*European Journal of Orthodontics* 25 (2003) 359–363 

# Bone turnover rate in rats does not influence root resorption induced by orthodontic treatment

Carlalberta Verna, Michel Dalstra and Birte Melsen
Department of Orthodontics, Royal Dental College, Aarhus University, Denmark

SUMMARY The aim of this study was to determine, in a rat model, whether a state of high or low bone turnover had an effect on the rate and type of tooth movement and on the incidence of root resorption induced by orthodontic treatment. The maxillary left first molar was moved mesially for 21 days in 52 6-month-old Wistar rats. They were divided into three groups: group 1 ($n$ = 19) with normal bone turnover, group 2 ($n$ = 16) with high bone turnover, and group 3 ($n$ = 17) with low bone turnover. The contralateral side was left untreated to act as a control. The different metabolic rates were created by inducing hyper- and hypothyroidism. The amount of tooth movement was measured using an electronic calliper and the location of the centre of rotation (CRot) was determined after microcomputer tomographic scanning and subsequent three-dimensional reconstruction. Histomorphometric evaluation of root resorption was performed on undecalcified 7 μm thick sections of the maxilla and the differences between treated and untreated sides were evaluated.

The results showed that high bone turnover increased the amount of tooth movement compared with the normal or low bone turnover state. There was no statistical difference in the location of the CRot. The treated side presented more root resorption than the untreated side, but this difference was not influenced by the metabolic rates. On the contrary, the untreated side in the low bone turnover group showed more root resorption, suggesting that in subjects where a decreased bone turnover rate is expected, the risk of root resorption could be increased.

## Introduction

External root resorption is a phenomenon that has been associated with orthodontic treatment (Thilander, 1992; Brezniak and Wasserstein, 1993a,b; Vlaskalic *et al.*, 1998). Numerous potential factors, related to both the individual patient and to treatment, have been suggested as risk factors for root resorption, but direct causal factors have not been identified (Vlaskalic *et al.*, 1998). This lack of consistent findings has led to the recent suggestion of a different approach to the analysis of the problem, where the primary effect is studied among patient factors rather than among treatment variables (Vlaskalic *et al.*, 1998). Among the patient-related factors, recent investigations have examined the role of genetics, the immune system and the patient's medical history (Wheeler and Stroup, 1993; Baumrind *et al.*, 1996; Harris *et al.*, 1997). The variability of the relationship between orthodontic treatment response and the host's metabolic condition is well known (Goldie and King, 1984; Bridges *et al.*, 1988; Engström *et al.*, 1988; Verna, 1999). The role of hard tissue physiology in the prevalence of root resorption, with and without orthodontic force, has also been investigated (Engström *et al.*, 1988; Robinson and Harvey, 1989; Poumpros *et al.*, 1994). Finally, the effect of drugs administered to control the rate of tooth movement on the incidence of root resorption has been evaluated (Wesselink and Beertsen, 1989; Brudvik and Rygh, 1991; Lasfargues and Saffar, 1993; Leiker *et al.*, 1995; Igarashi *et al.*, 1996; Boekenoogen *et al.*, 1996; Zhou *et al.*, 1997). Goldie and King (1984) reported that increased bone resorption due to secondary hyper-parathyroidism was associated with decreased root resorption during orthodontic treatment, while the opposite was found by Engström *et al.* (1988). Thyroid hormones, which in excess increase bone turnover (Melsen and Mosekilde, 1977), have been found to reduce root resorption during orthodontic tooth displacement in a rat model, and a clinical application has also been suggested (Loberg and Engström, 1994; Poumpros *et al.*, 1994). However, hypothyroidism has been associated with increased root resorption, in the absence of orthodontic loads (Becks and Cowden, 1942).

The aim of this study was to determine, in a rat model, whether a state of high or low bone turnover had an effect on tooth movement rate and type, and on the incidence of root resorption induced by orthodontic treatment.

## Materials and methods

The materials and methods related to the orthodontic force application and the animal model have been described previously (Verna *et al.*, 2000a).

Fifty-two 6-month-old outbred male Wistar rats were divided into three groups: group 1 ($n$ = 19) with normal bone turnover, group 2 ($n$ = 16) with high bone turnover, and group 3 ($n$ = 17) with low bone turnover.

High and low bone turnover were produced by inducing hyper- and hypothyroidism, according to the protocol of Gøtzsche and Ørskov (1994). The levels of total triiodothyronine ($TT_3$) and total thyroxine ($TT_4$) were evaluated in the serum before the administration of the drugs and at sacrifice, and body weight was checked weekly. Bone turnover was assessed by measuring the mineral appositional rate (MAR) index in the mid-diaphysal section of the left femur in the three groups at the end of the experiment.

After 4 weeks of drug administration, a 25 g Sentalloy® closed orthodontic coil spring (GAC, Ctr. Islip, New York, USA) was inserted between the upper left first molar and the upper incisors in all animals. After 21 days of loading, a mesial tipping of the molars was obtained. The influence of inter-animal variation in response to metabolic stimuli was controlled by the use of a split-mouth design, in which the untreated contralateral side served as the control. At the end of the observation period the animals were sacrificed with an overdose of $CO_2$ and the maxillae were excised.

The distance between the mesial surface of the first molar and the distal surface of the third molar was measured bilaterally with an electronic calliper and tooth movement was evaluated as indicated by Hong *et al.* (1992). Following microcomputed tomography scanning (Scanco Medical, Bassersdorf, Switzerland), a three-dimensional reconstruction of each hemi-maxilla was performed and for each first molar the image including the longest mesial root was selected. On this image the root length, defined as the linear distance between the apex and the furcation area along the pulp canal, was measured with the scanner's built-in image analysis tools on both the treated and untreated molars. The same image was used to evaluate tooth inclination and, in combination with the linear movement, the location of the centre of rotation (CRot) (Verna *et al.*, 2000a).

The undecalcified excised maxillae were then embedded in methylmethacrylate and cut at two levels 1150 μm apart: coronal and apical. At each level, two horizontal 7 μm thick histological sections (150 μm apart) were cut. Within each set, one section was cut and stained with modified Goldner trichrome (Figure 1).

For each animal the percentage of root resorption was measured as follows. On each section, the mesio-lingual root of the treated and untreated upper molars was identified and histomorphometric evaluation of the percentage of root resorption was performed. The image of the mesio-lingual root was projected onto a white field at ×12.5 magnification. A 17 mm interval grid was randomly positioned on the projected image of the root and the number of intersections of the root surface affected by resorption was counted (Figure 1). The percentage of root resorption was obtained by the ratio of this number and the total number of intersections with the root surface. Root resorption was defined as a scalloped surface with or without resorbing cells. A minimum of 200 intersections was evaluated for each root. The mean values, obtained in the four sections per root, were used as the dependent variable for statistical analysis.

### *Statistical analysis*

The analysis used for the evaluation of $TT_4$, $TT_3$, body weight, and MAR has been reported previously

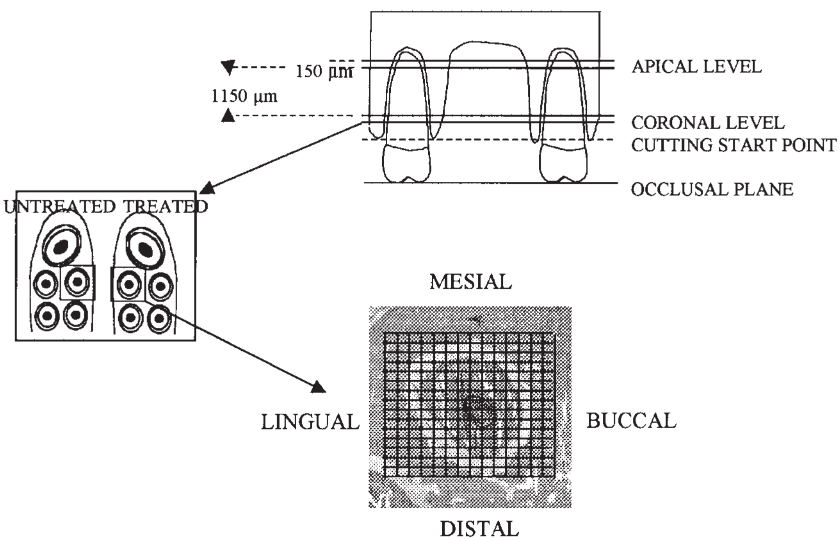


**Figure 1** Schematic illustration of the levels at which the histological sections were cut and the grid used to measure the percentage of root resorption.

(Verna *et al.*, 2000b). The effects of orthodontic treatment and bone metabolism on root resorption and root length were analysed by means of a repeated measurements ANOVA, considering the treated and the control side as repeated measurements. The Bonferroni and Student–Newman–Keuls tests showed that there was a significant difference between the metabolic groups. The treated and the untreated sides were also compared separately by means of a *t*-test for each group. Linear regression analysis was performed to explore the relationship between root resorption and root shortening and the amount and type of tooth movement (Armitage and Berry, 1995). The level of significance was set at 5 per cent.

## Results

The levels of $TT_4$, $TT_3$, body weight, and MAR found in this study confirmed the successful induction of high and low bone turnover rates (Verna *et al.*, 2000a).

Mean values and standard deviations indicating tooth movement in the three different bone turnover groups are illustrated in Figure 2. The amount of tooth movement in the high bone turnover group was larger than in the normal bone turnover group, while the low bone turnover group showed less tooth movement than the normal group. The difference in the amount of tooth movement was significant ($P < 0.05$) between both the high and low bone turnover groups, and the normal and high bone turnover groups. No significant difference was found in the location of the CRot, although the low bone turnover group showed a tendency towards a more coronal location and the high bone turnover group showed a more apical tendency.

No significant differences were found in root length between the treated and the control sides within or between groups (Table 1). The treated sides of all three groups showed a significantly higher percentage of root resorption. When the treated and untreated sides were compared separately, the low bone turnover group showed a significantly larger percentage of root resorption on the untreated side compared with the normal and high bone turnover groups (Figure 3). However, repeated measurement ANOVA analysis did not show any significant difference between the three bone turnover groups.

Regression analysis showed no relationship between root resorption and either tooth movement or tooth movement type.

**Table 1** Root length in microns (mean ± standard deviation) on the treated and control sides in the three bone turnover groups. No significant difference was found.

| | Normal | | High | | Low | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| Treated | 2495 | 423 | 2556 | 227 | 2451 | 213 |
| Control | 2532 | 248 | 2527 | 189 | 2386 | 172 |

SD, standard deviation.

## Discussion

The results of this study showed an influence of different bone turnover rates on orthodontic tooth movement. Bone turnover rate influenced the amount of root resorption, as the low bone turnover group showed more root resorption than the other groups. The mechanical load did not exert any synergistic effect, as root resorption increased on the treated sides by the same amount in the three groups.

The greater tooth movement found in the high bone turnover group and the smaller movement observed in

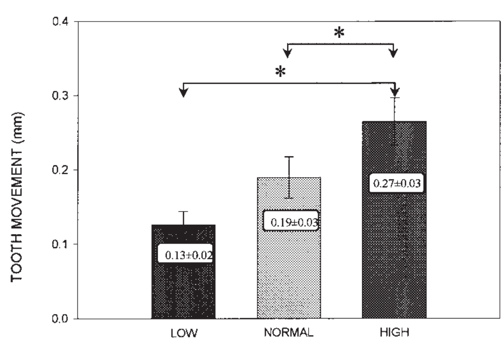


**Figure 2** Amount of tooth movement in millimetres (mean ± standard error of the mean) at the end of the experimental period in the three groups. * = significant at the 5 per cent level [previously published in Verna *et al*. (2000a). Published with kind permission of Società Italiana di Ortodonzia].

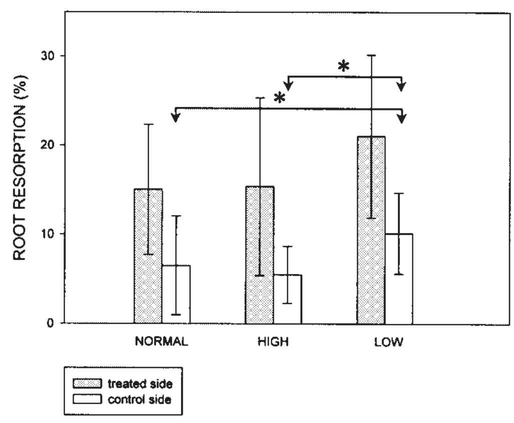


**Figure 3** The percentage of root resorption in the three groups. Note that the significant difference (* = significant at the 5 per cent level) is present only between the untreated sides of the hypothyroidic animals and the untreated sides of the normal and hyperthyroidic group.

the low bone turnover group is in line with previous findings (Midgett *et al.*, 1981; Ashcraft *et al.*, 1992).

Although the interaction between bone metabolism and tooth movement has been previously studied (Davidovitch *et al.*, 1972; Yamasaki *et al.*, 1984; Collins and Sinclair, 1988; Leiker *et al.*, 1995; Igarashi *et al.*, 1996; Kehoe *et al.*, 1996; Roche *et al.*, 1997), the influence on the type of tooth movement has not been considered previously. In this study, the location of the movement's CRot did not vary. The location of the CRot close to the apex is in agreement with previous findings of Macapanpan *et al.* (1954) and Gibson *et al.* (1992), while Katona *et al.* (1995) identified the CRot near the furcation area.

Orthodontic treatment produced an increase in the percentage of root resorption in all three groups and the treated sides had significantly more resorption than the untreated sides. However, this effect was not seen as a reduction in root length, with no difference found between the treated and the control sides. The latter observation is not in agreement with the literature, where root resorption is often found after radiographic evaluation (Levander and Malmgren, 1988; Lupi *et al.*, 1996). This can be ascribed to the short treatment time, which did not allow for radiographic detection of the root resorption observed at a histological level. Moreover, the uncontrolled tipping observed in this study induced a concentration of stresses at the coronal more than at the apical level (Nikolai, 1975).

As shown in Figure 3, the differences in root resorption between the treated and control sides were of the same magnitude in the three groups. It is also evident that low bone turnover induces a significantly larger amount of resorption on roots that are not submitted to mechanical loading. This is consistent with the finding of Becks and Cowden (1942), where a positive association between hypothyroidism and root resorption was found. The same has been observed during the administration of indomethacin, an inhibitor of prostanoid synthesis (Lasfargues and Saffar, 1993), whose important role as a mediator for mechanical stress-induced bone remodelling has been assessed (Yamasaki *et al.*, 1980). It can thus be suggested that in clinical situations where turnover of alveolar bone is delayed, root surfaces could already be affected by root resorption as a baseline condition. The protective effect against root resorption observed by Poumpros *et al.* (1994) was not confirmed by the results of the present study, as the rats with high bone turnover did not display less root resorption than those with low bone turnover.

The present investigation demonstrated an interaction between mechanical load and metabolism. Clinical situations where metabolic changes are to be expected, apart from specific metabolic bone disease, are often found when patients are undergoing pharmacological treatment, e.g. indomethacin (Giunta *et al.*, 1995), and steroid treatment given for various types of allergy (Ashcraft *et al.*, 1992). An increase in the rate of tooth movement could be expected in pregnant women, as demonstrated by Hellsing and Hammarström (1991), or in secondary hyperparathyroidism induced by lactation (Goldie and King, 1984). Although orthodontic treatment does not increase the rate of root resorption under different metabolic conditions, it should be taken into consideration that baseline root resorption rates could already be altered.

These results suggest that a thorough case history regarding possible pathophysiological conditions influencing bone metabolism should be performed on an individual patient basis. In subjects where increased bone turnover rates are expected, the reactivation of the appliance could be performed more frequently. However, in patients where decreased bone turnover rates are expected, the reactivation should be carried out less frequently and the risk of root resorption should be carefully evaluated.

## Address for correspondence

Carlalberta Verna
Department of Orthodontics
Royal Dental College
Vennelyst Boulevard
8000 Aarhus-C
Denmark

## Acknowledgements

This study was supported by the Aarhus University Research Fund no. F-1997-SUN-1-75 and F-1998-SUN-1-107. Thanks are due to the Laboratory of the Medical Department (Diabetes and Endocrinology, Aarhus University Hospital), Professor F. Melsen (Institute of Pathology, Aarhus University Hospital), Sussi Madsen for her laboratory assistance and Jørn Lund Pedersen. The coil springs were provided by GAC Company.

## References

Armitage P, Berry G 1995 Statistical methods in medical research. Blackwell Science, Oxford

Ashcraft M B, Southard K A, Tolley E A 1992 The effect of corticosteroid-induced osteoporosis on orthodontic tooth movement. American Journal of Orthodontics and Dentofacial Orthopedics 102: 310–319

Baumrind S, Korn E L, Boyd R L 1996 Apical root resorption in orthodontically treated adults. American Journal of Orthodontics and Dentofacial Orthopedics 110: 311–320

Becks H, Cowden R 1942 Root resorptions and their relationship to pathologic bone formation. Part II. Classification, degrees, prognosis and frequency. American Journal of Orthodontics and Oral Surgery 28: 513–526

Boekenoogen D I, Sinha P K, Nanda R S, Ghosh J, Currier G F, Howes R I 1996 The effects of exogenous prostaglandin $E_2$ on root resorption in rats. American Journal of Orthodontics and Dentofacial Orthopedics 109: 277–286

Brezniak N, Wasserstein A 1993a Root resorption after orthodontic treatment: Part 1. Literature review. American Journal of Orthodontics and Dentofacial Orthopedics 103: 62–66

Brezniak N, Wasserstein A 1993b Root resorption after orthodontic treatment: Part 2. Literature review. American Journal of Orthodontics and Dentofacial Orthopedics 103: 138–146

Bridges T, King G, Mohammed A 1988 The effect of age on tooth movement and mineral density in the alveolar tissues of the rat. American Journal of Orthodontics and Dentofacial Orthopedics 93: 245–250

Brudvik P, Rygh P 1991 Root resorption after local injection of prostaglandin $E_2$ during experimental tooth movement. European Journal of Orthodontics 13: 255–263

Collins M K, Sinclair P M 1988 The local use of vitamin D to increase the rate of orthodontic tooth movement. American Journal of Orthodontics and Dentofacial Orthopedics 94: 278–284

Davidovitch Z, Musich D, Doyle M 1972 Hormonal effects on orthodontic tooth movement in cats—a pilot study. American Journal of Orthodontics 62: 95–96

Engström C, Granström G, Thilander B 1988 Effect of orthodontic force on periodontal tissue metabolism. A histologic and biochemical study in normal and hypocalcemic young rats. American Journal of Orthodontics and Dentofacial Orthopedics 93: 486–495

Gibson J M, King G J, Keeling S D 1992 Long-term tooth movement response to short-term force in the rat. Angle Orthodontist 62: 211–215

Giunta D, Keller J, Nielsen F F, Melsen B 1995 Influence of indomethacin on bone turnover related to orthodontic tooth movement in miniature pigs. American Journal of Orthodontics and Dentofacial Orthopedics 108: 361–366

Goldie R S, King G J 1984 Root resorption and tooth movement in orthodontically treated, calcium-deficient, and lactating rats. American Journal of Orthodontics 85: 424–430

Gøtzsche L B, Ørskov H 1994 Cardiac triiodothyronine nuclear receptor binding capacities in amiodarone-treated, hypo- and hyperthyroid rats. European Journal of Endocrinology 130: 281–290

Harris E F, Kineret S E, Tolley E A 1997 A heritable component for external apical root resorption in patients treated orthodontically. American Journal of Orthodontics and Dentofacial Orthopedics 111: 301–309

Hellsing E, Hammarström L 1991 The effects of pregnancy and fluoride on orthodontic tooth movements in rats. European Journal of Orthodontics 13: 223–230

Hong R K, Yamane A, Kuwahara Y, Chiba M 1992 The effect of orthodontic retention on the mechanical properties of the periodontal ligament in the rat maxillary first molar. Journal of Dental Research 71: 1350–1354

Igarashi K, Adachi H, Mitani H, Shinoda H 1996 Inhibitory effect of the topical administration of a bisphosphonate (risedronate) on root resorption incident to orthodontic tooth movement in rats. Journal of Dental Research 75: 1644–1649

Katona T R, Paydar N H, Akay H U, Roberts W E 1995 Stress analysis of bone modeling response to rat molar orthodontics. Journal of Biomechanics 28: 27–38

Kehoe M J, Cohen S M, Zarrinnia K, Cowan A 1996 The effect of acetaminophen, ibuprofen, and misoprostol on prostaglandin $E_2$ synthesis and the degree and rate of orthodontic tooth movement. Angle Orthodontist 66: 339–349

Lasfargues J J, Saffar J L 1993 Inhibition of prostanoid synthesis depresses alveolar bone resorption but enhances root resorption in the rat. Anatomical Record 237: 458–465

Leiker B J, Nanda R S, Currier G F, Howes R I, Sinha P K 1995 The effects of exogenous prostaglandins on orthodontic tooth movement in rats. American Journal of Orthodontics and Dentofacial Orthopedics 108: 380–388

Levander E, Malmgren O 1988 Evaluation of the risk of root resorption during orthodontic treatment: a study of upper incisors. European Journal of Orthodontics 10: 30–38

Loberg E L, Engström C 1994 Thyroid administration to reduce root resorption. Angle Orthodontist 64: 395–399

Lupi J E, Handelman C S, Sadowsky C 1996 Prevalence and severity of apical root resorption and alveolar bone loss in orthodontically treated adults. American Journal of Orthodontics and Dentofacial Orthopedics 109: 28–37

Macapanpan L C, Weinmann J P, Brodie A G 1954 Early tissue changes following tooth movement in rats. Angle Orthodontist 24: 79–95

Melsen F, Mosekilde L 1977 Morphometric and dynamic studies of bone changes in hyperthyroidism. Acta Pathologica et Microbiologica Scandinavica Section A, Pathology 85A: 141–150

Midgett R J, Shaye R, Fruge J F J 1981 The effect of altered bone metabolism on orthodontic tooth movement. American Journal of Orthodontics 80: 256–262

Nikolai R J 1975 On optimum orthodontic force theory as applied to canine retraction. American Journal of Orthodontics 68: 290–302

Poumpros E, Loberg E, Engström C 1994 Thyroid function and root resorption. Angle Orthodontist 64: 389–393

Robinson P B, Harvey W 1989 Tooth root resorption induced in rats by diphenylhydantoin and parathyroidectomy. British Journal of Experimental Pathology 70: 65–72

Roche J J, Cisneros G J, Acs G 1997 The effect of acetaminophen on tooth movement in rabbits. Angle Orthodontist 67: 231–236

Thilander B L 1992 Complications of orthodontic treatment. Current Opinion in Dentistry 2: 28–37

Verna C 1999 The influence of different bone turnover rates on the tissue reactions to orthodontic load. Thesis, Aarhus University, Denmark

Verna C, Dalstra M, Melsen B 2000a Le variazioni della qualità e quantità ossea indotte dallo spostamento ortodontico sono influenzate dal metabolismo osseo. Ortognatodonzia Italiana 9: 199–209

Verna C, Dalstra M, Melsen B 2000b The rate and the type of tooth movement is influenced by bone turnover in a rat model. European Journal of Orthodontics 22: 343–352

Vlaskalic V, Boyd R L, Baumrind S 1998 Etiology and sequelae of root resorption. Seminars in Orthodontics 4: 124–131

Wesselink P R, Beertsen W 1989 The influence of 1-hydroxyethylidene-1,1-bisphosphonate (HEBP) on dental root resorption in the mouse. Calcified Tissue International 45: 104–110

Wheeler T T, Stroup S E 1993 Traumatic root resorption in dentine-immunized mice. American Journal of Orthodontics and Dentofacial Orthopedics 103: 352–357

Yamasaki K, Miura F, Suda T 1980 Prostaglandin as a mediator of bone resorption induced by experimental tooth movement in rats. Journal of Dental Research 59: 1635–1642

Yamasaki K, Shibata Y, Imai S, Tani Y, Shibasaki Y, Fukuhara T 1984 Clinical application of prostaglandin $E_1$ ($PGE_1$) upon orthodontic tooth movement. American Journal of Orthodontics 85: 508–518

Zhou D, Hughes B, King G J 1997 Histomorphometric and biochemical study of osteoclasts at orthodontic compression sites in the rat during indomethacin inhibition. Archives of Oral Biology 42: 717–726