Growth Modification of the mandible using Ultrasound in Monkeys: A preliminary report.

Abstract

Introduction: Previous studies have shown that growth modification in treating patients having a skeletal Class II malocclusion due to mandibular deficiency is still controversial. Also, it has been shown that low intensity pulsed ultrasound (LIPUS) can modify mandibular growth in growing rabbits. To apply such treatment to humans, it is essential to study its effect on mandibular growth in higher animal models. The objective of this study was to evaluate the effect of using LIPUS on mandibular growth in baboon monkeys. **Methods:** Fourteen juvenile male Hamadryas baboon monkeys were used and were divided into two groups of seven each. One group received bite jumping appliances (jumper group) and the other group did not (control group). In both groups, the left temporomandibular joint area received LIPUS (stimulated) and the right side served as the control (nonstimulated). Before euthanization, after four months of starting treatment, all monkeys were injected with 99-mTc MDP (methylene diphosphonate). The heads were decapitated and scanned for bone growth using a dual head gamma camera. The mandibles were then dissected, sectioned into two halves and radiographed for anthropometric measurement. The condyles were harvested and processed for histological examination. **Results:** Results showed that LIPUS increased mandibular length and growth activity in all treated sides ($P < .001$) especially in the jumper group.
Conclusions: LIPUS enhances mandibular growth in growing baboon monkeys, especially when combined with anterior mandibular jumping appliances.

Key words: Growth modification, ultrasound, primates, mandible, functional appliance.

Introduction

Class II malocclusion is mainly due to mandibular deficiency and not maxillary excess.[1,2] For many adult patients having a Class II malocclusion with mandibular deficiency, the optimal overall results are best obtained via a surgical-orthodontic approach.[3] However, treating such malocclusions in growing patients using bite jumping appliances is believed to produce satisfactory improvement in the facial esthetics and minimizes the need for surgical intervention later on.[4] There is evidence that compensatory growth occurs at the mandibular condyle in response to altered occlusal function in young, growing animals.[5-7] Long-term mandibular adaptation to protrusive function was studied in monkeys.[8] After 48 weeks, there was significant increase in condylar growth and in the overall mandibular length in the treated animals. At the end of the 144-week experimental period, the mandibles of the treated animals were 5 to 6 mm longer than those of the control animals. The McNamara and Bryan 1987 study did not support the hypothesis that the mandible has a genetically predetermined length.[8] This was confirmed by another study.[9] Also, it has been reported that the mandibular condyle is essential for mandibular ramus growth.[10] Studies on normal growth in monkeys reported that no major normal growth changes occur after 3.5 to 4 years of age in monkeys.[11]

Bone scan (bone scintigraphy) has been used to investigate growth activity and to diagnose temporomandibular joint (TMJ) disorders as it has the potential to detect active bone metabolism and remodeling.[12,13]

The biological effect of different types of ultrasounds was tested and it was concluded that the use of LIPUS has an optimal biological effect in promoting tissue healing and stimulation when applied for three weeks on a daily basis for 15-20 minutes with intensity ranging between 30 to 50 milliwatts per cm^2 of the transducer's surface area.[14,15] Many studies were conducted to evaluate the effect of LIPUS on calcified tissues. LIPUS was reported to stimulate proteoglycan synthesis in rat chondrocytes by increasing the aggrecan gene expression [16, 17] and to increase the platelet-derived growth factor (PDGF) secretion from bone cells.[18] Also, LIPUS was found to enhance the growth of the tibia of the young rat [19], bone growth in rabbits[20], bone fracture healing[21-22] and facilitate bone maturation after mandibular osteodistraction.[23,24] Also, LIPUS when applied to a growing rabbit's temporomandibular joint area, increases mandibular ramal height and stimulates mandibular condylar growth both clinically and histologically.[25] In order to apply such treatment to humans, it is essential to study its evidence and effect in an animal model that is close in phylogenetic affinity to humans. The objectives of this preliminary study were to evaluate the effects of therapeutic ultrasound on mandibular condylar and total mandibular growth with and without the use of bite jumping appliances in monkeys and to justify its future use in humans.

Materials and methods

This research was approved by the Research council at King Abdul Aziz University (KAAU). For this preliminary study, fourteen juvenile male Hamadryas baboon monkeys (Papio hamadryas hamadryas) were chosen and divided into two groups of seven each. One group received bite jumping appliances (jumper group) and the other group did not (control group). In both groups, one side of the mandible received LIPUS (stimulated) and the other side served as the self-control (nonstimulated). The average age of the animals was 2.6 ± 0.6 years at the beginning of the experiment as was determined by the presence of the deciduous dentition and the erupted first permanent molars. The skeletal growth status of these animals was assessed using hand-wrist radiographs that confirmed that the animals were still growing. These monkeys are similar to humans in their early childhood (pre-adolescence) stage.[26] For all experimental procedures, the animals were sedated with Ketamine HCl (7 to 15 mg/kg intramuscularly) and Rompun (Xylazine) (1 to 2 mg/kg intramuscularly). Mandibular and maxillary alginate impressions were taken for each animal, soaked in antiseptic solution (Cidex) and poured with hard dental stone to produce a study model with bite registration. The maxillary and mandibular midlines were checked at the beginning of the experiment. Anterior mandibular bite jumping appliances were constructed for each animal so as to produce a protrusive occlusal relationship as previously described by McNamara and Bryan (1987)[8] but with the addition of forward positioning Forsus springs (3M Unitek, Monrovia, California, USA). The Forsus springs were added to insure the forward positioning of the mandible, as it was felt that the bite jumping appliance alone did not produce adequate forward positioning of the mandible. The springs were activated once at the time of appliance cementation. The appliances were cemented to the lower teeth using light cured glass ionomer cement (Fuji, GC America Inc., Alsip, IL, USA). In a few animals the appliances were removed by the animals. Whenever this happened, the appliances were re-cemented and secured in place with transosseous wiring.

The appliances produced a vertical (inferior) displacement of 2 to 3 mm and a horizontal (anterior) displacement of 5 mm. At the same time all animals were receiving ultrasound treatment on the left side (stimulated) and the right side served as the control (nonstimulated) for the left side. The LIPUS was applied for 20-minutes/day for four months and the control groups had the transducer, inactively placed over the temporomandibular region. LIPUS was applied using a 2.5 cm lead zirconate-titanate transducer and consisted of a 200-microsecond burst of 1.5 MHz sine waves repeating at 1 kHz that delivers 30 mW/cm^2 incident intensity (Exogen Inc., West Caldwell, NJ, USA).[20-25] Ultrasound power for each ultrasound device was calibrated before and after each application using the method described by Rooney (1981).[27]

Bone scan:

All animals were sacrificed at the end of four months by perfusion with neutral buffered formalin. Before sacrificing the animals, all animals were sedated and injected with 4 millicurie per year of age of 99-mTc MDP (methylene diphosphonate). The heads were cut then planner and tomographic images were obtained and the region of interest (ROI) was chosen on the right and left TMJ areas of the bone scan images to determine the amount of 99-mTc MDP uptake by each side. Also, a curve in the amount of 99-mTc MDP uptake on serial slices was obtained using the ROI for the right and left TMJ areas.

Radiographic measurements of the mandible:

After bone scanning, the animals heads were fixed in 10 % buffered formalin for two weeks, and then the mandibles were carefully dissected with the TMJ intact. The mandibles were then split into two halves at the symphyseal sutures that were not fully ossified and therefore making it easy to split the mandibles. The two mandibular halves were fixed to a radiographic cassette and radiographed using the following X-ray machine setting (65 kVp [peak kilovoltage], 300 mA [milliampere], and 1/60 second).

The outlines for each hemi-mandible were traced using regular acetate tracing papers and the mandibular length for each hemi-mandible was measured from the condylion (most superior and posterior point on the convexity of the mandibular condyle) and the gnathion (a point on the contour of the mandibular length that is corresponding to the point of intersection of a tangent to the anterior outline of the mandibular symphysis and tangent to the inferior mandibular plane) (Figure 1).

Histological examination:

The right temporomandibular joints were removed and decalcified using 10% nitric acid. Tissue blocks were then embedded in paraffin, sectioned at 6µm thickness sections, and stained with hematoxylin and eosin (H&E). A histological study was performed using a light microscope for qualitative evaluation and histomorphometric analysis.

Histomorphometric analysis:

Histomorphometric analysis was performed as follows. For each specimen, a microscopic field was chosen in the cartilaginous area immediately under the fibrocartilaginous area. The total area, which is 20 mm^2, was selected. These fields were captured by a Panasonic Video Camera mounted on a light microscope (BX-60, Olympus, Japan). The microscopic field included in the analysis was focused on the condylar head region of each microscopic section. The total surface area of bone trabeculae was used to assess the histological results. This parameter was calculated using image analysis software (Image-J, version 1.31, NIH, Bethesda, MD, USA). Bone trabeculae were automatically color-coded after selection of the appropriate threshold that ensures count of trabeculae and excludes other non-desired structures. The color-code threshold of bone trabeculae was automatically calculated as a perimeter of trabecular structure that was more than 20 pixels and had a grayscale range of 50-130. The collected variables were analyzed and compared between groups by ANOVA test using SPSS software.

Error test

Subsets of five records for mandibular length, bone trabecular area and bone scan count were measured twice by two different investigators. The two measurements for each variable were compared using Pearson's correlation coefficient. The correlation coefficients between the two measurements were found to be r= 0.92, r=0.90 and r=0.87 for mandibular length, bone trabecular area and bone scan uptake percentage measurements respectively. This indicated that the measurement techniques were reproducible.

Results

Clinical findings:

Figure 2 shows the intraoral photographs of the animals in both groups after completion of the treatment where there is midline deviation in all animals to the right side (nonstimulated sides). This midline deviation is more prominent in the animals that had both bite jumping appliances and LIPUS (Figure 2a) than those with LIPUS only (Figure 2b).

Bone scan findings:

Figures 3a and 3b and table I show the findings of the bone scan slices of the animals in the bite jumping appliance and control groups respectively with the curve representing the 99-mTc MDP uptake by the ROI in the right and left sides of the TMJ. It can be seen that the 99-mTc uptake is more in the jumper group than in the control group.

Radiographic measurements:

Table II shows the comparison between both groups. It can be seen that both groups had almost similar mandibular lengths as there was no statistical difference between the nonstimulated sides (right side) in both groups. However, it can be seen that there was a statistical difference between both nonstimulated (right) and stimulated (left) sides in both groups, which shows clearly the stimulatory effect of LIPUS in mandibular growth in both groups. Also, it can be seen that the anteriorely jumping appliance promoted the stimulatory effect of the ultrasound in the jumper group (appliance and LIPUS) more than in the LIPUS-only group as there was a statistically significant difference between the stimulated (left) mandibular lengths in both groups.

Histological/histomorphometric analysis

Figures 4 and 5 show the histological sections of the right (nonstimulated) and left (LIPUS stimulated) treated condyles of the jumper group. It is clearly seen that the condylar cartilage on the right side (nonstimulated side) showed bone replacement as indicated by bony trabecular invasion of the condyles and also decreased thickness of the condylar cartilage when compared with the normal condyle (nonstimulated side of the control group) (figure 6). Similar findings can be noticed in comparing figures 5 and 7 as more bone formation and maturation can be noted in the jumper group stimulated side than in the control group stimulated side (figure 7) that shows more thickening of the condylar cartilage and less bone formation when compared with figure 5.

Table III shows the comparisons between the histomorphometric analysis of the right (nonstimulated) and left (stimulated) condyles in both groups and also between both groups.

Discussion

This study evaluated the effect of LIPUS on mandibular condylar growth with and without the use of anterior bite jumping appliances in growing baboon monkeys. A previous report showed that LIPUS of similar parameters can stimulate mandibular growth in rabbits. Before conducting clinical trials in humans, the technique had to be evaluated on higher animals that have more phylogenetic affinity to humans, such as monkeys.[28] Previous studies have shown that the maximum adaptive response in the condylar cartilage occurs after delivering functional protrusive appliances in monkeys reached at 4-6 weeks.[8] Also, previous research has shown that the maximum stimulatory effect of ultrasound can be achieved after 3-4 weeks of application.[15] However, the clinical evidence of the stimulatory effect was not evident until after four months of ultrasound application in monkeys compared to the results obtained in four weeks in rabbits.[25] This may be due to the different phylogenetic response and life span of each animal species.[28] The nonsignificant difference between the mandibular lengths on the nonstimulated (right) sides in both groups should not be explained as that the functional appliances alone have no stimulatory effect on growth modification, as might be inferred. A better explanation may be that the jumping appliances have produced some effect as evidenced from the histological changes in the condyles when compared to the stimulated (right) side in the control group (Figures 4 and 6). However, no significant anthropometric changes in mandibular length can be noted over the experimental period. This evidence justifies performing more studies to evaluate the long-term effect of LIPUS and with and without the use of anteriorly jumping appliances. Another possible explanation to that is the increased mandibular shift in the jumper group due to the synergetic effect of the bite jumping appliance could potentially adversely affect the growth of the non stimulated sides. Again, this could be studied better if a non treatment control group was used. The increased thickness of the condylar cartilage in the control group compared to the jumper group indicates the advent of bony tissue replacing hypertrophic cartilaginous zone in the jumper group that had more stimulation with the bite jumping appliance. This is in agreement with Shen et al., 2005 [29] and with Tang and Rabie, 2005 [30] who pointed out that once the condyle undergoes more endochondoral bone formation and replacement, the condylar cartilage decreases in thickness. Also, it can be noticed that the formed endochondoral bone replacement in the jumper group (Figure 5) is more mature than the woven bone in the control (Figure 7). The whole qualitative histological findings are parallel to the quantitative histomorphometric analysis in that in the jumper group more bone formation was noted when compared to the control group, especially in the LIPUS stimulated sides. The difference between the nonstimulated sides in both groups is parallel to those of mandibular length measurements.

Excessive bone formation detected in all LIPUS treated condyles, is in agreement with the previous research that suggested the stimulatory effect of LIPUS on the bone cells and osteogenesis in UMR – 106 cells.[31] It was also found that LIPUS has an enhanced anabolic effect on mouse bone – marrow cells, possibly due to its thermal effect.[32] The biological effects of the LIPUS may have been caused by the pressure waves of ultrasound mechanical perturbation. These pressure waves could mediate biological activity of the cell membrane by changing the influx and efflux of potassium ions through the cell membrane.[33] The increased blood vessels shown in the LIPUS-treated condyles' bone marrow spaces in both groups (figures 5 and 7) suggested that LIPUS stimulated bone formation through increased angiogenesis. This is in agreement with a previous report on the stimulatory effect of LIPUS on bone formation as mediated by the increase in new blood vessels formation.[34] Also, this is in agreement with other studies which showed that bone growth, repair, and remodeling are dependent on the formation of new blood vessels (angiogenesis).[35,36]

The results obtained in this study show that combining the ultrasound and functional appliances produces a more synergetic effect on promoting mandibular growth. This was evidenced by the increase in the growth activity indicated by bone scan, total mandibular length, and bony trabeculae as compared to the animals treated by ultrasound only. Even though the results obtained with the control group that received ultrasound only was small, relative to that in the jumper group, it was still significant and in agreement with the previous rabbit study that showed LIPUS can induce growth modification of the mandibular condyle by itself, although with different treatment durations.[25]

Conclusion and recommendation

The results of this research show that LIPUS can stimulate and modify the growth pattern of the mandible in growing baboon monkeys especially when it is used with functional appliances. More studies are needed to evaluate the long-term stability and the molecular basis of the obtained clinical results.

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Figure legends:

Figure 1: Cephalometric tracing of half of the monkey mandible showing the linear measurement of the mandibular length.

Figure 2: Clinical photograph showing midline deviation of a monkey in the jumper appliance group (a) and in the control group (b) after the experiment.

Figure 3: Bone scan of the monkey skulls showing the ROI for the right and left TMJ areas in the jumper appliance group (a) and in the control group (b). It can be seen that more uptake is noticed in the stimulated sides than in the nonstimulated sides, and this is also greater in the jumper appliance group than in the control group.

Figure 4: (Jumper appliance group, nonstimulated side) Light photomicrograph of the articular surface of the condyle after using the bite jumping appliance showing dense fibrous covering, fibrocartilaginous layer, and bony trabeculae (H&E stain X200).

Figure 5: (Jumper appliance group, stimulated side) Light photomicrograph of the condyle after using LIPUS and bite jumping appliance treatment showing thin dense fibrous covering, mineralized fibrocartilaginous layer, and highly matured/organized bony trabeculae with few marrow spaces (H&E stain X200).

Figure 6: (Control group, non-stimulated side) Light photomicrograph of normal condyle showing thick dense fibrous covering, fibrocartilaginous layer and normal bony trabeculae. (H&E stain X200)

Figure 7: (Control group, stimulated side) Light photomicrograph of the articular surface of one of the LIPUS-treated condyles showing thicker layer of dense fibrous covering, fibrocartilaginous layer, and bony trabeculae noticeably more lined by active osteoblasts than in the normal condyle (H&E stain X200).

Figure2a

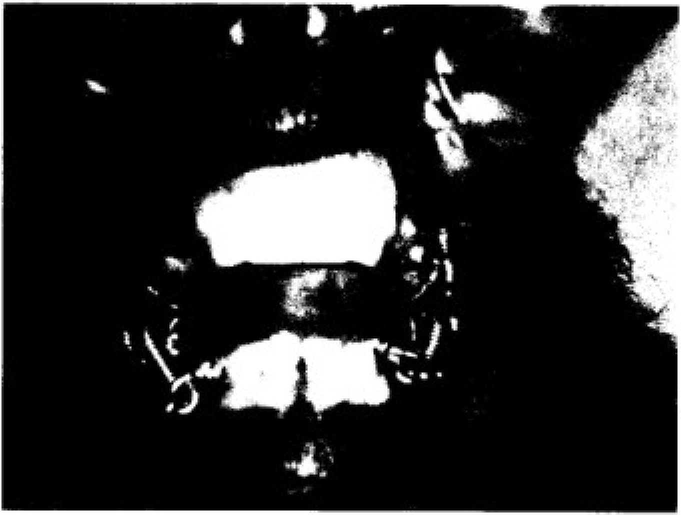
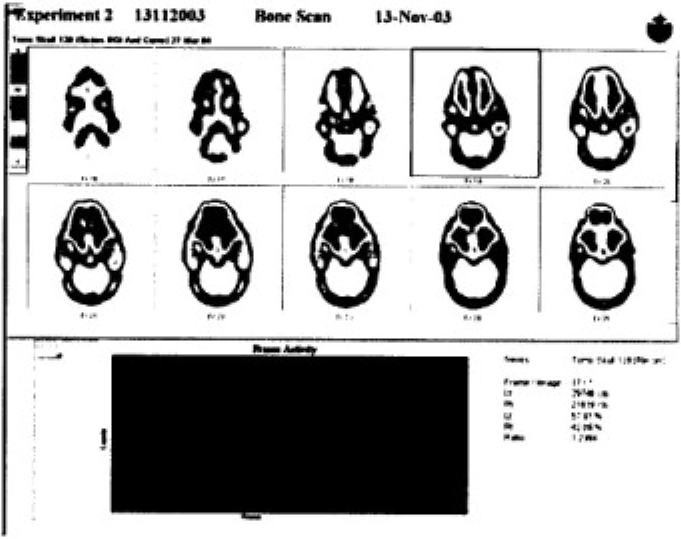


Figure2b



Experiment 2 13112003 Bone Scan 13-Nov-03

Figure3b

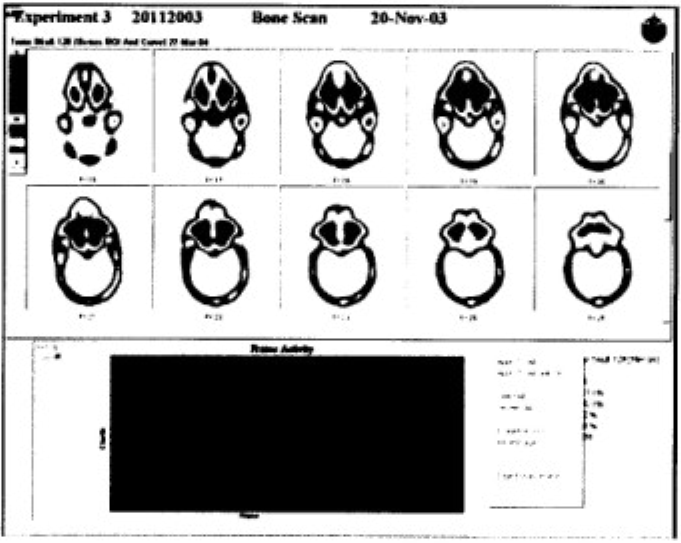


Figure4

Figure5

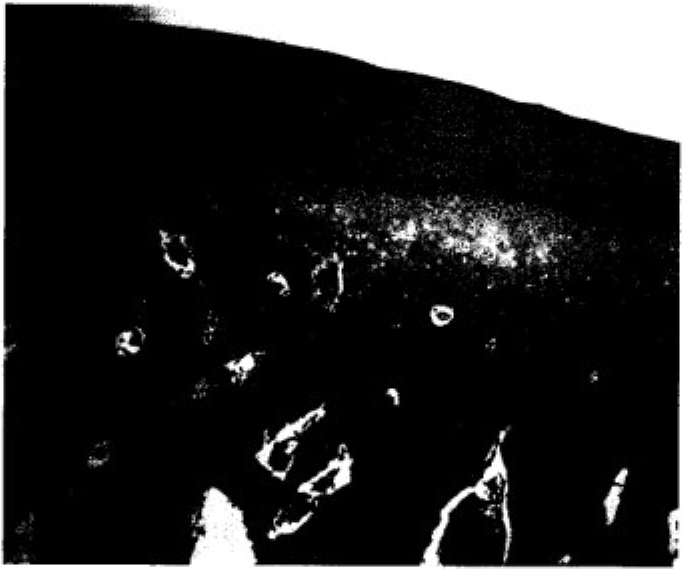


Figure6

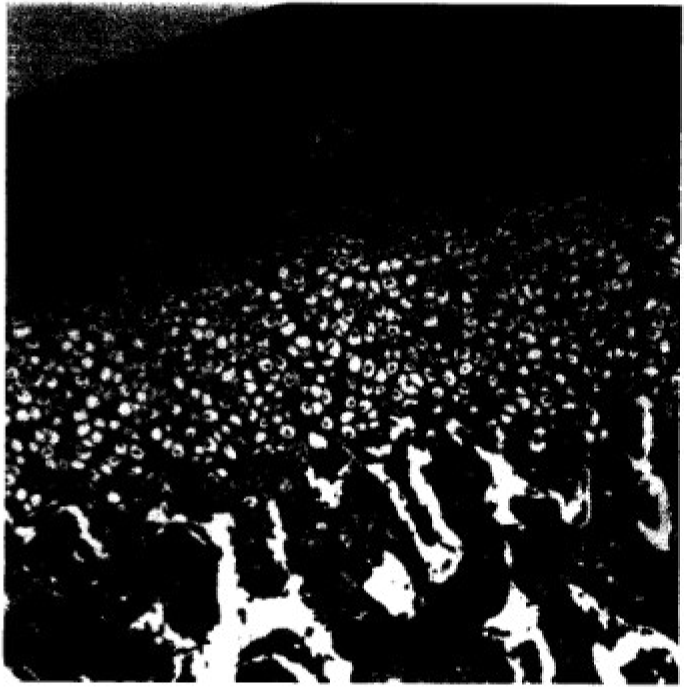


Figure7

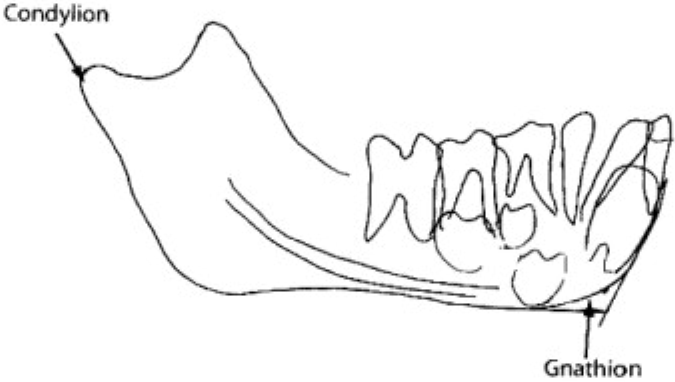

Condylion
Gnathion

Table I. Comparison by ANOVA test of bone scan represented as the percentage of the radionuclide uptake by the right and left condylar area between both sides in each group and between both groups.

Jumper Appliance Group (G1)			Significance	Control Group (G2)			Significance
Right	Left	Diff		Right	Left	Diff	
43.8 ± 1.6	56.6 ± 2.2	12.8± 3.4	$P < .001$	46.07 ± 1.6	53.2 ± 1.9	6.1 ± 2.5	$P < .001$
Right side (nonstimulated)				Left side (stimulated, LIPUS)			
G1	G2	Diff		G1	G2	Diff	
43.8 ± 1.6	46.07 ± 1.6	-2.2 ± 2.6	$P < .05$	56.6 ± 2.2	53.2 ± 1.9	3.5 ± 3.8	$P < .005$

Table II. **Comparison by ANOVA test of the anthropometric measurements of the right and left mandibular lengths in mm between both sides in each group and between both groups in mm.**

Jumper Appliance Group (G1)			Significance	Control Group (G2)			Significance
Right	Left	Diff		Right	Left	Diff	
76.2 ± 1.7	79.3 ± 1.7	3.1 ± 0.7	P< .001	76.2 ± 2.1	77.9 ± 1.6	1.7 ± 0.6	P < .001
Right side (nonstimulated)				Left side (stimulated, LIPUS)			
G1	G2	Diff		G1	G2	Diff	
76.2 ± 1.7	76.2 ± 2.1	0.07 ± 2.5	P>.05	79.3 ± 1.7	77.9 ± 1.6	1.4 ± 2.2	P<.05

Table III. **Comparison by ANOVA test of the total surface area in mm^2 of bone trabeculae in the right and left mandibular condyles between both sides in each group and between both groups.**

Jumper Appliance Group (G1)			Significance	Control Group (G2)			Significance
Right	Left	Diff		Right	Left	Diff	
2.5 ± 0.3	3.8 ± 0.3	1.3 ± 0.3	P< .001	2.3 ± 0.4	3.1 ± 0.5	0.7 ± 0.3	P < .001
Right side (nonstimulated)				Left side (stimulated, LIPUS)			
G1	G2	Diff		G1	G2	Diff	
2.5 ± 0.3	2.3 ± 0.4	0.23 ± 0.59	P> .05	3.8 ± 0.3	3.1 ± 0.5	0.78 ± 0.6	P< .001