Influence of dietary n-3 polyunsaturated fatty acid on experimental tooth movement in rats

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Abstract: This study was conducted to investigate the influence of dietary n-3 polyunsaturated fatty acid on experimental tooth movement. This acid substantially reduces the production of arachidonic acid. Sixty 4-week-old male Wistar strain rats were divided into experimental and control groups. Animals in the experimental group were fed a purified diet containing 10% refined fish oil (rich in n-3 fatty acid); control animals were fed a diet containing 10% corn oil (rich in n-6 fatty acid). After 6 weeks, the maxillary first molars were moved buccally with an initial force of 20 g for periods of 0, 3, 7, or 14 days. Tooth movement in the experimental group was 80% of that seen in the controls. The number of osteoclasts on the pressure side during tooth movement was nearly 60% of that seen in controls, and the degree of bone resorption was 80%. The data suggest that a diet enriched with fish oil reduces osteoclastic activity and subsequent alveolar bone resorption that is the key to experimental tooth movement.

Key Words: Arachidonic acid, Bone resorption, n-3 polyunsaturated fatty acid, Experimental tooth movement, Prostaglandins

Dietary lipids contain two types of polyunsaturated fatty acids essential to the human body. The n-6 (also called as ω-6) and n-3 (ω-3) series are derived from linoleic and α-linolenic acids, respectively. The n-6 fatty acids produce arachidonic acid, which is released from cell membrane phospholipids mainly by the action of phospholipase A_2. Arachidonic acid can be converted to series 2 prostaglandins (PGs) or series 4 leukotrienes (LTs) by the cyclooxy-genase or lipoxygenase pathways. On the other hand, dietary supplementation of n-3 fatty acids, which are abundant in fish oil, reduces the production of arachidonic acid[1-3] and the products derived from it.[4-7] Thus, levels of arachidonic acid in phospholipids can be modulated by the type of dietary lipids ingested. It has also been reported that a diet enriched with fish oil (n-3 fatty acids) suppresses the inflammatory response in humans[8,9] and in animal models,[10,11] similar to non-steroidal anti-inflammatory drugs (NSAIDs).

Although the fatty acid composition of plasma and soft tissue influenced by dietary lipids has been studied extensively, very little information is available for bone. Alam et al.[12] investigated the fatty acid composition and levels of arachidonic acid in the alveolar bone of rats fed different lipids and indicated that the intake type of dietary lipids altered the fatty acid composition of bone lipids and the intake of the fish oil enriched diet significantly decreased the concentration of arachidonic acid.

Orthodontic tooth movement is accompanied by the appearance of osteoclasts and subsequent alveolar bone resorption, which may be mediated through the local production and action of PGs.[13-16] Inhibitors of PG synthesis, such as NSAIDs, could also inhibit the appearance of osteoclasts[13] and reduce the rate of tooth movement.[17,18] Since dietary n-3 fatty acids have actions similar to those of NSAIDs, it may be assumed that the intake of dietary lipids would affect bone remodeling and subsequent orthodontic tooth movement. Kokkinos et al.[19] demonstrated that arachidonic acid and PGE_2 concentration in the alveolar bone and orthodontic tooth movement were significantly lower in

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Submitted: April 1998, **Revised and accepted:** July 1998
Angle Orthod 1999;69(4):365-371.

rats fed a fish oil enriched diet compared with those fed a corn oil diet. However, the effect of a fish oil enriched diet on bone resorption incident to orthodontic tooth movement has not been elucidated.

The purpose of this study was to investigate the influence of dietary n-3 polyunsaturated fatty acids on experimental tooth movement in terms of histological changes in the periodontium.

Materials and methods

Animals

Sixty 4-week-old male Wistar strain rats, weighing from 63 to 78 g (mean weight 70.5 ± 4.8 g), were used. The rats were kept in cages in a room kept at 25°C with an alternating 12-hour light-dark cycle. They were given food and water ad libitum. The animals were divided into an experimental and a control group, each of which consisted of 30 rats. The experimental group was fed a purified diet containing 10% refined fish oil (rich in n-3 fatty acid), and the controls were given a diet containing 10% corn oil (rich in n-6 fatty acid).

Diet protocol

The basic diet (Clea Japan Co Ltd, Tokyo, Japan) was 24.5% casein, 41.5% cornstarch, 10.0% sucrose, 5.0% cellulose, 7% balanced mineral mixture, and 1% essential vitamin mixture, and was fat free. The diet was mixed with 10% corn oil or 10% refined fish oil (EPA-28, Tama Biochemical Co, Tokyo, Japan). The corn oil contained 50.5% linoleic acid, and the refined fish oil contained 28.3% eicosapentaenoic acid (EPA) and 13.4% docosahexaenoic acid (DHA). The remaining ingredients in both oils were saturated or monounsaturated fatty acids. The diets were administered daily for 6 weeks before experimental tooth movement, and weight was measured once a week.

Experimental tooth movement

Six weeks after initiation of the diets, the right and left maxillary first molars were moved buccally using a lateral expansion spring. Under general anesthesia with pentobarbital (40 mg/kg i.p.) a standardized expansion spring, fabricated with 0.012" inch nickel titanium wire (Nitinol, Unitek, Monrovia, Calif), was placed between the right and left maxillary first molars.[20] The spring was adjusted to deliver an initial force of 20 g on each side and was held in the mouth by its own expansive force (Figure 1). The force was applied continuously for 0, 3, 7, or 14 days (12, 16, 16, and 16 rats, respectively) without adjustment. Animals that lost the expansion spring during the experiment were excluded. The rats were fed continuously during the experimental period.

Measurement of tooth movement

To measure tooth movement, a silicone impression was taken of the maxillary dentition at the beginning and end of tooth movement. The distance between the crests of the mesiopalatal cusps of the maxillary first molars was measured on plaster models using a Measurescope (UM-2, Nikon, Tokyo) with a precision of 0.001 mm under 20x magnification. The difference in the distances before and after the experiment determined the amount of orthodontic tooth movement. Twenty randomly selected samples were measured by a single investigator in a blind test, the measurement error was found to be 0.012 mm. Errors were calculated as Error = $\sqrt{\Sigma d^2/2n}$, where d=difference between two measurements and n= number of samples.

Morphological examinations

At the end of the experiment, the maxillary bones were dissected and fixed in 10% neutral buffered formalin for 24 hours. Specimens in each group were decalcified in 14% EDTA solution (pH=7.4) for 2 weeks and embedded in paraffin. Tissue blocks were cut into serial cross-sections 5 mm thick. The sections were stained alternatively with hematoxylin and eosin, or for tartrate-resistant acid phosphatase (TRAP), by the method of Burstone[21] using naphthol AS-MX phosphate as a substrate. Hematoxylin was used for counterstaining. Periodontal tissues around the mesiobuccal root of the maxillary first molar at the bifurcation level were examined using a light microscope.

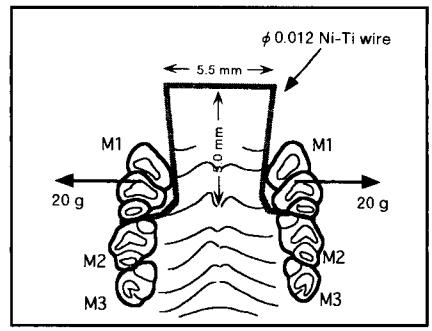

Figure 1
Schematic diagram of appliance used for experimental tooth movement. The expansion spring, fabricated with 0.012" nickel titanium wire, was placed between the right and left maxillary first molars. Arrows denote direction of force.

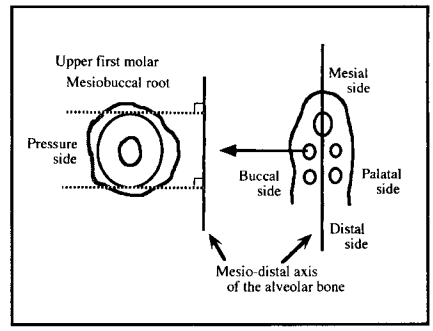

Figure 2
Schematic diagramof areas examined microscopically. Histological exam focused on pressure side of mesiobuccal roots of maxillary first molars. Bone resorption parameters measured in meshed area.

Measurement of bone resorption

In order to evaluate the degree of bone resorption on the pressure side, the following parameters

were measured on the TRAP-stained sections using a semiautomatic image-analyzing computer system linked to a light microscope (SP500F, Olympus, Tokyo, Japan). A measurement area was defined on the pressure side of the alveolar bone surface between two parallel lines perpendicular to the mediolateral axis of alveolar bone and tangent to the mesial and distal sides of the root (Figure 2). TRAP-positive multinucleated cells with resorption lacunae on the alveolar bone surface were identified as osteoclasts. Osteoclast surface was defined as bone surface perimeter (mm) where osteoclasts bordered. Eroded surface was defined as resorptive cavities perimeter (mm) whether or not osteoclasts could be seen. The parameters were (1) number of osteoclasts per measurement area (N.Oc), (2) osteoclast surface/bone surface (Oc.S/BS, %), (3) eroded surface/bone surface (ES/BS, %), and (4) TRAP-positive area/bone surface (TRAP$^+$S/BS, %).[22] Oc.S/BS, ES/BS, and TRAP$^+$S/BS were calculated as the rate to total bone surface for each measurement area. These parameters were measured on the five sections selected every four sections above and below the section 120 μm apart from the bifurcation level, and the means were obtained for each animal. The sections were taken from the anatomic area between the interradicular crest and one-sixth of the root length to the apex.

Statistical evaluation

The amount of tooth movement and the parameters of bone resorption for the two groups were subjected to two-way analysis of variance. If the analysis showed a significant difference between the two groups, the unpaired two-tailed *t*-test was applied to examine the mean differences between both groups for each experimental period.

Results

Changes in body weight

Changes in body weight during the experiment are shown in Figure 3. No significant differences in body weight were found between the groups at the beginning of feeding and experimental tooth movement. The different diets did not substantially affect general growth of the rats. In both groups, mean weight gain decreased slightly 3 days after the application of orthodontic force, and then gradually increased until the end of the experiment. At the end of the experiment, mean body weight was slightly greater in the fish oil group than in the controls, but the difference was not significant.

Experimental tooth movement

The amount of tooth movement as a function of time is shown in Figure 4. Three days after force application, the experimental teeth in the control group experienced movement of 0.542 ± 0.083 mm. The amount of tooth movement was slightly less in the fish oil group, but not significantly different from the controls. Then, tooth movement in both groups progressed relatively slowly up to 7 days. At 14 days, the amount of tooth movement in the control group increased to 1.082 ± 0.170 mm on average. The amount of tooth movement in the fish oil group was significantly less than in the controls (82.0% and 80.0% at 7 and 14 days, respectively).

Histological findings

Figures 5 and 6 show changes in the periodontal tissues on the pressure side around the mesiobuccal root of the maxillary first molar when examined using a light microscope. Before the application of force, almost no osteoclasts were induced on the smooth alveolar bone surface in either the fish oil or control group (Figures 5a and 6a). Three days after force application,

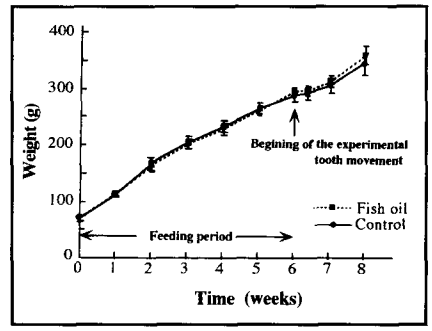

Figure 3
Mean changes in body weight in the control (solid line) and fish oil (dotted line) groups during experimental period. No significant differences between groups were found by means of Student's unpaired *t*-test.

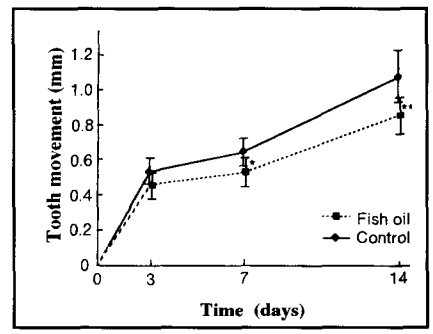

Figure 4
Amount of tooth movement in control (solid line) and fish oil (dotted line) groups. *$p<0.05$; **$p<0.01$ after two-way ANOVA. Day 0, n=6. Days 3, 7, and 14, n=8 for each group.

the periodontal space was compressed on the buccal side of the root and hyalinized degeneration of the periodontal ligament (PDL) appeared. Numerous osteoclasts were observed on the irregular bone surface around the hyalinized tissue and subsequent undermining bone resorption appeared in the control group (Figure 5b). In the fish oil group, little bone resorption and few osteoclasts were observed, and the hyalinized area of the PDL was prominent, compared with the controls (Figure 6b). At 14 days, it seemed that advanced bone resorption became more prominent in the control group, and the elimination of the hyalinized tissue was delayed in the fish oil group (Figures 5c and 6c). On the tension side, new

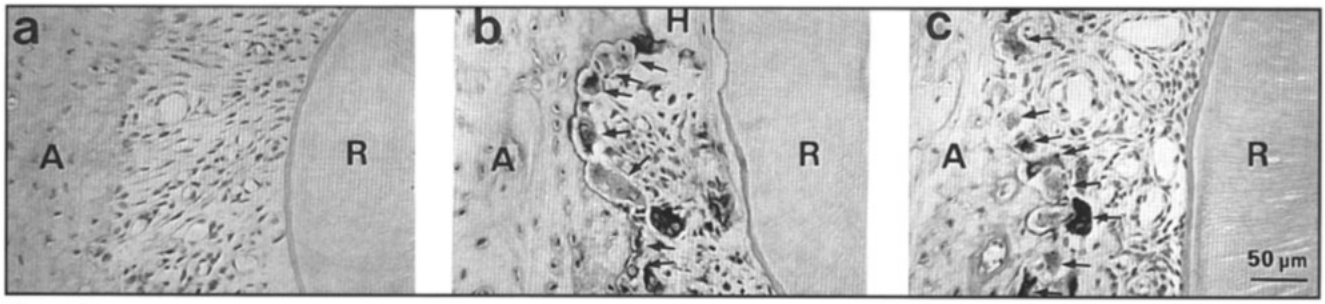

Figure 5

Histological changes of periodontal tissues on pressure side of mesiobuccal roots of maxillary first molars in control group: (a) before the application of force (day 0), (b) three days, and (c) 14 days after the application of force. Before application of force, there were almost no osteoclasts on the alveolar bone surface. On day 3, hyalinized degeneration of the periodontal ligament appeared and alveolar bone resorption by numerous osteoclasts was observed. Bone resorption of the mesial side was advanced on day 14. Arrows: osteoclast; A: alveolar bone; H: hyalinized tissue; R: mesiobuccal root of the first molar. TRAP stain. x 66.

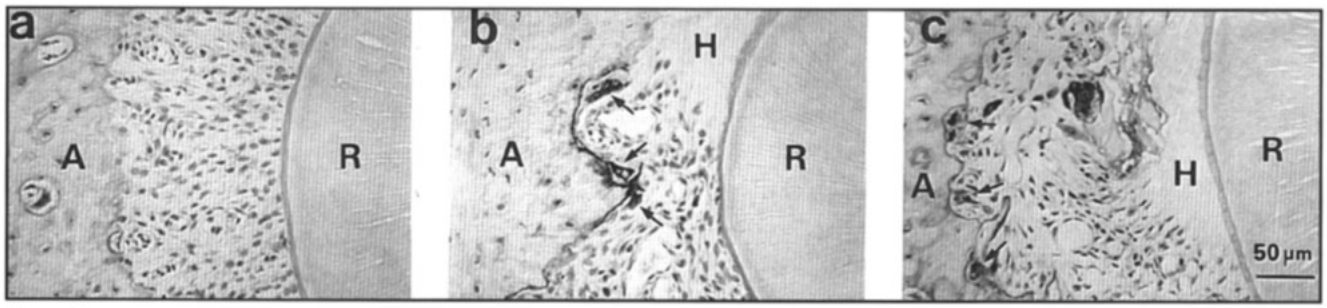

Figure 6

Histological changes of periodontal tissues on pressure side of mesiobuccal roots of maxillary first molars in fish oil group: (a) before the application of force (day 0), (b) three days, and (c) 14 days after the application of force. With the feeding of fish oil diet, the appearance of osteoclasts and bone resorption incident to tooth movement were decreased as compared with the controls. The hyalinized area of PDL was prominent on day 3 and the elimination of the hyalinized tissue delayed on day 14. Arrows: osteoclast, A: alveolar bone; H: hyalinized tissue; R: mesiobuccal root of the first molar. TRAP stain, x 66.

bone formation was observed along the stretched PDL fibers in association with increased osteoblasts in both groups. However, there were no distinct differences between two groups during the experimental period.

Changes in bone resorption parameters on the pressure side

Figure 7 shows longitudinal changes in the parameters of bone resorption on the pressure side during experimental tooth movement. Before the application of force, the mean values of all parameters were very low and exhibited no significant differences between groups. Three days after force application, the mean values of all parameters increased rapidly and were almost constant or slightly increased up to 7 days, although a slight decrease was found at 14 days. In the fish oil group, the mean value of N.Oc decreased significantly to 55.8%, 60.8%, and 58.2% of the controls at 3, 7, and 14 days, respectively. Oc.S/BS was also significantly less in the fish oil group on each day. ES/BS in the fish oil group showed significant decrease to 79.7%, 87.2% and 80.0% of the controls at 3, 7, and 14 days, respectively. $TRAP^{+}S/BS$ was slightly less in the fish oil group than in the control group, but no significant differences were found between the two groups.

Discussion

The present results demonstrate that fish oil diets, rich in n-3 polyunsaturated fatty acids, reduce experimental tooth movement. In the model used in this experiment, "the amount of tooth movement" might also include normal growth. However, the increase in intermolar distance due to normal growth would be negligible for a short experimental period because the animals used in this study were no longer growing rapidly.

Orthodontic tooth movement is produced by a repeated process of bone resorption and deposition. It has been demonstrated that bone resorption caused by the application of orthodontic force may be mediated through the local production and action of PGs. PGs have been shown to be induced in the periodontal tissues around teeth that are orthodontically moved.[15] It has also been found that local injection of PGE_1 or PGE_2 results in a dose-dependent increase in the appearance of osteoclasts at the site of tooth movement in rats[13] and accelerates the rate of tooth move-

ment in monkey models and humans.[23,24] The mechanisms by which mechanical forces induce PGE_2 production are speculated as follows. Mechanical stress may cause mechanical perturbation of the cell membrane phospholipids and increase the activity of phospholipase A_2, which stimulates the release of arachidonic acid, a precursor of PGs and LTs.[25] PGs produced by orthodontic stimuli cause intracellular cyclic AMP accumulation,[24] which further leads to cellular responses, such as cell transformation of osteoclast progenitor cells into osteoclasts.[26]

Dietary supplementation with n-3 fatty acids reduces the production of PGE_2, similar to the effect of NSAIDs. NSAIDs inhibit the synthesis of PGs by inhibiting cyclooxygenase, an enzyme that produces PGs from arachidonic acid. On the other hand, inhibition of PGE_2 synthesis by dietary n-3 fatty acid may be due to a competitive action between n-3 and n-6 fatty acids for the desaturation/chain elongation of linoleic acid to arachidonic acid.[4-6] Arachidonic acid, a precursor of PGE_2 in cell membrane phospholipids, was replaced by EPA, a precursor of PGE_3, PGI_3, LTB_5 and LTC_5, which have low biological activity. PGE_3 has been shown to have a potential for bone resorption. However, the relative proportion of PGE_3 was very small because the effect of EPA was one-tenth as a substrate for PGE_3, compared with arachidonic acid for PGE_2.[27] Consequently, dietary supplementation of n-3 fatty acids can result in the production of eicosanoids with altered or diminished biological activity.

Although the influence of plasma and soft tissue on fatty acid composition has been studied extensively, there are few studies that examine the influence of dietary lipids on bone remodeling or orthodontic tooth movement. Alam et al.[12] compared the effect of dietary lipids on fatty acid composition in the alveolar bone of rats and found that arachidonic acid concentrations in phospholipids in the maxillae of rats fed a fish oil enriched diet decreased to 38.3% of that of controls. Kokkinos et al.[19] demonstrated in rats fed a 10% fish oil diet that arachidonic acid and PGE_2 concentration in the maxillae decreased to nearly 30%, and the rate of tooth movement became nearly 80% of that of the rats fed a 10% corn oil diet. The inhibition of orthodontic tooth movement by feeding n-3 fatty acid-rich diet in a previous study[19] is consistent with the present results. Both studies conclude that diet-induced changes of arachidonic acid levels in the alveolar bone can influence bone resorption—the key to tooth movement—and the effect seems to be mediated by changes in PGE_2 levels in bone. Furthermore, this study demonstrated that the prevalence of osteoclasts and amount of bone resorption on the pressure side were inhibited by the fish oil diet. At day 0, the mean values of all parameters were low and exhibited no significant differences between groups; almost no osteoclasts were observed on the smooth alveolar bone surfaces in either group before the application of force. TRAP+S/BS exhibited no significant decrease in the fish oil group, but TRAP-positive surface, including both the resorption phase and the reversal phase of the bone remodeling cycle, does not always represent bone resorptive surface.

Dietary n-3 fatty acids also reduce the amount of LTB_4 derived from arachidonic acid by the lipoxygenase pathway.[7] It has been suggested that LTs are produced by bone tissue[28] and that inhibition of LT synthesis combined with mechanical stress reduces bone resorption and enhances bone

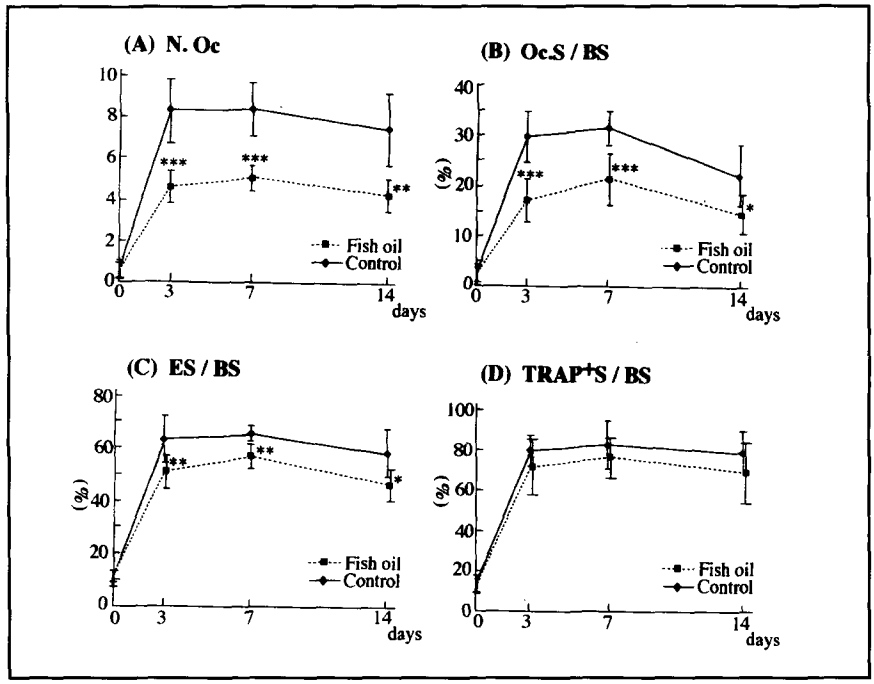

Figure 7
Changes in bone resorption parameters on pressure side of mesiobuccal roots of maxillary first molars in control (solid line) and fish oil (dotted line) groups. (A) number of osteoclasts, (B) osteoclast surface / bone surface, (C) eroded surface / bone surface, (D) TRAP positive area / bone surface. *$p<0.05$; **$p<0.01$; ***$p<0.001$ after two-way ANOVA. Day 0, n=6; days 3, 7, and 14, n=8 for each group.

formation.[29,30] Mohammed et al.[18] found that the oral administration of leukotriene synthesis inhibitor AA861 caused a significant inhibition of LTB_4 production and orthodontic tooth movement in rats. These findings emphasize that LTs might play a role in mediating bone resorption and subsequent orthodontic tooth movement. Moreover, Endres et al.[31] demonstrated that synthesis of interleukin (IL)-1α and IL-1β was suppressed by dietary supplementation with n-3 fatty acids. IL-1 induced bone resorption in connection with conversion of arachidonic acid into PGs.[32,33] The suppression of LTB_4 and IL-1 by dietary n-3 fatty acid may also be relevant to an inhibitory mechanism in bone resorption in addition to the suppression of PGE_2.

In this study, the difference in dietary lipids did not affect weight gain of the animals. However, it has been reported that the administration of NSAID on PG synthesis inhibitor altered bone growth.[34] It has also been suggested that PGE_2 may increase osteoblastic activity of bone in addition to stimulating osteoclasts.[35-37] Therefore, dietary lipids may affect systemic bone remodeling. The systemic effects of dietary n-3 polyunsaturated fatty acids on bone remodeling are not elucidated and thus further extensive studies are needed to clarify the issue.

Conclusions

The present study was conducted to investigate the influences of dietary n-3 polyunsaturated fatty acids on experimental tooth movement. The following results were obtained.

1. The amount of tooth movement in the fish oil group was 80% of that seen than in controls.

2. The number of osteoclasts and the degree of bone resorption on the pressure side during tooth movement was significantly lower in the fish oil group, nearly 60% and 80%, respectively, of the levels observed in controls.

From these results, it has been shown that a fish oil enriched diet reduces osteoclastic activity and the subsequent alveolar bone resorption that is key to experimental tooth movement.

Acknowledgments

This investigation was supported in part by a Grant-in-Aid for Scientific Research (B) from the Ministry of Education, Science, and Culture of Japan (No. 07457509).

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