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STRAIN INDUCED OSTEOGENESIS OF THE CRANIOFACIAL SUTURE UPON CONTROLLED DELIVERY OF LOW-FREQUENCY CYCLIC FORCES

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1. ABSTRACT

Static forces have been used for more than a century to modulate osteogenesis of craniofacial sutures in not only laboratory research, but also clinical practice. Whether cyclic forces more effectively stimulate sutural osteogenesis than static forces is unknown. Here, the premaxillomaxillary sutures of growing rabbits received *in vivo* exogenous static forces with peak magnitude of 2 Newtons, or cyclic forces also at 2 Newtons but with frequencies of 0.2 Hz and 1 Hz. The static force and two cyclic forces did not evoke significant differences in the peak magnitude of static bone strain (506 µstrain±182; mean±S.D.), 0.2-Hz cyclic strain (436 µstrain±191) or 1-Hz cyclic strain (461 µstrain±229). However, cyclic forces at 0.2 Hz delivered to the premaxillomaxillary suture for 10 min/d over 12 days (120 cyclic per day) induced significantly more craniofacial growth ($p < 0.01$), marked sutural separation, and islands of newly formed bone, in comparison with both sham controls and static force of matching peak magnitude. The bone strain threshold of approximately 500 µstrain for inducing sutural osteogenesis is lower than the minimum effective strain capable of inducing bone apposition in long bones. These data demonstrate, for the first time, that application of brief doses of cyclic forces induces sutural osteogenesis more effectively than static forces with matching peak magnitude.

2. INTRODUCTION

The macroscopic morphology of craniofacial bones differs among themselves and from long bones. In contrast to the longitudinal growth of long bones by endochondral ossification of growth plate cartilage, the great majority of craniofacial bones elongate by osteogenesis in sutures, most of which consist of fibrous connective tissue rather than growth cartilage. Functionally, sutures are “growth plates” between craniofacial bones and are of fundamental importance not

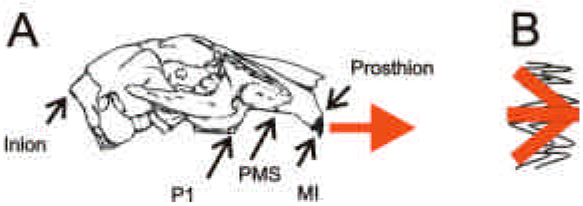

Figure 1. A: Diagrams illustrating the rabbit skull in the sagittal plane and a segment of the premaxillomaxillary suture (PMS). The premaxillomaxillary suture has a wavy, complex course, extending from the oral cavity between the premaxilla and maxilla rostrally towards the nasal bone. The strain gage/rosette was placed in the intraoral portion of the PMS. The horizontal arrow indicates the direction of tensile forces applied to the maxillary incisors (MI). P1: the alveolar bone mesial to the first premolar. Prosthion: the most anterior point of the maxillary alveolar bone; Inion: the most posterior point of the occipital bone. B: Enlargement of the strain gage rosette bonded to the premaxillomaxillary suture. The rosette's center gage was perpendicular to the sutures's longitudinal (vertical) course.

only to biologists with broad interests from paleontological form to genetic control of sutural development, but also clinical practitioners who attempt to correct skeletal defects due to premature closure of craniofacial sutures (1). Driven by these broad interests, a number of approaches have been used to study sutural osteogenesis. Among these, biomechanical modulation of craniofacial osteogenesis has received the most enduring attention and yet has yielded many conflicting reports (2,3).

Sustained exogenous forces applied to the craniofacial skeleton are capable of changing an otherwise undisturbed natural growth rate of sutures (2). However, little is known about 1) threshold forces above which sutural osteogenesis is accelerated beyond its natural amount, 2) optimal forces at which sutures can be accelerated to grow, and 3) the maximum forces at which sutural osteogenesis arrests. These logical issues are not trivial and cannot be addressed until pertinent experiments are performed.

There is general consensus that cyclic forces, i.e. forces with rapidly oscillating magnitude, applied to the appendicular skeleton in a number of species such as the turkey, dog, rat, and sheep lead to increased periosteal and endosteal bone apposition rates (4-8). This remarkably increased osteogenesis in the postcranial skeleton upon application of cyclic forces appears to be modulated by force frequencies: the higher the frequency, the greater the amount of bone apposition (4,8-12). It is not known whether cyclic forces induce more effective craniofacial osteogenesis. In two recent preprints (13,14), we have demonstrated the modulation of the peak magnitude of sutural bone strain by exogenous forces and the increase of sutural width upon applying exogenous forces. The present study extends the work described in these preprints and tests the hypothesis that cyclic forces more effectively evoke sutural growth than static forces of matching peak magnitude and duration.

3. MATERIALS AND METHODS

A total of 21 male, 6-weeks-old, New Zealand White rabbits were used: 8 were for acute experiments, and 13 for chronic experiments.

3.1. Rabbit model and surgical procedures

In 8 rabbits, general anesthesia was induced by intramuscular injection of a cocktail containing 90% ketamine (100 mg/ml; Aveco, Fort Dodge, IA) and 10% Xylazine (20 mg/ml; Mobay, Shawnee, KS). An incision was made intraorally to remove approximately 5 × 8 mm (height×length) of oral mucosa lateral to the right premaxillomaxillary suture (PMS). This incision extended from the cementoenamel junction of the maxillary incisor to 5 mm dorsal to the suture (cf., Figure 1A). The mucoperiosteum was cut and stripped to expose the cortical bone adjacent to the premaxillomaxillary suture. The present experimental procedures were approved by the University of Illinois at Chicago's Animal Care Committee.

3.2. Bone strain

Installation of strain gages and strain rosettes followed procedures described in detail elsewhere (15-17). Briefly, the cortical bone across the premaxillomaxillary suture was degreased, abraded with sandpaper, and neutralized with M-Prep Neutralizer (Measurements Group, Raleigh, NC). Care was taken to remove as little cortical bone as possible with sandpaper and to maintain the anatomical integrity of the suture. After local moisture reduction, uniaxial strain gages (EA-06-062AQ-350, Measurements Group) were installed with catalyst and cyanoacrylate (M-Bond 200, Measurements Group) over each suture with approximately half of the strain gage on each side of the suture. Each strain gage was kept perpendicular to the suture's longitudinal course. After bone strain recordings were made with the uniaxial gage in two rabbits, three-element strain rosettes (WK-06-030WR-120; Measurements Group) were installed to replace the above-described uniaxial strain gage over the premaxillomaxillary suture. The orientation of the rosette's center gage was aligned with the pre-existing uniaxial strain gage and perpendicular to the suture's longitudinal course (Figure 1B). Once installed, each strain gage or strain rosette was coated with polyurethane (M-Coat A). All strain gages/rosettes were then excited with 1000 mV DC in 1/4 bridge circuits, and the output signals were conditioned with a sampling rate of 10 Hz and digitally recorded with computer data acquisition (Model 6000, Measurements Group, Raleigh, NC). Compressive strain was expressed as negative values, whereas tensile strain as positive values. Experimental stress analysis was performed, following methods described in detail elsewhere (15-19).

3.3. Acute application of exogenous mechanical stimuli

Once strain gages were installed and secured, the rabbit was placed in a supine position in a custom-made resin body holder. The premaxilla was secured tightly to the resin body holder by restraining the oral commissure with stainless steel wires wrapped in a plastic sheath. An O

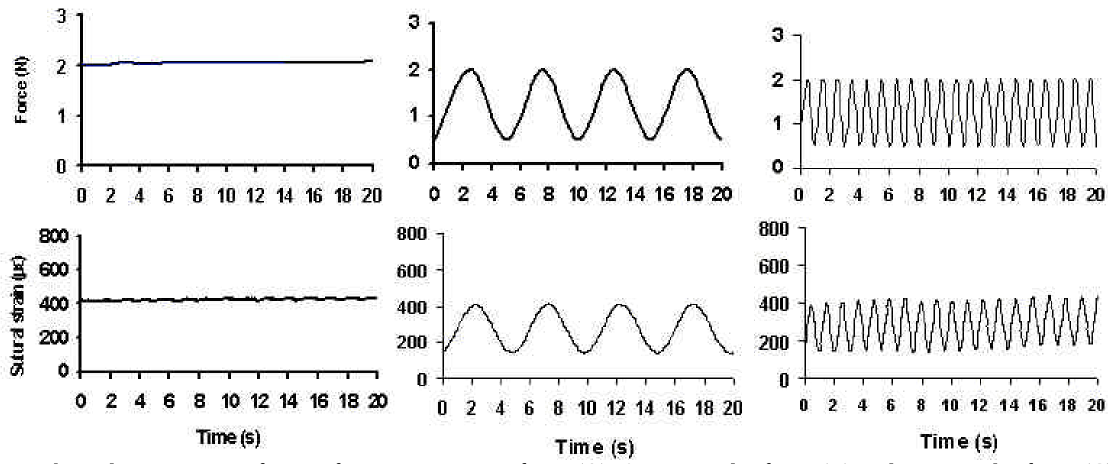

Figure 2. Top three diagrams: waveforms of exogenous static force (A), 0.2 Hz cyclic force (B) and 1 Hz cyclic force (C), all with the same peak magnitude of 2 Newtons applied to the maxilla. Despite the same peak magnitude at 2 N, static force lacked any appreciable oscillation in force magnitude over time. In contrast, 0.2 Hz cyclic force oscillated at 1 cycle every 5 s, whereas 1 Hz cyclic force oscillated at 1 cycle every 1 s. Bottom three diagrams: corresponding profiles of bone strain of the premaxillomaxillary suture (PMS) closely mimicked the waveforms of exogenous forces. A': Static bone strain in response to static force; B': 0.2 Hz cyclic bone strain in response to 0.2 Hz cyclic force; C': 1 Hz cyclic bone strain in response to 1 Hz cyclic force. The profiles of bone strain were modulated by waveforms of exogenous forces, despite similar peak bone strain amplitude at approximately 400 :strain.

ring was used to connect the maxillary central incisors to the handset of a computerized force workstation (Synergie 200, MTS, Eden Prairie, MN). Tensile forces with the same peak magnitude of 2 Newtons were pre-programmed and delivered via the O ring placed on the maxillary incisors (cf., horizontal arrow in Figure 1A) with two different waveforms: static forces with a frequency of 0 (cf., Figure 2A) and sine-wave cyclic forces with frequencies of 0.2 and 1 Hz (cf., Figure 2B and 2C respectively). Once each force was delivered to the premaxilla as verified by real-time display, bone strain was continuously recorded for up to 40 s.

3.4. Chronic delivery of mechanical stimuli

A total of 13, six-week-old, male New Zealand White rabbits were used in a chronic experiment protocol with all procedures performed under general anesthesia as described above. Tensile cyclic forces at 0.2 Hz (120 cycles per day) and 2 Newtons were applied to the rabbit premaxilla *in vivo* in the same fashion as described above for 10 min/d over 12 days (N = 5). Static forces of matching peak magnitude and daily duration were applied in age- and sex-matched rabbits (N = 4). Additional age- and sex-matched rabbits (N = 4) served as sham controls. On Day 1 and Day 12 of force delivery, standardized cephalometric X-ray images were taken (Phillips Oralix 70), maintaining a constant distance between the film and the object. The images were computer-scanned and superimposed on amalgam markers that were previously installed in calvarial bones to measure the overall and fractional craniofacial lengths in the sagittal plane. The anterior craniofacial length (ACL) was measured as the linear distance from prosthion to the P1 (the alveolar bone mesial to the first premolar; cf., Figure 1A), whereas the total craniofacial length (TCL) was measured as the linear distance from the P1 to inion (cf., Figure 1A). Both the ACL and TCL were measured independently by two investigators (MPM and JJM) with inter-observer variability accounted for. On Day 12, the rabbits were euthanized by overdosage of sodium pentobarbital. The right half of the skull was dissected. The hemimaxilla with the premaxillomaxillary suture was trimmed, demineralized in 50% formic acid and 20% sodium citrate, and embedded in paraffin. Sequential sections, each 8 micron thick, were cut in the parasagittal plane, and stained with hematoxylin and eosin. All experimental procedures were performed in sham control rabbits except for chronic, repetitive applications of exogenous forces.

3.5. Data analysis and statistics

Kruskal-Wallis and Mann Whitney U tests were used to compare the peak bone-strain amplitude induced by static and cyclic forces, as well as increases in the ACL and TCL among sham control, static loading and cyclic loading groups. $P < 0.05$ was considered to indicate statistical significance.

4. RESULTS

4.1. Characterization of sutural bone strain responses to cyclic and static forces

Sutural bone strain magnitudes of the premaxillomaxillary suture in response to 2-Newton tensile forces with static (0 Hz) and cyclic profiles (0.2 Hz and 1 Hz) are summarized in Table 1. The average peak bone strain evoked by static forces (506 µstrain±182; mean±S.D.) was not statistically different from the average peak bone strain induced by 0.2-Hz cyclic forces (436 µstrain±206) and 1-Hz cyclic force (461 µstrain±229). Representative profiles of exogenous forces with the same

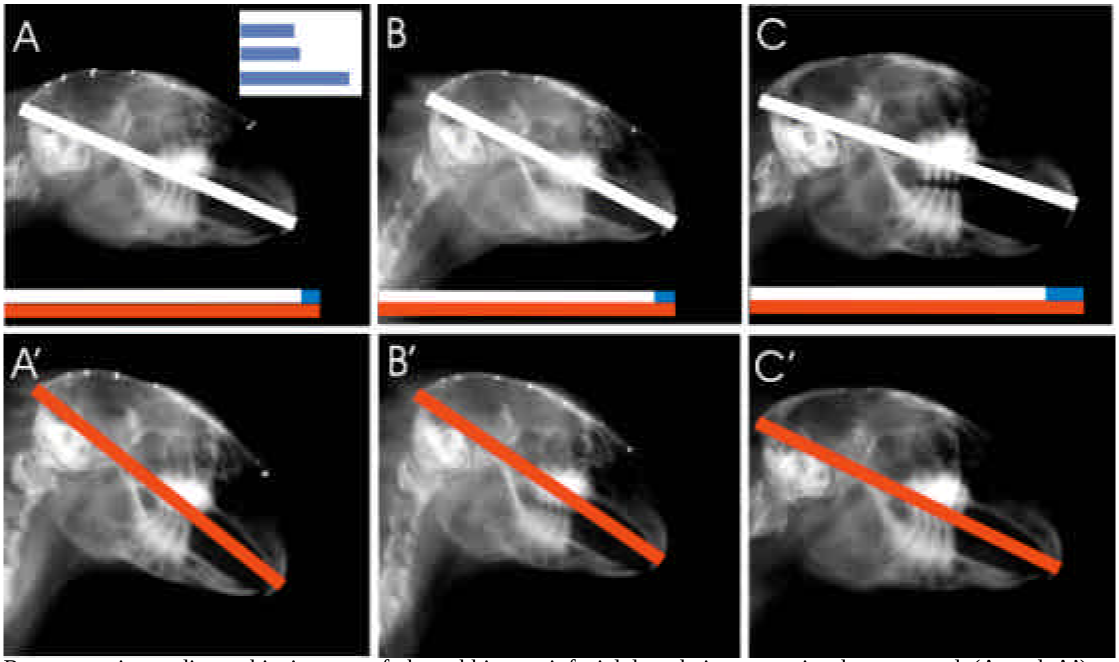

Figure 3. Representative radiographic images of the rabbit craniofacial length increases in sham control (A and A'), static loading (B and B') and cyclic loading (C and C'). The total craniofacial length (TCL) was defined as the linear distance from prosthion (the most anterior point of the skull) to inion (the most posterior point of the skull) (cf., Figure 1A). The top 3 images (A, B, C) represent the TCL on Day 1, whereas the bottom 3 images represent the TCL on Day 12 of the same corresponding rabbits (cf., amalgam markers implanted in calvarial bones in the same rabbits). By comparing the TCL on Day 1 and Day 12, the before (white) and after (red) linear lines near the bottoms of each of Figure 3A, B and C demonstrated the corresponding differences (green) in the amount of linear craniofacial growth. These differences in the TCL (green) were proportionally enlarged and demonstrated in the small window in Figure 3A. Cyclic forces (the bottom green line in the small window in A) induced marked increases in craniofacial length, in comparison with than static forces (the middle green line in the small window in A) and sham control (the top green line in the small window in A).

peak magnitude of 2 Newtons, but with different waveforms and their corresponding sutural bone strain patterns, are demonstrated in Figure 2. Despite the same peak magnitude at approximately 2 Newtons, waveforms of exogenous forces differed between static force (Figure 2A), 0.2 Hz cyclic force (Figure 2B) and 1 Hz cyclic force (Figure 2C). Accordingly, despite the similar peak magnitude of the elicited bone strain in the range of 436 to 506 µstrain, bone strain patterns differed among the static loading (Figure 2A'), 0.2 Hz cyclic loading (Figure 2B') and 1 Hz cyclic loading (Figure 2C'). Comparison of the waveforms of exogenous forces (Figure 2A, B, and C) and the corresponding bone strain patterns (Figure 2A', B' and C') revealed that the evoked bone strain was a function of the frequency of exogenous forces.

4.2. Chronic delivery of exogenous cyclic forces evoked more craniofacial growth and osteogenesis of the premaxillomaxillary suture

Figure 3 illustrates radiographic images of the rabbit crania taken before daily delivery of exogenous forces (Figure 3A, B, and C), and 12 days after delivery of exogenous forces (Figure 3A', B', and C'). Craniofacial length was measured as an oblique line connecting the most ventral point (prosthion) and dorsal point (inion) of the skull. By comparing the total craniofacial lengths (TCL) measured as the linear distance from prosthion to inion of the rabbits on Day 1 (white lines in Figure 3) and Day 12 (red lines in Figure 3), two adjacent white and red lines near the bottoms of each of Figure 3A, B and C illustrate the differences in the amount of linear craniofacial growth, as shown in green. These differences in the TCL are proportionally enlarged and demonstrated in the small window in Figure 3A. Cyclic forces at 0.2 Hz (the bottom green line in the small window in Figure 3A) induced marked increases in craniofacial length, in comparison with static forces (the green middle line in the small window in Figure 3A) and sham control (the top green line in the small window in Figure 3A).

Quantitatively, there was a significant increase in the TCL of rabbits exposed to cyclic forces than static forces and sham controls. The average TCL of rabbits exposed to cyclic forces (vertically hatched histogram in Figure 4A) was significantly longer ($p < 0.01$) than that of either sham controls (open histogram in Figure 4A) or those exposed to static forces of matching peak magnitude and duration (obliquely hatched histograms in Figure 4A). Interestingly, no statistically significant differences were found in the TCL between sham controls and static loading. The same trend was observed for the gain in the ACL: i.e., a significant increase in response to cyclic loading (p <

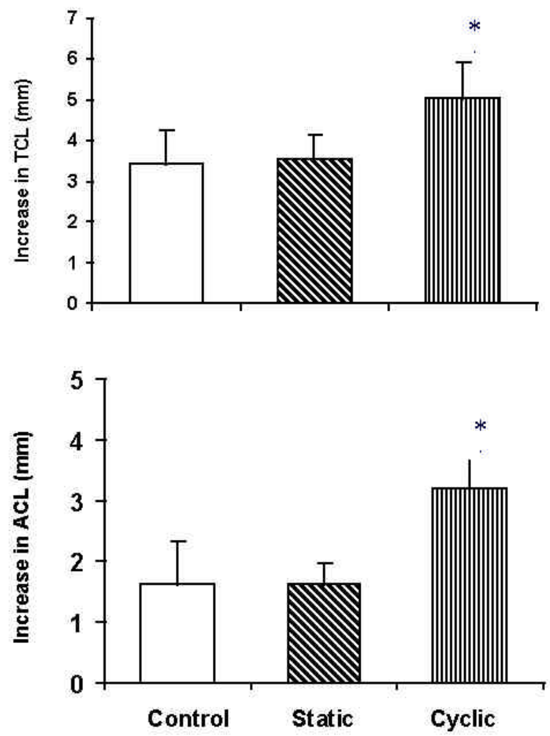

Figure 4. Quantification of craniofacial growth in the sagittal plane. A: Increases in the total craniofacial length (TCL) measured as the linear distance from prosthion to inion (cf., Figure 1A) on Day 1 and Day 12 of sham control (open histogram), static loading (obliquely hatched histogram) and cyclic loading (vertically hatched histogram). Craniofacial length of cyclic loading was significantly higher than both static loading and sham controls. B: Increases in the anterior craniofacial length (ACL) as measured by the linear distance from prosthion to P1 (cf., Figure 1A) on Day 1 and Day 12 of sham control (open histogram), static loading (obliquely hatched histogram) and cyclic loading (vertically hatched histogram) showed the same trend. The increases in the ACL of cyclic loading were significantly higher than both static loading and sham controls. N = 4 for sham controls, 4 for static loading and 5 for cyclic loading.

0.01) than sham controls or static loading (Figure 4B), and a lack of significant differences in the ACL between sham controls and static loading.

Representative photomicrographs of the premaxillomaxillary sutures in association with sham control (Figure 5A), static force (Figure 5B) and cyclic force (Figure 5C) illustrate the remarkable differences in structural characteristics of the premaxillomaxillary suture. The premaxillomaxillary suture treated with cyclic loading showed wide sutural separation with islands of new bone formation (NB in Figure 5C). In contrast, the premaxillomaxillary sutures treated with sham control (Figure 5A) and static loading (Figure 5B) had regular sutural width and lacked islands of new bone formation.

5. DISCUSSION

Despite the same peak magnitude of 2 Newtons, waveforms of cyclic forces at both 0.2 Hz and 1 Hz differed between themselves and from static force. Different waveforms of exogenous force evoked corresponding bone strain profiles of the premaxillomaxillary suture. This is the first time exogenous forces are shown to evoke corresponding bone strain profiles in craniofacial sutures. Clearly, bone strain profiles are modulated by waveforms of exogenous forces, providing the basis for using different types of exogenous forces to modulate sutural osteogenesis. The present data suggest a potential that sutural cells are likely exposed to different sutural bone strain profiles. This *in vivo* model therefore could be potentially valuable for studying responses of sutural connective tissue cells to a cascade of mechanical stresses.

Exogenous forces are a crude function of bone apposition, for the mechanobiological effects of the same 10 Newton force applied to the horse tibia and rat tibia would clearly differ due to scale. Bone strain, on the other hand, is a universal measure of exogenous forces that transmit as mechanical stresses on cortical bone. The present data demonstrate that threshold bone strain for inducing sutural osteogenesis appears to be approximately 500 µstrain. This threshold bone strain differs drastically from at least 1500 µstrain that has been found to induce periosteal and endosteal bone apposition rates in long bones in several animal species that have been studied (4,7-11,20). The present bone strain data suggest that the threshold for sutural osteogenesis in intramembranous bones is lower than that for periosteal and endosteal osteogenesis in appendicular bones. This potentially lower threshold of sutural osteogenesis can perhaps be attributed to two factors. First, bones of the face and cranial vault are articulated by sutures and differ from are long bones (9) and even the cranial base (21), both of which develop from endochondral bone formation. It is probable that sutural osteoblasts respond to bone strain differently from osteoblasts in long bones due to their different origins. This argument is supported by *in vitro* data showing differential responses to mechanical strain between osteogenic cells that originate from craniofacial bones and from long bones (22). Second, the present application of tensile forces differs from the typical four-point bending model in long bones (c.f. 4,10,11,20).

The present data demonstrate that cyclic forces at 0.2 Hz delivered for 10 min/d over 12 days (120 cycles per day) evoke more craniofacial growth and osteogenesis in the premaxillomaxillary suture than static forces of matching peak magnitude and duration. This is the first time that cyclic forces have been shown to modulate growth and osteogenesis in craniofacial bones. The significantly greater increase in craniofacial length in response to 2-Newton cyclic loading for 10 min/d over 12 days than sham controls or static loading demonstrates that sutural osteogenesis is modulated by a short dose of cyclic forces. Short doses of static forces, on the other hand, fail to stimulate substantial craniofacial growth. These data are

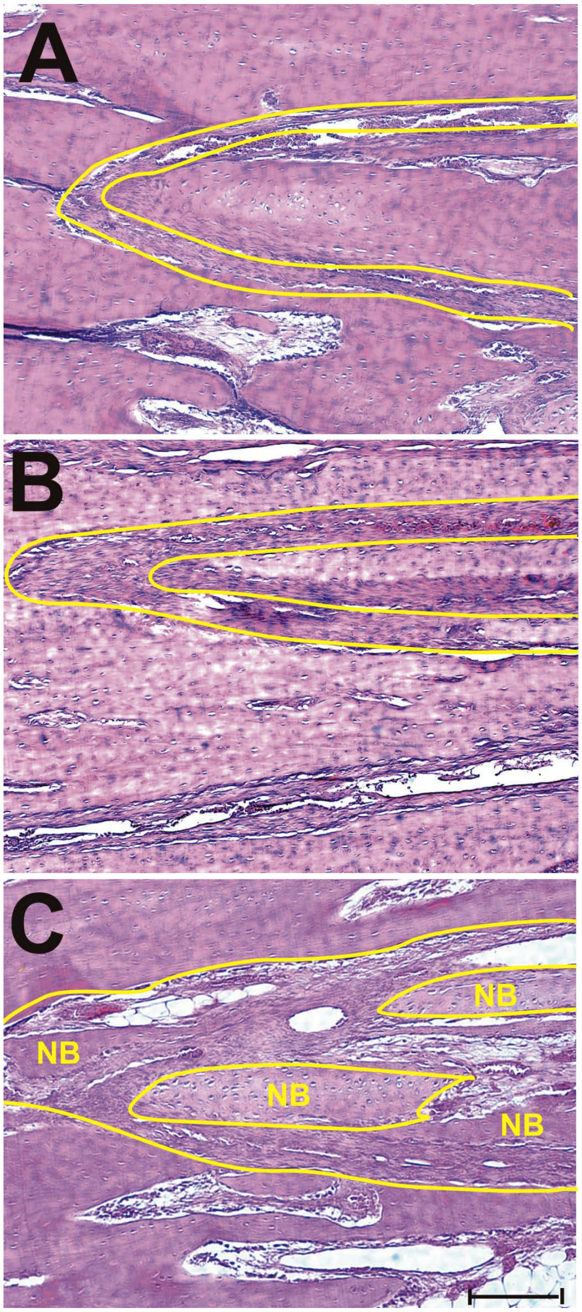

Figure 5. Representative photomicrographs of the premaxillomaxillary sutures in association with sham control (A), static force (B) and cyclic force (C) demonstrated remarkable differences. The premaxillomaxillary suture treated with cyclic forces showed wide sutural separation along with two islands of new bone formation (NB in C). In contrast, the premaxillomaxillary sutures treated with sham control (A) and static force (B) had regular sutural width and lacked islands of new bone formation.

in general agreement with findings in appendicular bones that osteogenic modeling is threshold driven, responding to cyclic forces with varying frequencies instead of static forces with constant magnitude (4,11,23-25). If parallelism can be drawn between craniofacial bones and appendicular bones, delivery of cyclic forces with higher frequencies wi1l likely lead to increasing amounts of sutural bone apposition in craniofacial bones.

The present study was designed with an eventual goal of applying the principles of biological findings in a human model, given that craniofacial orthopedics has applications in patients with craniofacial anomalies, dentofacial deformities, and those receiving orthodontic treatments, which respectively constitute 1.2%, 11.6% and 36% of the U.S. population (26). The present observation that sutural osteogenesis of craniofacial sutures can be accelerated by cyclic mechanical stimuli offers the possibility that conventionally used static forces in craniofacial orthopedics can perhaps be replaced or intermingled with cyclic forces to achieve optimal therapeutic effects. The following considerations can be taken into account for attempts of potential data extrapolation. First, intrinsic and exogenous forces evoke bone strain in both animal models and human skulls (17,27-29). The presence of bone strain in craniofacial sutures and the modulation of sutural bone strain patterns by exogenous forces, as shown in the present work, further implies that sutural osteogenesis is strain driven. Second, the bone strain threshold capable of inducing periosteal and endosteal osteogenesis in long bones applies across species such as the turkey, rat and sheep (4,6,8,11,23). Thus, there is no *a priori* reason to rule out the possibility that bone strain threshold in craniofacial bones also applies across species such as between the rabbit and human models. Third, the mechanisms of bone apposition evoked by cyclic forces likely involve activation of stress-sensitive genes, leading to measurable macrostructural changes. *In vivo* cyclic loading changes the properties of long bones such as Young's modulus (30), and enhances the expression of osteogenic molecules such as osteopontin and *c-fos* (31). These genetic and structural changes are likely to take place in craniofacial bones (32), leading to a morphologically measurable amount of osteogenesis and growth as observed in the present study.

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7. REFERENCES

1. Cohen, M.M. Jr., & R. MacLean. Craniosynostosis Diagnosis, Evaluation, and Management. Second edition. New York:Oxford University Press, 3-10 (2000)

2. Wagemans, P.A.H.M., J.-P. van de Velde, & A.M. Kuijpers-Jagtman: Sutures and forces: A review. *Am J Orthod Dentofac Orthop* 94, 129-141 (1988)

3. Herring, S.W., & S.Y. Teng: Strain in the braincase and its sutures during function. *Am J Phys Anthropol* 112, 573-593 (2000)

4. Rubin, C. T. & L.E. Lanyon: Regulation of bone formation by applied dynamic loads. *J Bone Joint Surg* 66A, 397-415 (1984)

5. Rubin, C.T., G.W. Pratt, Jr., A.L. Porter, L.E. Lanyon, & R. Poss: Ultrasonic measurement of immobilization-induced osteopenia: an experimental study in sheep. *Calcif Tissue Int* 42, 309-312 (1988)

6. Turner, C.H., M.R. Forwood, J.-Y. Rho, & T. Yoshikawa: Mechanical loading thresholds for lamellar and woven bone formation. *J Bone Min Res* 9, 87-97 (1994)

7. Turner, C.H.: Three rules for bone adaptation to mechanical stimuli. *Bone* 23, 399-407 (1998)

8. Rubin, C.T., A.S. Turner, S. Bain, C. Mallinckrodt, & K. McLeod: Low mechanical signals strengthen long bones. *Nature* 412, 603-604 (2001)

9. Burr, D. B. & R.B. Martin: Mechanisms of bone adaptation to the mechanical environment. *Triagnle:Sandoz J Med Sci* 31, 59-76 (1992)

10. Rubin, C.T., K.J. McLeod, T.S. Gross, & H.J. Donahue. Physical stimuli as potent determinant of bone morphology. In Carlson Ds, Goldstein SA (Eds) Bone Biodynamics and Orthodontic and Orthopedic Treatment. Craniofacial Growth Series 27, University of Michigan, Ann Arbor. 77-91 (1992)

11. Turner, C.H., I. Owan, & Y. Takano: Mechanotransduction in bone: role of strain rate. *Am J Physiol* 269 (*Endocrinol Metab* 32), E438-E442 (1995)

12. Fritton, S.P., K.J. McLeod, & C.T. Rubin: Quantifying the strain history of bone: spatial uniformity and self-similarity of low magnitude strains. *J Biomech* 33, 317-325 (2000)

13. Kopher, R.A., & J.J Mao: A model of controlled compressive loading of craniofacial sutures in the rabbit. *J Dent Res* 80, 133 (2001)

14. Nudera, J.A., X. Wang, & J.J. Mao: Characterization of sutural bone strain upon controlled tensile forces in the rabbit premaxillomaxillary suture. *J Dent Res* 80, 132 (2001)

15. Mao, J.J., & J.W. Osborn: The direction of bite force determines the ratios of EMG activity in jaw closing muscles. *J Dent Res* 73, 1112-1120 (1994)

16. Mao, J.J., P.W. Major, & J.W. Osborn: Coupling electrical and mechanical outputs of human jaw muscles undertaking multi-directional bite-force tasks. *Arch Oral Biol* 41, 1141-1147 (1996)

17. Mao, J.J., M. Oberheim, R.A. Cooper, & M. Tassick. Stress patterns of craniofacial bones upon orthopedic headgear loading in dry human skulls. In: Growth Modification. McNamara JA, Jr. (Ed.), Craniofacial Growth Series 35, Center for Human Growth and Development, The University of Michigan, Ann Arbor, 87-104 (1999)

18. Dally, J.W., & W.F. Riley: Experimental Stress Analysis. McGraw-Hill:New York, 453-505 (1991)

19. Osborn, J.W., & J.J. Mao: A thin bite-force transducer with three-dimensional capabilities reveals a consistent change in bite force direction during human jaw-muscle endurance tests. *Arch Oral Biol* 38, 139-144 (1993)

20. Forwood, M.R., & C.H. Turner: Skeletal adaptations to mechanical usage: result from tibial loading studies in rats. *Bone* 17(Suppl), 197s-205s (1995)

21. Wang, X., & J.J. Mao: Accelerated chondrogenesis of the rabbit cranial base growth plate upon oscillatory mechanical stimuli. *J Bone Miner Res* (In press) (2002)

22. Rawlinson, S.C.F., J.R. Mosley, R.F.L. Suswillo, A.A. Pitsillides, & L.E. Lanyon: Calvarial and limb bone cells in organ and monolayer culture do not show the same early responses to dynamic mechanical strain. *J Bone Min Res* 10, 1225-1232 (1995)

23. Rubin, C.T., & L.E. Lanyon: Osteoregulatory nature of mechanical stimuli: Function as determinant for adaptive remodeling in bone. *J Orthop Res* 5, 300-310 (1987)

24. Martin, A.D., & R.G. McCulloch: Bone dynamics: stress, strain and fracture. *J Sports Sci* 5, 155-163 (1987)

25. Lanyon, L.E.: Using functional loading to influence bone mass and architecture: objectives, mechanisms, and relationship with estrogen of the mechanically adaptive process in bone. *Bone* 18, 37S-43S (1996)

26. Brunelle, J.A., M. Bhat, & J.A. Lipton: Prevalence and distribution of selected occlusal characteristics in the US population, 1988-1991. *J Dent Res* 75(Spec Iss), 706-713 (1996)

27. Hylander, W.L., & K.R. Johnson: *In vivo* bone strain patterns in the zygomatic arch of Macaques and the significance of these patterns for functional interpretations of craniofacial form. *Am J Phys Anthropol* 102, 203-232 (1997)

28. Rafferty, K.L., & S.W. Herring: Craniofacial sutures: morphology, growth, and *in vivo* masticatory strains. *J Morphol* 242, 167-179 (1999)

29. Oberheim, M.C., & J.J. Mao: Bone strain patterns of the zygomatic complex in response to simulated orthopedic forces. *J Dent Res* (In press) (2002).

30. Raftopoulos, D., E. Katsamanis, F. Saul, W. Liu, & S. Saddemi: An intermediate loading rate technique for the determination of mechanical properties of human femoral cortical bone. *J Biomed Eng* 15, 60-66 (1992)

31. Owan, I., D.B. Burr, C.H. Turner, J. Qui, Y. Tu, J.E. Onyia, & R.L. Duncan: Mechanotransduction in bone: osteoblasts are more responsive to fluid forces than mechanical strain. *Am J Physiol (Cell Physiol 42)* 273, C810-C815 (1997)

32. Mao JJ: Mechanobiology of craniofacial sutures. *J Dent Res* (In press) (2002)

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