insulin. Clinical assessment of this alteration is usually performed based on bone mineral density (BMD) determinations. It is a fast, noninvasive, painless method to determine the amount of calcium in the spinal column, wrist, arm, or leg and therefore provides an estimate of fracture risk in individual patients.

Studies of type 2 diabetes report decreased, normal, and increased BMD values compared with nondiabetic patients. Most studies of type 1 diabetic patients show decreased BMD values, even when the onset of the disease is after peak bone mass has been achieved.

Insulin therapy reverses damage to organs associated with hyperglycemia, or at least it partially does. Nevertheless, there are reports that show significant changes in bone in diabetic patients, even in those receiving intensive insulin treatment, and in young patients.

The need for orthodontic treatment in adult diabetic patients is usually associated with a number of occlusal problems related to periodontal degradation and tooth loss, which in turn lead to nutritional deficiencies, psychological consequences and impaired quality of life. Another reason for orthodontic treatment in young diabetic patients is the occurrence of abnormalities in
the development of the jaws. There are clinical and experimental studies\textsuperscript{15–17} reporting a decrease in cephalometric parameters and delayed skeletal development in individuals with type 1 diabetes, all of which are predisposing factors for dental malpositions.

Orthodontic treatment is based on the principle that a prolonged application of pressure to the tooth will result in movement, which is a consequence of remodeling of the surrounding bone. The success of orthodontic treatment largely depends on bone response to the application of forces; hence, bone health is fundamental in order to obtain tooth movements as they were planned.

According to Bensch et al.,\textsuperscript{1} Burden et al.,\textsuperscript{2} and Proffit and Fields,\textsuperscript{13} if diabetes is properly controlled, the periodontal and bone response to orthodontic forces is almost normal, and a satisfactory orthodontic result can be obtained. Conversely, if diabetes is poorly controlled, it is accepted that there is a real risk of accelerating periodontal degradation, therefore contraindicating orthodontic treatment until the metabolic disorder is compensated. However, there is no scientific evidence or experimental or clinical studies that support these principles used in the clinical practice of orthodontics, as there are scant reports in the literature combining principles used in the clinical practice of orthodontics, or experimental or clinical studies that support these compensated.

Therefore, the aim of this study was to analyze bone response of insulin-treated and untreated diabetic rats after applying orthodontic forces.

**MATERIAL AND METHODS**

**Experimental animals**

Twenty-four male Wistar rats weighing 100 to 130 g were used. They were divided into 3 groups of 8 animals each:

1. Experimental orthodontics (ORT)
2. Experimental diabetes and orthodontics (DBT + ORT)
3. Experimental diabetes treated with insulin and experimental orthodontics (DBT + INS + ORT)

The animals were kept in groups of 4 in each cage. They were given regular rat chow and water *ad libitum*. The animals were kept under 12-hour light-dark cycles (light 7:00 AM to 7:00 PM), at room temperature (18–22°C) and relative humidity (40%–60%). All procedures for treatment and maintenance of the animals were conducted in keeping with internationally accepted guidelines (“Guidelines for the care and use of laboratory animals”, NIH Publication, 1985) and were approved by the Ethics Committee of the School of Dentistry of the University of Buenos Aires.

**Experimental diabetes induction**

Diabetes was induced on day 1 of the experiment in groups II and III by administering a single intraperitoneal injection of streptozotocin (STZ) 60 mg/kg body weight (Sigma-Aldrich, Inc., St. Louis, Mo), dissolved in freshly prepared citrate buffer pH 4.0. STZ is a cytoxic agent that is relatively selective against pancreatic β cells in animals, causing an irreversible diabetic state. Therefore, diabetes induced by this method is similar to type 1 diabetes, which is insulin-dependent or insulin-requiring diabetes. The animals in the nondiabetic group (group I) received an equivalent volume of citrate buffer. The orthodontic device was applied 6 weeks after diabetes was induced.

**Sampling and determination of blood glucose**

Twenty-four hours after administration of STZ in the diabetic groups and administration of the vehicle in the control group, blood samples were obtained from all animals for quantitative determination of blood glucose using the glucose oxidase method with Accu-Chek Sensor Comfort test strips (Roche Products Ltd, Santiago, Chile) in the Accu-Chek Sensor (Roche Diagnostics GmbH, Mannheim, Germany). Only animals with blood glucose levels higher than 250 mg/dL were included in the different groups of experimental diabetes, and only those with blood glucose levels less than 120 mg/dL were included in group I.

Determinations were repeated once a week in non-insulin treated groups, to ensure that glycemic levels were as expected, and every 48 hours in insulin-treated rats. All the animals included in the study met the inclusion criteria corresponding to their particular group.

**Insulin treatment**

Insulin therapy was initiated in the insulin-treated diabetic group the day after STZ injection by daily administration of human NPH insulin subcutaneously (Humulin N, Eli Lilly y Compañía de México S.A. de C.V., Mexico D.F., Mexico) according to the requirements of each animal.

**Installation of the orthodontic appliance and sample collection**

Orthodontic forces were applied to all animals the first day of the seventh week of the experiment. The orthodontic appliance was specifically designed for the rat and meets the basic standards applied in the clinical setting. It consists of 2 stainless steel molar bands with tubes welded to their palatal aspect. These bands are cemented to the upper first molars of rats with glass...
ionomer cement. A 0.014-in circular cross-section stainless steel wire was shaped into a helical spring to exert a force magnitude of 120 ± 15 g toward the vestibular plate (Fig 1).

The animals were anesthetized with a xylazine and ketamine solution (Kensol, Laboratorios König SA, Buenos Aires, Argentina; Ketamine 50, Holliday-Scott SA, Buenos Aires, Argentina) in order to place the device. Forty-eight hours after placement of the orthodontic device and after measuring blood glucose levels, all the animals were killed (day 44 of the experiment) by an intraperitoneal overdose of sodium thiopental (150 mg/kg) (Pentovet, Richmond Laboratorios, Buenos Aires, Argentina) and acepromazine maleate (3 mg/kg) (Acedan, Holliday-Scott SA, Buenos Aires, Argentina).

Immediately after death, body weight was recorded and the maxillae were excised and fixed in formalin buffer (pH 7.0) for 48 hours. After this time, the samples were carefully dissected and decalcified by immersion in a solution of 10% EDTA (ethylenediaminetetraacetic acid), pH 7, for 30 days. The samples were then processed using routine histologic techniques. Buccopalatal and longitudinally oriented sections of the mesial root of the upper first molar were obtained under stereoscopic microscope; the blocks were then reoriented under stereoscopic microscope, changing the angulation of the platens of the microtome to obtain sections of the distal roots, which were also buccopalatal and longitudinally oriented. All the sections were oriented in an apicocoronal direction, parallel to the long axis of the corresponding root. All the sections were stained with hematoxylin and eosin.

Digital photographs were taken of the histologic sections with a digital camera Canon Powershot A640 10.0 megapixel 4x optical zoom lens (Canon Inc., Tokyo, Japan) mounted on an optical microscope Carl Zeiss Axioskop 2 (Carl Zeiss Mikroskopie, Jena, Germany) with a 5x objective. The images obtained were used for the histomorphometric study using the Image-Pro Plus 4.0 image analysis program (Media Cybernetics, Inc, Bethesda, Md) (Fig 2).

**Body weight record**

Body weight of each animal was recorded periodically with a digital laboratory balance Ohaus CS 2000 (Ohaus Corp, Pine Brook, NJ) to draw a curve of weight gain.

**Histologic and histomorphometric studies**

The following histomorphometric parameters were assessed on the dental alveolus according to stereologic principles and using current nomenclature as stated by Parfitt et al:

- **Bone activity**

On digital images of sections of the mesial root, static bone activity was evaluated by determining the percentage of the following parameters on the surface of the periodontal cortical of alveolar bone in the studied area:

- Areas covered by osteoblasts (Obs/BS)
- Total erosive surfaces (ES/BS)
- Erosive surfaces covered with osteoclasts (ES (Oc+)/BS)
- Erosive surfaces not covered with osteoclasts (ES(0c−)/BS)
- Resting surfaces (rest)
Surfaces facing hyalinized areas (nec): percentage of the surface of the periodontal cortex of alveolar bone in the studied area presenting surfaces facing necrotic periodontal ligament (hyalinized areas). These areas were defined as areas of necrosis.

- **Number of osteoclasts**
  Total number of cells that are morphologically recognizable as osteoclasts found lining the periodontal cortex of alveolar bone measured at the mesial root.

- **Bone volume of the interradicular bone (BV/TV)**
  Fraction of bone tissue in total volume. Total volume was taken as bone tissue plus bone marrow evaluated at the level of the distal roots.

**Statistical analysis**

The values are expressed as mean ± standard deviation. The statistical analysis of the results was performed using 1-way analysis of variance (ANOVA). Multiple comparisons were made using the Bonferroni test. The level of statistical significance was set at a value of \( P < 0.05 \).

**RESULTS**

At the onset of the experiment, body weight was similar in all the animals. As of the third week of the experiment, body weight gain in non–insulin treated diabetic animals was lower than that observed in control animals and in insulin-treated diabetic animals. At the time of sacrifice, a statistically significant difference in body weight was observed between diabetic animals \((180 ± 21 \text{ g})\) and healthy \((291 ± 42 \text{ g})\) and insulin-treated diabetic animals \((299 ± 38 \text{ g})\).

The microscopic sections of the alveolus and mesial root of the upper first molar of the rats evidenced an inclination of the tooth toward the vestibular plate in response to the application of the orthodontic forces, with the consequent compression of the periodontal ligament in the cervical third of the vestibular plate of the alveolus and the apical third of the palatal plate; the characteristic hyalinization areas were observed in these areas. No substantial differences in the histologic image were observed among the different groups in regard to the periodontal ligament, dental tissue, bone tissue, or vasculature.

The results of the histomorphometric study of bone activity in the periodontal cortex of the alveolus are presented in Figure 3 and show a statistically significant decrease in Obs/BS in diabetic animals compared with controls and recovery of this parameter after insulin
treatment. In addition, a significant decrease in ES/BS was observed in the diabetic group compared to ORT and DBT + INS + ORT animals.

A significant decrease in the number of osteoclasts in the periodontal cortex of the dental alveolus was observed in the DBT + ORT group (1.78 ± 1.99) compared with ORT (11.00 ± 2.74) and DBT + INS + ORT rats (6.89 ± 3.55). The number of osteoclasts was also significantly lower in the DBT + INS + ORT group as compared with the ORT group.

Histomorphometric evaluation of BV/TV showed no statistically significant differences among groups (ORT 0.79 ± 0.11; DBT + ORT 0.77 ± 0.08; DBT + INS + ORT 0.71 ± 0.09).

**DISCUSSION**

The results presented in this article clearly show the significant alteration in bone response of diabetic animals to the application of an orthodontic force. A significant decrease in Obs/BS and ES/BS was observed in animals with chronic diabetes subjected to orthodontic forces in comparison with healthy animals that were exposed to the same mechanical stimulus. In addition, an increase in rest was also observed in these animals. However, bone response of insulin-treated diabetic animals to the application of orthodontic forces was no different from that observed in healthy animals.

The alteration in bone response of diabetic animals to orthodontic forces outlined above leads to discussion on several issues that may have significant implications in the orthodontic treatment of diabetic patients.

The experimental model of chronic diabetes used here is a model that closely resembles the clinical situation observed in patients with type 1 diabetes. The experimental time point used in this experiment is appropriate to elicit the known long-term effects of diabetes on bone tissue.20, 21 The bone alterations associated with the chronic condition of the disease are clinically diagnosed primarily based on BMD determinations.

![Graph](image-url)
Thus, bone metabolism is assessed at the spine, wrist, arm, and leg. However, no information is gathered concerning jaw bone metabolism.

The considerations for orthodontic treatment in diabetic patients are based on rational principles and concepts of the dental management of patients with systemic disorders.22 However, there are scant experimental23 or clinical studies providing evidence to support such behavior in diabetic patients.

To our knowledge, the only report associating experimental diabetes with orthodontics is a histologic study by Holtgrave and Donath18 published in 1989 involving molar movement of the upper first molar of the rat. The authors observed periodontal ligament thinning and microangiopathies in the gingiva and a delay in bone regeneration in diabetic rats subjected to an orthodontic stimulus.

The decrease in bone formation in diabetes has been reported in a large number of studies,25-30 based on serum measurements, bone density studies, and histomorphometric analysis of long bones and the axial skeleton. According to our results, bone formation in the dental alveolus of diabetic animals in response to the application of an orthodontic stimulus was also lower than that observed in healthy animals subjected to the same stimulus.

In previous studies, we showed that osteocytes of alveolar bone respond early to hyperglycemia, as shown by the alteration in lacunar shape and a decrease in osteocyte density.31 As these cells are involved in the mechanotransduction that regulates osteogenesis in the tension areas of bone formation,12 early alterations of these cells caused by hyperglycemia may be associated with the decrease in bone formation observed in diabetic animals subjected to the application of an orthodontic force.

With regard to the decrease in ES/BS and in the number of osteoclasts in response to the application of orthodontic forces observed in diabetic animals, it is valid to say that there is some controversy in the literature as to whether or not diabetic osteopenia is associated with an alteration in osteoclastogenesis and bone resorption.33-36

In our study, the number of cells morphologically recognizable as osteoclasts on the periodontal cortex of the dental alveolus was significantly lower in diabetic animals compared with controls and increased significantly with insulin treatment. These results concerning the number of osteoclasts correlate with the significant decrease in erosive areas observed in these diabetic animals as compared with the control group and insulin-treated diabetic animals.

The histomorphometric evaluation of BV/TV showed no differences among groups. The results concerning tissue response to the application of orthodontic forces in diabetic rats indicate that even though activity in the cortical alveolar bone of the tooth may be altered, the alteration is not severe enough to affect the amount or volume of alveolar bone at the studied time point. Alteration of bone quality is considered today another possible cause of bone abnormalities found in diabetic patients.24 There are experimental studies reporting a decrease in bone quality, even when BMD was not significantly altered.37 Studies in BB rats (spontaneously diabetic) report decreased bone strength.38 A decrease in torque and angular deflection for the femur of diabetic rats has also been reported. Therefore, it should be considered that the quality of the interradicular bone formed in diabetic animals might not be the same as that of healthy animals, even when interradicular bone volume remains unchanged.

CONCLUSIONS

The results of the present experimental study allow the suggestion that diabetic patients who are not strictly monitored should not receive orthodontic treatment, even when needed, until their metabolic status normalizes, since the application of strong orthodontic forces have been shown to cause unwanted effects. The present experimental study also provides scientific evidence supporting the hypothesis that bone response to orthodontic forces in insulin-treated diabetic subjects does not differ significantly from that observed in healthy subjects, even when strong forces are used.

REFERENCES

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