Original Article

Evaluation of systemic Omega-3 PUFAs effect on orthodontic tooth movement in a rabbit model: RCT

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ABSTRACT

Objectives: To evaluate the effect of systemic administration of omega-3 fatty acids on orthodontic tooth movement (OTM) with histological analysis.

Materials and Methods: OTM was induced in 20 adult albino New Zealand rabbits, divided into omega-3 and control groups, with nickel-titanium coil springs for 21 days. Omega-3 or saline was given every day via oral gavage during the experimental period. Animals were sacrificed for histomorphometric analysis of alveolar bone remodeling after 21 days of OTM.

Results: A significant difference in OTM amount was found in the third week of OTM with means of 1.445 ± 0.13 and 1.72 ± 0.15 for the experimental and control groups, respectively. Histomorphometric analysis showed a significant reduction in the area of active bone-resorptive lacunae and a significant increase in osteoblastic activity in the omega-3 group after 3 weeks.

Conclusions: Strong evidence of the osteoclastic inhibitory effect of systemic omega-3 was found, which reduced the percentage and amount of OTM. (*Angle Orthod.* 2023;93:476–481.)

KEY WORDS: Omega-3; Rabbit; Systemic; OTM; Histology

INTRODUCTION

Orthodontic tooth movement (OTM) is characterized by remodeling changes involving dental and paradental tissues. At the compression side of the periodontal ligament (PDL), bone resorption is performed by osteoclasts and, at the tension side, bone deposition is achieved by osteoblasts.[1] Maintaining tooth anchorage during orthodontic treatment has challenged orthodontists since the beginning. Conventional methods for improving tooth anchorage aim at redirecting such forces to skeletal structures or distributing them over a larger number of teeth. Franzen et al [2] found that orthodontic relapse and OTM are associated with similar cellular adaptations, such as increased osteoclastic differentiation in compression areas. Given this background, it could be argued that endogenous or pharmacologic bone modulation to inhibit osteoclast resorption and promote osteoblast neoformation may have clinically relevant effects on the regulation of OTM and relapse. Recently, retention strategies are aimed at increasing alveolar bone density after cessation of OTM or controlling alveolar bone remodeling around tooth roots by influencing osteoblast and/or osteoclast activity to prevent tooth relapse.

For nearly four decades, the polyunsaturated fatty acids (PUSFs) family has been studied extensively for prevention and treatment of cardiovascular disease.[3] The health-promoting effects of omega-3 may be partially due to their immune-modulating and anti-inflammatory actions.[4] Although this was first described in cardiovascular disease, the potential role that inflammatory mediators play in metabolic bone diseases such as osteoporosis has caused investigators to extend studies of omega-3 to include skeletal outcomes.[5] Different mechanisms contribute to these effects, including conditioning cell membrane function and composition, eicosanoid production, and gene expression.[6] Omega-3, a polyunsaturated fatty acid, is composed of α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Omega-3 shows anti-inflammatory effects via decreasing the level of proinflammatory cytokines and inflammatory mediators such as arachidonic acid–derived eicosanoids ([Prostaglandin E2–(PGE2]).[4] In a rat study

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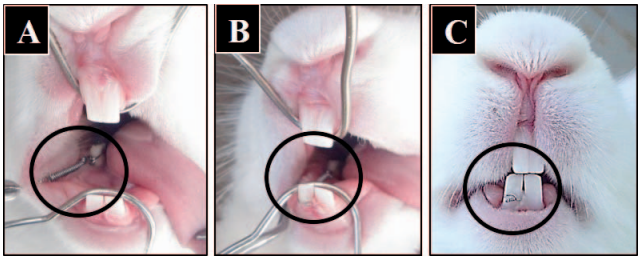

Figure 1. Intraoral photographs: (A) ligation of coil spring to first molar tooth; (B) ligation of coil spring to the incisor; and (C) light cure flowable composite application.

model, it was shown that omega-3 inhibited osteoclast activity and bone resorption while stimulating osteoblast activity and new bone formation.[7] Another review declared that omega-3 and omega-6 played a role in bone development and that omega-3 may improve bone health by increasing calcium absorption in the gut, increasing osteoblast differentiation and activity, reducing osteoclast activity, and promoting deposition of mineral in developing bones.[8]

There are few studies in the literature investigating the effect of omega-3 on OTM. Iwami-Morimoto et al.[9] found that a diet containing high omega-3 ratios decreased experimental tooth movement in rats more than other rich in omega-6. Kokkinos et al.[10] reported that the concentration of PGE2 and arachidonic acid in alveolar bones of rats fed with fish oil was lower than rats fed with corn oil. In addition, Ogrenim et al.[11] concluded that systemic administration of omega-3 showed antioxidant and anti-inflammatory effects with deceleration of OTM. This paucity of information about the effect of omega-3 in orthodontics indicates the need for a more structured research approach, in animal models and humans, to provide clinicians with more evidence-based results. In this study, an experimental rabbit model was used to explore the effect of systemic administration of omega-3 on OTM. It was hypothesized that the osteoinductive effects of omega-3 on the dental supporting tissues as well as the inhibition of osteoclastic activity might decrease OTM in rabbits. The null hypothesis was that omega-3 supplement would have no effect on OTM.

MATERIALS AND METHODS

Twenty adult male New Zealand albino rabbits (12 to 16 weeks old with body weight about 2.8 to 3.2 kg) were used for the experiment in the animal house of the Institute of Graduate Studies and Research, Alexandria University, Egypt. Throughout the study period, animals were examined daily by the veterinarian staff for evaluation of the general health status of each animal, weight loss, appliance breakage, gingival or soft tissue inflammation. The animals were maintained at a room temperature between 20° and 25° C with constant humidity, fed with standard ground ration and water. All procedures involving animals were in strict accordance with ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines[12] for conducting animal studies and this study was approved by the ethics committee of the Faculty of Dentistry, Kafr el Sheikh University, Egypt, which includes the institutional experimentation committee. Sample size was calculated using the formula for studies comparing paired continuous data[13] after a pilot study yielding a standard deviation of 0.55 mm at a study power of 90% and a significance level of 0.05.

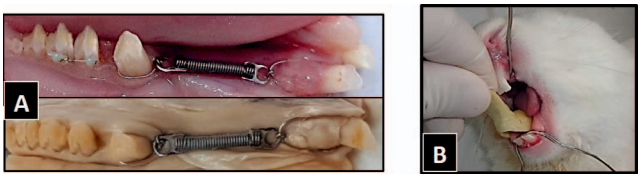

Figure 2. (A) Dissected rabbit mandible showing the appliance design and the diastema between molars after 21 days of active orthodontic tooth movement and (B) Impression using light body vinyl polysiloxane with custom special tray.

Rabbits were divided into two groups: the experimental group (Appliance + omega-3) and the control (Appliance + normal saline). All experimental procedures were performed under general anesthesia to maximize accessibility during operation. Intramuscular Ketamine was injected at a dose 50 mg/kg (Ketamine Alfasan 10%; Alfasan, Woerden, The Netherlands) and Xylazine (Xyla-Ject Injectable Solution; ADWA, 10th of Ramadan City, Egypt), a muscle relaxant, was administered in the same manner at a dose of 5 mg/kg for appliance placement. Adequate depth of anesthesia was determined by visual inspection of the tongue reflex when a dental mirror was inserted in the oral cavity.

Ligature wire (0.09 mm) was passed interdentally between the first and second molars and wrapped around the first molar. Similarly, ligature wire was also figure eight-tied around incisors, and twisted with artery forceps until they fit into the grooves. Then, nickel-titanium coil springs were tied to ligature wire between mandibular molar and incisors with 100 gram force, measured using a tensiometer (Morelli Orthodontic Tension Meter Force Gauge Intra/Extra Oral Elastics, Brazil). [14]A thin coat of flowable composite (Z350 XT flow, 3M ESPE, Calif, USA) was applied and light cured to avoid dislodgement of the appliance and to lessen irritation of any wire projections (Figure 1).

A piece of ligature wire was used to ligate the second molar to the third molar to prevent any possible movement of the second molar mesially by the effect of gingival interseptal fibers (Figure 2A).

Table 1. Comparison of Amount of OTM Between Groups[a]

OTM (mm)	Experimental (n = 10)	Control (n = 10)	*t*	*P*
1st wk				
Min.–Max.	0.5–0.75	0.5–0.8	2.260	.12
Mean ± SD	0.63 ± 0.20	0.69 ± 0.10		
Median	0.65	0.74		
2nd wk				
Min.–Max.	0..9–1..32	0.9–1.2	2.26	.65
Mean ± SD	1.07 ± 0.16	1.12 ± 0.15		
Median	1.1	1.1		
3rd wk				
Min.–Max.	1.2–1.7	1.32–1.9	2.2	.003*
Mean ± SD	1.44 ± 0.13	1.72 ± 0.15		
Median	1.43	1.73		

* Statistically significant at $P \leq .05$.
[a] OTM indicates orthodontic tooth movement.

Rabbits were equally and randomly assigned to: a control group receiving saline or an experimental group receiving omega-3 by oral gavage daily (200 mg/kg)[15] from day one of OTM.

Impressions of experimental teeth were performed on the 7th, 14th, and 21st days using injection of silicone vinyl polysiloxane impression material (3M ESPE Express Vinyl Polysiloxane Impression Material—Fast Set; 3M ESPE Dental Products, St. Paul, Minn) loaded into previously fabricated custom trays (Figure 2B). The impressions were then poured with the use of an improved die stone (Elite Rock Dental Stone, Zhermack, Badia Polesine, Rovigo, Italy).

The intermolar distance (IMD) was measured manually from the mesioocclusal margin of the second molar to the disto-occlusal margin of the first molar using a digital caliper with accuracy of 0.01 mm. Measurements were performed in a blinded fashion by a single investigator. The intraexaminer error for tooth movement measurements was assessed by repeating the measurements 2 weeks apart by the same investigator.

After 21 days of OTM, animals were sacrificed and their mandibles were dissected, cut into halves, fixed, and decalcified. Parasagittal serial sections of 6-mm thickness were obtained, and five randomly selected sections per specimen were processed. The sections were stained with hematoxylin and eosin. Sections along the mesial aspect of the root of the mandibular first premolar in each group were evaluated under a light microscope (Zeiss Primo Star Light Microscope; Carl Zeiss, Oberkochen, Germany) equipped with a 5-megapixel digital camera. Images of representative areas were captured and described.

Statistical Analysis

Statistical analysis was accomplished using Statistical Package for Social Sciences SPSS software (IBM SPSS Statistics for Windows, version 23; IBM, Armonk, NY). For comparisons between groups, Kolmogorov-Smirnov and Shapiro-Wilk tests were used to verify the normality of the distribution. Once verified, paired-samples *t*-test was conducted to compare mean values between groups. Otherwise, Wilcoxon signed-rank test was conducted.

Quantitative data were described using range (minimum and maximum), mean, standard deviation, and median. Significance of the obtained results was judged at the 5% level; differences with *P* values less than .05 were considered significant.

Table 2. Comparison of Percentage of OTM per Week Between Groups[a]

IMD (%)	Experimental	Control	t	*P*
1st wk of OTM				
Mean ± SD	43.75 ± 1.1	40.1 ± 2.63	0.600	.554
2nd wk of OTM				
Mean ± SD	30.55 ± 1.01	25.5 ± 3.61	2.270	.022
3rd wk of OTM				
Mean ± SD	25.69 ± 1.50	34.88 ± 2.90	2.362*	.017*

* Statistically significant at $P \leq .05$.
[a] IMD indicates intermolar distance; OTM, orthodontic tooth movement.

RESULTS

Clinical Results

The spring appliance was well tolerated and there was no statistically significant changes in body weight or food intake between groups.

After 3 weeks of force application, tooth movement of the first molars ranged from 1.3 mm to 1.9 mm, with a mean movement of 1.6 ± 0.3 mm. The amount and percentage of OTM through the 3 weeks are demonstrated in Tables 1 and 2:

In the first week, the amount and percentage of OTM in control and experimental groups were nearly the same: 0.69 ± 0.1 mm, 40.01%; and 0.63 ± 0.07 mm, 43.75%, respectively.

In the second week, the amount and percentage of OTM in control and experimental groups were nearly the same: 1.1 ± 0.01 mm, 25.5%; and 1.07 ± 0.15 mm, 30.55%, respectively.

In the third week, the amount and percentage of OTM between control and experimental groups were significantly different at 1.72 ± 0.15, 34.88%; and 1.44 ± 0.13, 25.69%, respectively.

Histological Results

Presence of a large number of active osteoclasts on the mesial surface of the socket was observed in the control group in contrast to the experimental group that showed a decrease in the osteoclast count (Figures 3,

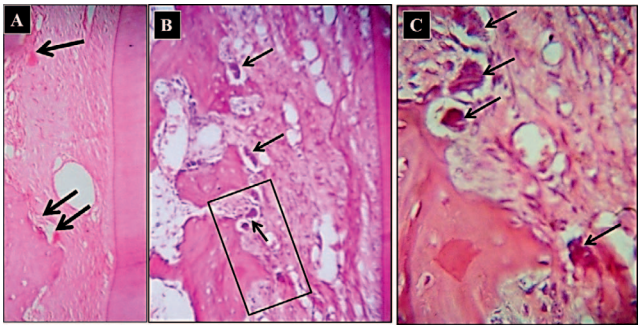

Figure 3. Light microscopic images from the mesial surface (compression) of the first mandibular molar in control group showing: (A) irregular surface of the alveolar bone with extensive bone-resorptive lacunae, (H&E stain, ×100), and (B, C) highly active voluminous osteoclasts, (H&A, ×400). AB indicates alveolar bone; PDL, periodontal ligament; R, root of mandibular first molar.

4, and 5) and the extent of bone resorption lacunae (Table 3).

DISCUSSION

Omega-3 acids are essential to normal growth and health. Recently, a strong relationship was found between these acids, bone health, and bone formation. Omega-3 acids were found to affect bone formation, bone resorption, serum calcium, and inflammatory mediators, but the exact mechanism of action has not yet been determined.[16]

The rabbit is one of the most widely used models for studying bone remodeling. In the current study, the rabbit model was chosen because rabbits have faster skeletal change and bone turnover (significant intracortical, Haversian remodeling) compared to other species such as primates and some rodents.[17]

Tissue reactions to orthodontic forces in adult humans start within 2 days after force application. However, in rodents, tissue reactions start within 30 minutes of force application.[18] Kilic et al.[19] showed that tooth movement in

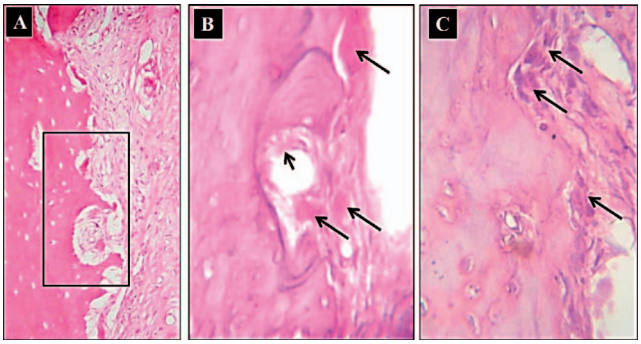

Figure 4. Light microscopic images from the mesial surface (compression) of the first mandibular molar in experimental group showing: (A) irregular surface of the alveolar bone with shallow bone-resorptive lacunae, (H&E stain, ×100), and (B, C) small osteoclast (arrows), (H&A, ×400).

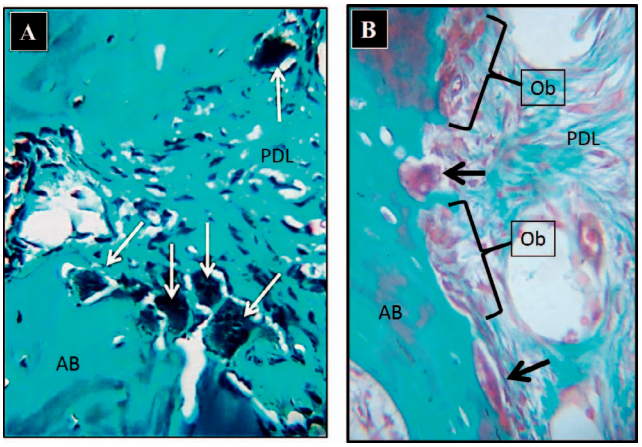

Figure 5. Light microscopic images from the mesial surface (compression) of the first mandibular molar comparing between: (A) large number of voluminous osteoclasts in the control group, and (B) small scattered osteoclasts (arrows) enclosed between active osteoblasts (Ob) in the experimental group, (Trichrome Stain, ×400).

rabbits occurred in three phases: an initial phase, an arrest or lag phase, and an acceleration or progressive movement phase within the first 20 days of OTM. Therefore, the study period chosen was for 21 days.

The spring design with a 100 cN orthodontic force was chosen in accordance with other studies.[14] Intermolar distance (IMD) was measured with a digital caliper on stone casts so it would be repeatable, easier, and more accurate than measuring directly while the animal was under anesthesia.

The daily omega-3 dosage (200 mg/kg) was selected based on a previous animal study by Clubb et al.[15] as an optimal minimal dose. However, Karunia et al.[20] used higher doses of omega-3 (750–1500 mg/kg), which decreased OTM, but another study on rats by Al-Hashemi et al.[21] found bone resorption and bone mineral imbalance at higher doses.

In the first week of OTM, almost half of the total OTM percentage was found in both groups (experimental group: 43.75% and control group: 40.01%), followed by 30.55% and 25.5%, respectively. The initial increase in the amount of tooth movement during the first week, followed by a decrease in the amount of tooth movement during the second week, was in agreement with previous studies by Iwami-Morimoto et al.[9] and Kokkinos et al.[10]

Table 3. Comparison of Osteoclast Count Between Groups After 21 days of OTM

Parameter	Control	Experimental	Test of sig	*P*
Osteoclast count	4.33 ± 0.49	2.33 ± 0.98	2.14	.0008*
Area of active bone-resorptive lacunae	0.33 ± 0.11	0.21 ± 0.09	2.1	.002*

* Statistically significant at $P \leq .05$.

A significant decrease of OTM was found in the experimental group compared to the control group after 3 weeks of OTM. This delay of action was due to oral gavage administration, which was in agreement with Azuma et al.[22] and Al-Hashemi et al.[21]

Histologically, the appearance of osteoblasts in the experimental group either on mesial or distal surfaces of the socket was noticeably greater than their appearance in the control group. On the other hand, osteoclasts were noticeably smaller, less frequently encountered, and less active than those observed on the same surfaces of the control teeth. Iwami-Morimoto et al.[9] showed that fish oil enriched diets reduced osteoclastic activity and the amount of alveolar bone resorption on the pressure side, which was in agreement with the current study and other studies in mice,[23] rats,[22,24] rabbits,[20] dogs,[25] and humans.[26]

Previous studies that had examined the effect of dietary lipids on alveolar bone remodeling or OTM were consistent with results of the current study on the inhibitory effect of omega-3 on alveolar bone resorption. Alam et al.[24] found a relationship between the type of dietary lipids and the fatty acid composition of bone lipids. Kokkinos et al.[10] revealed that an omega-3 enriched diet had an inhibitory effect on OTM. Both studies concluded that lipid diets induced changes in archidonic acid level in alveolar bone with accompanied changes in prostaglandin levels. Additionally, the current study used oral gavage to deliver and assure intake of the proper amount of omega-3 supplement.

The current study revealed that osteoclast count and OTM were directly correlated, highlighting the importance of bone remodeling during the OTM and relapse phases. This was in agreement with a study done by Dolci et al.[27] that concluded that statin-induced osteoprotegerin (OPG) overexpression reduced relapse after OTM, by a phenomenon correlated with decreased osteoclast counts. However, considering the overall osteoclast number and OTM findings, the control group had a high osteoclast count with high activity in the compression site, whereas the omega-3 group exhibited the opposite profile. Once the essential elements (osteoclastic activity, number, and diminished cellular infiltrate) for bone remodeling are reduced, OTM or even relapse is affected.

More studies on the effect of systemic omega-3 on OTM and on relapse are needed. This may be helpful during long-term retention after orthodontic treatment. Additionally, a longer-term study lasting more than a 3-week period is required, with histologic analysis of tissues at multiple timepoints to overcome some of the current study's limitations.

CONCLUSIONS

- The results of the present study indicate that systemic administration of omega-3 polyunsaturated fatty acids could reduce the amount of OTM due to its osteoclastic inhibitory effect.

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