J Oral Res
research in oral sciences

Article

Effects of ambient oxygen pressure on orthodontic tooth movement.

Efectos de la variación en la presión de oxígeno ambiental sobre el movimiento dentario ortodóntico.

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Receipt : 10/25/2020 Revised: 04/26/2021
Acceptance : 12/31/2021

Abstract: Objective: To evaluate the effects of variation in ambient oxygen pressure on orthodontic tooth movement in guinea pigs. Material and Methods: Seventy-two guinea pigs randomly distributed into two groups (A and B) were evaluated in the study. All specimens were fitted with orthodontic appliances to distalize maxillary incisors. Group A was controlled under conditions of oxygen pressures at sea level (150 masl, 157 mm Hg) and Group B under conditions of oxygen pressures at altitude (3405 masl, 107 mm Hg). The clinical (distance between the distal-incisal angles of the maxillary incisors), biochemical (serum alkaline phosphatase), and histopathological characteristics (osteoblast and osteocyte count) were evaluated before placing the orthodontic devices and after 24 and 72 hours. Results: In the clinical evaluation, the distance between the distal-incisal angles of the maxillary incisors, on day one and three, was significantly higher in group B compared to group A (p=0.002 and p=0.001, respectively). In the biochemical evaluation, the level of serum alkaline phosphatase on the first and third days was significantly higher in group B compared to group A (p=0.001 and p=0.001, respectively). In the histopathological evaluation, the osteoblasts and osteocytes count on day one and three was significantly higher in group B compared to group A (p<0.05). Conclusion: Oxygen pressure at high altitude positively influenced orthodontic tooth movement in guinea pigs, improving its clinical, biochemical, and histopathological characteristics.

Keywords: *guinea pigs; oxygen; hypoxia; osteocytes; orthodontic tooth movement.*

Resumen: Objetivo: Evaluar los efectos de la variación en la presión de oxígeno ambiental sobre el movimiento dentario ortodóntico en cobayos. Material y Métodos: Participaron 72 cobayos distribuidos aleatoriamente en dos grupos (A y B). A todos se les colocó dispositivos ortodónticos para distalizar los incisivos maxilares. El Grupo A fue controlado en condiciones de presiones de oxígeno a nivel del mar (150 msnm, 157 mm Hg) y el Grupo B en condiciones de presiones de oxígeno en altura (3405 msnm, 107 mm Hg). Se evaluaron las características clínicas (distancia entre los ángulos incisodistales

Cite as: Chumpitaz-Cerrate V, Chavez-Rimache L, Aguirre-Siancas E, Franco-Quino C, Ruiz-Ramirez E & Caldas-Cueva V. Effects of ambient oxygen pressure on orthodontic tooth movement. J Oral Res 2021; 10(6):1-11. doi:10.17126/joralres.2021.077

de los incisivos maxilares), bioquímicas (fosfatasa alcalina sérica) e histopatológicas (conteo de osteoblastos y osteocitos) antes de colocar los dispositivos ortodónticos y después de 24 y 72 horas. **Resultados:** En la evaluación clínica, la distancia entre los ángulos incisodistales de los incisivos maxilares, al primer y tercer día fue significativamente superior en el grupo B en comparación al grupo A (p=0,002 y p=0,001; respectivamente). En la evaluación bioquímica, el nivel de fosfatasa alcalina sérica, al primer y tercer día fue significativamente mayor en el grupo B en comparación al grupo A (p=0,001 y p=0,001; respectivamente). En la evaluación histopatológica, el conteo de osteoblastos y osteocitos, al primer y tercer día fue significativamente mayor en el grupo B en comparación al grupo A (p<0,05). **Conclusion:** La presión de oxígeno en altura influyó positivamente sobre el movimiento dentario ortodóntico en cobayos, mejorando las características clínicas, bioquímicas e histopatológicas.

Palabras Clave: *cobayos; oxígeno; hipoxia; osteocitos; movimiento dental ortodóntico.*

INTRODUCTION.

Orthodontic tooth movement (OTM) is produced by a combination of bone apposition in the tension zone and bone resorption in the compression zone, after the application of mechanical forces on a tooth.[1]

The periodontal ligament provides physiological mobility to the tooth under occlusal loads.[1,2] The main function of the periodontal ligament is to prevent the concentration of mechanical stress when the occlusal forces generated during chewing are transferred to the alveolar bone.[3] In addition, it increases the cellular reactions that promote bone remodeling during OTM.[3,4]

The cascade of biological events that induce OTM is initiated by mechanical stresses in the periodontium that are transferred to the teeth with the use of orthodontic mechanical devices.[5] An active orthodontic device produces a system of forces in the crown of the tooth, which consists of a combination of moments and controlled forces, which at the moment of creating tension in the periodontal ligament maintains or increases the blood flow in the alveolar crest on the side towards which the force is directed.[5] While in the compression zone, morphological changes are induced at the vascular level, reducing blood flow and producing ischemia.[6,7] This causes changes in the partial pressure of oxygen, modifying the chemical environment by releasing biologically active agents such as prostaglandins and cytokines.[7,8] These changes cause local hypoxia,[9] which has an effect on the cells of the periodontal ligament, through the release of chemical mediators related to resorption (IL-1 beta, IL-6, IL-8, TNF-alpha, substance P) or bone apposition (osteocalcin and leptin) and can be found in crevicular gingival fluid. This has been evidenced by the analysis of crevicular gingival fluid.[7-9]

Studies such as those conducted by Lie et al.12 reported that both compressive force and hypoxia play an important role in the initiation of osteoclastogenesis during OTM.

Furthermore, Kitase *et al.*,[6] demonstrated that mechanical loading under conditions of mild hypoxia allows the cells of the periodontal ligament to produce important adaptive responses for bone remodeling. One point to consider is that most of the studies have been performed *in vitro* under simulated hypoxic conditions, unlike the present study, which was performed *in vivo* under natural hypoxic environmental conditions. Considering that altitude variability *in vivo* can generate different degrees of hypoxia at the tissue level, compensatory mechanisms are produced that promote the formation of hypoxia-induced

ISSN Print 0719-2460 - **ISSN Online** 0719-2479.

factor (HIF-1α).[13] HIF-1α stimulates angiogenesis and osteogenesis, potentially influencing the magnitude of the OTM.[9,13] Therefore, it is important to consider, during the planning of orthodontic treatment, to what extent ambient oxygen conditions may influence OTM in populations located at different altitude levels.

Furthermore, the present study is an initial exploration of the effects of low ambient oxygen pressures (hypoxia) on OTM in an experimental guinea pig model. The aim of the present study was to evaluate the effects of variation in ambient oxygen pressure on orthodontic tooth movement in guinea pigs.

MATERIALS AND METHODS.

Ethical considerations

The present study followed the recommendations of the World Medical Association (WMA) on the use of animals in biomedical research.[10] Likewise, during the procedure, the recommendations of the Institutional Animal Care and Use Committee (IACUC, ILAR) and the current regulations of the Animal Protection Law (Law No. 27265) were complied with.[11] This study was authorized by the research institute of the School of Dentistry of Universidad Nacional Mayor de San Marcos, Peru.

Experimental animals

The sample consisted of 72 Andean guinea pigs, 8 weeks old, weighing 1000 +/- 100 g. The specimens were obtained from the Animal Facility of the National Institute of Agrarian Innovation (INIA, Lima-Peru). Animals were selected through a simple random sampling of 36 guinea pigs for each group (A and B). The guinea pigs of Group A were conditioned and acclimatized for 7 days in the Animal Facility of the School of Medicine of the Universidad Nacional Mayor de San Marcos (150 masl, 157 mm Hg).

Group B guinea pigs were transferred to the INIA Experimental Animal Facility Station in Zurite-Anta (Cusco) (3405 masl, 107 mm Hg), where they were conditioned and acclimatized for 7 days to avoid potential stress caused by transport. All guinea pigs were kept at room temperature, in alternating light/dark periods of 12 hours beginning at 8 am and were given water and balanced food ad libitum.

Treatment protocol

The 72 guinea pigs were anesthetized intramuscularly with Ketamine (Ketalar®) 30 mg/Kg. All guinea pigs underwent baseline clinical evaluation (distance between the distal-incisal angles of the maxillary incisors), using a 0.01 mm precision caliper (Mitutoyo®). The distance between the distal-incisal angles of the maxillary incisors was evaluated as the horizontal measurement from the distal-incisal angle of the crown of the upper left incisor to the distal-incisal angle of the crown of the upper right incisor.

Then, 12 guinea pigs from each group (A and B) were randomly selected for baseline biochemical evaluation (serum alkaline phosphatase) of blood samples (5 mL drawn *per* animal). Subsequently, these guinea pigs were euthanized with an overdose of Pentobarbital (Halatal®) 1 mL/250 g administered intraperitoneally, to take samples from the maxilla for baseline histopathological evaluation in the area of interradicular alveolar bone tension (osteoblast and osteocytes count).

The samples were fixed in formalin (10%) and decalcified in nitric acid (5%) for 15 days. Later they were embedded in paraffin, cut into sections, and adhered to a slide for hematoxylin-eosin staining.

Finally, they were covered with a coverslip. A pathologist prepared the sample using sagittal cuts of the tooth and alveolar bone, following the longitudinal axis of the tooth. Subsequently, the analysis of the cell count was carried out using a light microscope (MCX100 LCD Crocus II, Micros®).

Four random fields were observed focusing on the area of tension of the interradicular alveolar bone of the maxillary incisors. Then the cell count of osteoblasts and osteocytes was performed clockwise, taking an average of the four observations.

Each microscopic area observed was 0.458 mm^2 with a total magnification of 400X. The histological characteristics of the osteoblasts corresponded to cells located in the region of bone formation with basophilic cytoplasm and a single highly developed nucleus located towards the other end of the bone apposition. Osteoclasts were recognized as larger multinucleated cells with a ruffled border.

Subsequently, the vestibular surface of the maxillary incisors of the remaining 24 guinea pigs from each group (A and B) were etched with 37% O-phosphoric acid on for 30 seconds. A bracket (Generus® STD U1® .018 Slot) was used for the upper incisor, it was cut longitudinally into two halves and each half was placed on the maxillary right and left incisors, respectively.

For the adhesion of the brackets, a light-curing resin (Transbond XT 3M®) was used, photoactivated with an LED lamp (3M®) for 5 seconds. Finally, the orthodontic device was placed in the brackets to distalize the maxillary incisors. The orthodontic device was made with 0.014 beta-titanium alloy (TMA) wire bent twice, with a diameter of 2 mm and arms of 4 mm, with a reciprocal action force of 35 g, regulated with a dynamometer. (Figure 1A and Figure 1B) The arms of the device were placed in the slot and kept fixed with an elastic in each bracket. The orthodontic device was kept in that position for 3 days.

Twenty four hours after installing the orthodontic devices (day 1), 12 guinea pigs were randomly selected from each group (A and B). They were anesthetized with ketamine (Ketalar®) to perform the clinical evaluation (Figure 1C). Then, blood samples were taken from the heart of guinea pigs (5 mL) for biochemical evaluation. Subsequently, the guinea pigs were euthanized with an overdose of pentobarbital (Halatal®) and samples from the maxilla were obtained for histopathological evaluation.

After 72 hours of installing the orthodontic devices (day 3), the 12 remaining guinea pigs in each group underwent the same procedures for clinical (Figure 1D), biochemical, and histopathological evaluation. The evaluations were performed at 24 and 72 hours considering the protocol followed by Yabumoto *et al.*,[12]

Statistical analysis

The statistical package SPSS version 22.0 was used for the statistical analysis. For the quantitative variables, measures of central tendency (mean) and dispersion (standard deviation) were used. For the bivariate analysis, the Student's t-test was used for independent samples, considering a confidence level of 95% ($p<0.05$).

RESULTS.

Clinical Evaluation (distance between distal-incisal angles of the maxillary incisors)

In the present study, no differences were found in baseline values between the group evaluated at sea level and the one evaluated at a given altitude ($p=0.10$). On the first day, at sea level the distance between the distal-incisal angles of the maxillary incisors was 5.59±0.47 mm and at altitude it was 5.93±0.18 mm ($p=0.002$). On the third day, at sea level, the distance was 5.72±0.24 mm and at altitude it was 6.31±0.16 mm ($p=0.001$),(Table 1).

Biochemical evaluation (serum alkaline phosphatase)

There were no differences in the baseline values between the group evaluated at sea level and at altitude ($p=0.95$). On the first day, the serum concentration of alkaline phosphatase at sea level was 106.61±11.55 U/L, and at altitude it was 172.66±5.61 U/L ($p=0.001$). On the third day, at sea level, the serum concentration of alkaline phosphatase was 131.57±12.35 U/L, and at altitude it was 196.08±8.89 U/L ($p=0.001$), (Table 2).

Histopathological evaluation (count of osteoblasts and osteocytes)

Osteoblasts

There were no differences in the baseline values between the group evaluated at sea level and at altitude ($p=0.39$). On the first day, at sea level the number of osteoblasts was 20.08±3.34, and at altitude it was 24.25±4.24 ($p=0.015$). On the third day, at sea level, the number of osteoblasts was 24.91±4.23, and at altitude it was 30.66±3.77 ($p=0.002$), (Table 3 and Figure 2).

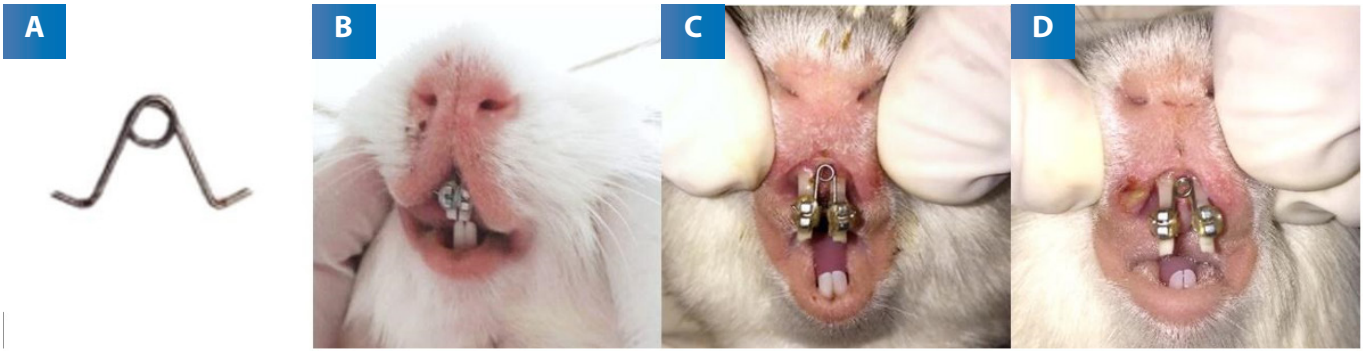
Osteocytes

There were no differences in the baseline values between the group evaluated at sea level and at altitude ($p=0.754$). On the first day, at sea level the number of osteocytes was 15.66±2.74, and at altitude it was 20.16±3.01 ($p=0.001$).

On the third day, the number of osteocytes at sea level was 17.08±3.57, and at altitude, it was 21.25±3.69 ($p=0.01$), (Table 3 and Figure 2).

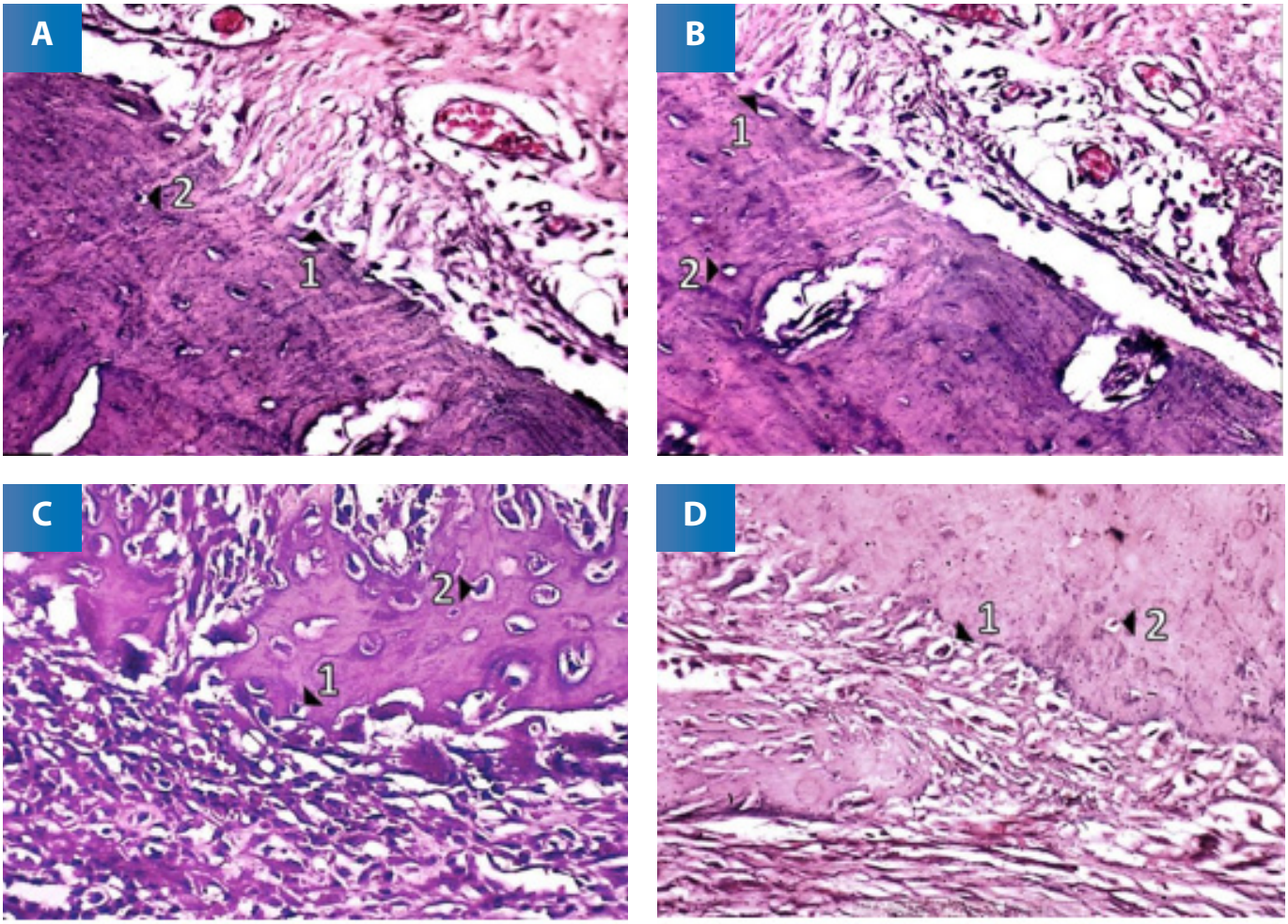
ISSN Print 0719-2460 - **ISSN Online** 0719-2479.

Figure 1. Orthodontic device evaluation sequence.



A: Initial placement of the orthodontic device. **B:** Initial placement of the orthodontic device. **C:** Front view of the activated orthodontic device on the first day of evaluation. **D:** Front view of the activated orthodontic device on the third day of evaluation.

Figure 2. Microphotographs of representative areas of each study group are observed at 400x magnification and stained with hematoxylin and eosin; with the presence of 1: osteoblasts, 2: osteocytes.



A: Group A (day 1). Osteoblasts are observed around bone trabeculae, a large number of fibroblasts and blood vessels. **B: Group A (day 3).** A moderate trabecular density with osteocytes and osteoblasts is observed. **C: Group B (day 1).** Large numbers of osteoblasts are seen around the bone trabeculae. **D: Group B (day 3)**. A high trabecular density with osteocytes and osteoblasts is observed.

Table 1. Distance between distal-incisal angles of the maxillary incisors in the groups evaluated at sea level (150 masl) and at altitude (3405 masl).

Distance between distal-incisal angles (mm)	Group	Mean	SD	CI 95%		*p**
				Min	Max	
Basal	At sea level	3.36	0.14	3.31	3.40	0.10
	At altitude	3.44	0.11	3.40	3.48	
Day 1	At sea level	5.59	0.47	5.29	5.89	0.002
	At altitude	5.93	0.18	5.81	6.04	
Day 3	At sea level	5.72	0.24	5.62	5.83	0.001
	At altitude	6.31	0.16	6.24	6.38	

***:** *p*-value of Student's t statistical test for independent samples. **SD:** Standard Deviation. **CI:** Confidence interval.

Table 2. Serum concentration of alkaline phosphatase in the groups evaluated at sea level (150 masl) and at altitude (3405 masl).

Alkaline Phosphatase (U/L)	Group	Mean	SD	CI 95%		*p**
				Min	Max	
Basal	At sea level	103.01	9.56	96.94	109.09	0.95
	At altitude	102.83	7.34	98.16	107.50	
Day 1	At sea level	106.61	11.55	99.27	113.95	0.001
	At altitude	172.66	5.61	169.09	176.23	
Day 3	At sea level	131.57	12.35	123.72	139.42	0.001
	At altitude	196.08	8.89	190.42	201.73	

***:** *p*-value of Student's t statistical test for independent samples. **SD:** Standard Deviation. **CI:** Confidence interval.

Table 3. Count of osteoblasts and osteocytes in the groups evaluated at sea level (150 masl) and at altitude (3405 masl).

Alkaline Phosphatase (U/L)		Group	Mean	SD	CI 95%		*p**
					Min	Max	
Osteoblasts	Basal	At sea level	13.16	0.71	12.71	13.01	0.39
		At altitude	12.83	1.11	12.12	13.54	
	Day 1	At sea level	20.08	3.34	17.90	22.25	0.015
		At altitude	24.25	4.24	21.55	26.94	
	Day 3	At sea level	24.91	4.23	22.22	27.60	0.002
		At altitude	30.66	3.77	28.26	33.06	
Osteocytes	Basal	At sea level	8.83	1.64	7.78	9.87	0.754
		At altitude	8.66	0.77	8.17	9.16	
	Day 1	At sea level	15.66	2.74	13.92	17.49	0.001
		At altitude	20.16	3.01	18.25	22.07	
	Day 3	At sea level	17.08	3.57	14.80	19.35	0.01
		At altitude	21.25	3.69	18.90	23.59	

***:** *p*-value of Student's t statistical test for independent samples. **SD:** Standard Deviation. **CI:** Confidence interval.

ISSN Print 0719-2460 - **ISSN Online** 0719-2479.

DISCUSSION.

In the present study, it was evidenced that, under high altitude conditions, hypoxia favored osteogenesis, increasing the number of osteoblasts and osteocytes in the tension area of the interradicular alveolar bone, the serum concentration of alkaline phosphatase, and the distance between distal-incisal angles of the maxillary incisors, at 24 and 72 hours, compared to what was found at sea level.

These results show a positive modulatory effect of altitude on the clinical, biochemical, and histopathological characteristics of OTM.

Many studies have shown the relationship between the variation in ambient oxygen pressure and bone formation. It has been observed that the decrease in ambient oxygen pressure increases the expression of mediators that promote bone formation.[13-15]

Zhang *et al*.,[16] suggest that hypoxia in high altitude conditions generates a marked expression of various molecules, among which hypoxia-inducible factor 1 (HIF-1) and vascular endothelial growth factor (VEGF) stand out. VEGF participates in angiogenesis and osteogenesis, in which it fulfills different biological functions such as increasing vascular permeability, promoting monocyte chemotaxis, and regulating endochondral ossification.

HIF-1 is a heterodimeric transcription factor, and it is the main transcriptional regulator of adaptive responses to hypoxia. It is composed of two subunits: alpha (α) and beta (β). Under hypoxic conditions, the α subunit activates HIF-1 and stabilizes it. In addition, it promotes angiogenesis, stimulates cell proliferation, and prevents cell death.[17,18]

Wei *et al*.,[18] studied a preclinical model in rats subjected to orthodontic forces to evaluate the expression of HIF-1α and VEGF. It was found that when VEGF was inactivated, it decreased angiogenesis and trabecular bone formation. This evidences that VEGF plays an important role in bone formation and orthodontic tooth movement.

These results were similar to previous studies, which indicated that HIF-1α activated VEGF, which in turn increased bone remodeling.[19,20] HIF-1 is a sensitive factor that responds to hypoxia. It presents high activity when tissue oxygen is between 0-2%.[10]

Wei *et al*.,[18] suggest that the increase in HIF-1α is not only caused by hypoxic conditions, but it can also occur under normoxic conditions. Some studies such as the one conducted by Murshid *et al*.,[21] reported that, during orthodontic tooth movements, osteocytes participate as sensitive bone mechanoreceptors, directing the bone apposition process in the tension zone and modulating bone resorption in the pressure zone. Lerner *et al*.,[22] reported the importance of osteo-blasts in the production of bone extracellular matrix and in the mineralization processes on the tension side of alveolar bone.

The regulatory role played by osteoblasts on the osteoclastic function on the pressure side of the alveolar bone through the formation of osteocalcin was also highlighted. In the present study, a higher osteogenic expression (osteoblasts and osteocytes) and a greater distance between the distal-incisal angles of the maxillary incisors were found under high altitude conditions, on day one and three, compared to the group evaluated at sea level. These findings agree with those reported by Wei *et al*.,[18] who applied 30 g of force between the first molar and the maxillary incisor in rats and found a marked increase in HIF on the first, third, and seventh days, and at 2 weeks. This increase in HIF is involved in osteogenic differentiation.

On the other hand, Zhang *et al*.,[17] found that applying 50 g of force between the first molar and the maxillary incisor in rats produced a high expression of HIF in the periodontal ligament. Likewise, in human cells of the periodontal ligament, HIF-1 is associated with osteogenic differentiation under hypoxic conditions.

Mingyuan *et al*.,[7] found that hypoxia promoted the signaling of transforming growth factor β1 by the action of HIF-1α, and both substances promote collagen deposition. Yu *et al*.,[23] and Niklas *et al*.,[24] suggested that the hypoxic microenvironment caused by the orthodontic mechanical forces applied between the alveolar bone and the root favors the appearance of HIF-1.

Furthermore, Yu *et al*.,[23] found that HIF-1α may be

involved in osteoblastic differentiation of primitive mesenchymal bone stem cells, which modulate osteogenic metabolism during the application of a mechanical force in a hypoxic environment.

Regarding alkaline phosphatase (ALP), an important osteogenic marker, Sun *et al*.,[25] showed that ALP decreased significantly in rats with streptozotocin-induced diabetes, finding that the rats had a high risk of bone fracture and decreased osteogenesis. Pujari-Palmer *et al*.,[26] demonstrated that chronic exposure to pyrophosphate stimulates genes involved in osteoblast differentiation, specifically those associated with ALP activity. On the other hand, it has been determined that ALP increases its expression and activity under hypoxic conditions.[27,28]

Alkaline phosphatase is an important marker in osteoblastic activity. A study carried out by Nizet *et al*.,[29] highlights the critical role of alkaline phosphatase in the processes of biomineralization and bone turnover, emphasizing its low variability over time and greater reliability for the longitudinal evaluation of bone turnover compared to parathormone.

On the other hand, in 2020 Vimalraj[30] reported the high expression of alkaline phosphatase in mineralized tissues and its importance in bone formation, promoting osteogenesis by increasing local rates of inorganic phosphorus, and the reduction of an extracellular inhibitor of mineralization such as pyrophosphate.

In this study, a higher expression of alkaline phosphatase was found in the group evaluated at altitude, on the first and third days, compared to the group evaluated at sea level. This coincides with the findings of Yu *et al*.,[23] who reported that the enzyme increased in a hypoxic environment *in vitro* at 2 and 6 hours of evaluation.

Although the hypoxic environment in this study was induced by natural conditions and in the study by Yu *et al*.,[23] it was induced in vitro, in both hypoxic conditions the response was similar, resulting in an increase in ALP.

Some of the limitations of the present study are that it is a preclinical study with guinea pigs in which the orthodontic device was placed in the incisors, because these teeth, due to their continuous growth, could have influenced the tooth movement. However, incisor growth in guinea pigs is slow (1-2 mm per week) and all evaluations were performed within the first 72 hours.

It is recommended that clinical research using varying magnitudes of orthodontic forces, from weak to strong, be conducted to induce tooth movement and to assess how force magnitude influences HIF expression. In addition, it is advisable to carry out an evaluation for a longer period. Likewise, it would be useful to evaluate the effect of paracetamol and NSAIDs on orthodontic tooth movement in hypoxic conditions.

In addition, the present study could be considered exploratory in nature, so it is recommended that further studies evaluate other biochemical and immunohistochemical parameters. Finally, the use of guinea pigs as experimental models at altitude is adequate, due to their genetic adaptation and tolerance to altitude. In addition, the anatomy and size of their teeth provide significant advantages for the installation of orthodontic devices.

CONCLUSION.

Based on the results of this study, it can be concluded that the group that was exposed to high-altitude oxygen pressure conditions presented an increase in orthodontic tooth movement.

This was evidenced by the increase in the distance between the distal-incisal angles of the maxillary incisors and the increase in serum alkaline phosphatase levels and the number of osteoblasts and osteocytes in the area of interradicular alveolar bone tension.

ISSN Print 0719-2460 - **ISSN Online** 0719-2479.

Conflict of interests: No authors have a conflict of interest.

Ethics approval: study protocol approved by the research institute of the School of Dentistry of Universidad Nacional Mayor de San Marcos, Perú.

Funding: Subsidized 160501011 (RAIS UNMSM).

Authors' contributions: Chumpitaz-Cerrate V: conception, design of the study and writing of the original draft. Aguirre-Siancas E: writing of the original draft. Franco-Quino C: writing of the original draft. E. Ruiz-Ramirez: writing of the original draft. Chavez-Rimache L: Analysis and interpretation of the data, writing of the original draft. Caldas-Cueva V: Bibliographic review. All authors participated in the execution of the study.

Acknowledgements: Our most sincere thanks to the Universidad Nacional Mayor de San Marcos for providing us with financial support to carry out this research work.

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