Quantifying the effects of mechanical vibration on the volume of the midpalatal suture

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Dedication

To my partner Yueyue, for your unwavering patience, support and understanding.

To my parents Kenny and Lynette, for your encouragement and inspiration.

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List of Abbreviations

2D	Two-dimensional
3D	Three-dimensional
3H-Thymidine	Tritiated thymidine
aBMD	Areal bone mineral density
BMC	Bone mineral content
BMD	Bone mineral density
BUA	Broadband ultrasound attenuation
BV/TV	Bone volume to tissue volume ratio
CBCT	Cone beam computed tomography
CD1	Cluster of differentiation 1
COX-2	Cyclo-oxygenase-2
CP	Cerebral palsy
CT	Computed tomography
DSST	Daily stimulus stress theory
DNA	Deoxyribonucleic acid
EMF	Electromagnetic field
GCF	Gingival crevicular fluid
GMCS	Gross motor classification system
HFLMV	High-frequency, low-magnitude vibration
Hz	Hertz
IL-1B	Interleukin 1-B
ISS	Inter-sphenoidal synchondroses
Micro-CT	Micro computed tomography
MPa	Megapascal
mm	Millimeter
MLO-Y4	Murine long bone osteocyte Y4
MRI	Magnetic resonance imaging
Mvt	Movement
N-S-Ar	Nasion-Sella-Articulare
N-S-Ba	Nasion-Sella-Basion
NiTi	Nickel titanium
NFS	Nasofrontal suture
OPG	Osteoprotegerin
PDL	Periodontal ligament
PEMF	Pulsed electromagnetic field
PGE2	Prostaglandin E2
PMS	Premaxillomaxillary suture
QCT	Quanta cloud technology
RANK	Receptor activator of nuclear kappa-B
RANKL	Receptor activator of nuclear kappa-B ligand
RPE	Rapid maxillary expansion

SARME	Surgically assisted rapid maxillary expansion
SEM	Scanning electron microscopy
SES	Spheno-ethmoidal synchondroses
SOS	Spheno-occipital synchondroses
TIFF	Tagged image file format
TMA	Titanium molybdenum alloy
TNF	Tumour necrosis factor
TRAP	Tartrate-resistant acid phosphatase
vBMD	Volumetric bone mineral density
Vib	Vibration

1 Aims and Objectives

To evaluate the effects of whole body high-frequency low-magnitude vibration (HFLMV) on the volume of the midpalatal suture (MPS) in a hypofunction and orthodontic tooth movement sample.

2 Introduction

Since its proposition by Julius Wolff in the 19th century, the concept 'form follows function' has clarified the importance of mechanical loading on skeletal morphology. Although Wolff's law could not predict the exact reactions of bone to a mechanical load, it demonstrated that externally induced strains can modify bone remodeling and subsequently have an effect on skeletal architecture (1). This concept provided the foundation for treatment methodologies aimed at improving bone quality through induction of a mechanical strain on the skeleton. An example is the induction of HFLMV through a whole-body vibration platform in children with motor disabilities (2-6). HFLMV activates mechanotransduction in bone, resulting in stimulation of osteogenesis which counteracts the deficiency in skeletal development (3, 4, 6, 7).

Several bony unisons of the cranium also experience bone turnover and remodeling during growth or as a result of orthodontic manipulation. Growth of the craniofacial sutures is heavily influenced by the external environment. In natural growth, sutures of the cranium rely on mechanical strains to modify and promote growth and lie dormant until an external signal such as the pressure of soft tissue growth is experienced. Accordingly, any additional external mechanical strains are likely to modify the rate of bone remodeling at the interface of the two maxillary bones and affect natural growth (8-12).

Natural midpalatal suture (MPS) growth relies on bone deposition at the interface of the two maxillary bones (12). Although static forces have been used for centuries in the modification of these joints, the effects of HFLMV on bone remodeling and deposition on the MPS and CBS have not been investigated.

2 Bone

2.1 Definitions

Bone is a special connective tissue, which also acts as an organ within higher vertebrates (197). It functions as structural support, protection of organs and systems, acid-base balance, locomotion, haematopoiesis within marrow spaces, mineral storage, homeostasis, and as a reservoir for growth factors and cytokines (198). Bone marrow is responsible for the differentiation of osteoprogenitor cells from self-renewing pluripotent mesenchymal stem cells. These osteoprogenitor cells then become osteoblasts, osteocytes and bone lining cells. Mononuclear monocyte-macrophage precursor cells are also derived in the bone marrow and eventually give rise to osteoclasts (197, 198).

2.2 Endochondral Ossification

Endochondral ossification is responsible for bone formation in the extremities of the long bones, vertebrae, ribs and mandibular condyle. If mesenchymal cells proliferate and form condensations of cartilage templates there is a subsequent replacement by mineralised bone. Cartilage cells stratify into layers of proliferative and mature hypertrophic phenotypes that cause zones of initial mineralisation to appear. Following maturation, chondrocytes release non-collagenous proteins and X collagen and metalloproteinases concurrently break down cartilage ECM. These processes combine to create an environment that promotes mineralisation and replacement of cartilage by bone (199).

2.3 Intramembranous Ossification

At the sites of intramembranous bone formation there is an increase in vasculature and proliferation of mesenchymal cells. These cells condense and differentiate into osteoblasts

and directly secrete bone specific extracellular matrix onto an organic matrix membrane. During differentiation, mesenchymal cells display alkaline phosphatase activity that results in mineralisation and bone strengthening (199).

2.4 Physical Properties of Bone

Bone demonstrates properties of viscoelasticity and anisotrophy in order to facilitate its role of locomotion and support. Another way that bone provides optimal support and function is by macroscopic and microscopic adaptation to the mechanical stimulation of the external environment. Every day, there is an infinite number of stresses and strains on bone. As a response to these external signals, there is a cascade of chemical and cellular events that instigate turnover and remodelling.

2.4.1 Stress

Stress is defined as force per unit area equal in magnitude but opposite in direction to the applied load. Sheer stress occurs as a result of two forces acting parallel to each other but not along a different line, compressive stress occurs if two forces act on the same line in the same direction and tensile stress occur following the application of two forces along the same line but in opposite directions.

2.4.2 Strain

Strain refers to the mechanical deformation in bone caused by a mechanical stimulus and is a quantified measure of the change in length over the original length (200).

2.4.3 Strength and Stiffness

Strength and stiffness of a bone depends on the internal trabecular alignment and

arrangement. This internal arrangement varies between dissimilar bones, as well as between different sites within the same bone. This results in an increase in strength that is not accompanied by an increase in mass, referred to as Anisotrophy (94).

2.4.4 Viscoelasticity

Viscoelasticity is a property which allows bone to demonstrate different properties relative to the rate of force applied. Under high loads, bone demonstrates low viscoelasticity and behaves as a brittle object. However at low loads, bone demonstrates a lower modulus of elasticity and behaves as a more viscous material(94).

2.4.5 Bone Remodelling

Remodelling refers to morphological changes in bone as a response to physiological or mechanical stimuli. The primary goals of this process are mineral haemostasis, maintaining bone strength and prevention of micro-damage (90, 198). Osteoblasts and osteoclasts work independently to achieve this and their cumulative effect results in resorption of old bone and deposition of new bone through initial matrix deposition and subsequent mineralisation (90, 198).

2.4.6 Structural adaptation of bone on a cellular level

Mechanotransduction occurs following an external stimulus that induces a biochemical and cellular response, acting to maintain the optimal strain environment. Burger and Klein-Nulend conducted a review of several studies and concluded that osteocytes residing in lacunae are the predominant mechanosensitive cells in bone (201). In these cases, morphological cellular adaptations allow communication to other cells. The osteocytes penetrate through the bone's canaliculi, resulting in communication between osteoblasts and bone lining cells via gap junctions and cell processes within the lacuno-canalicular system.

The amount a bone can deform is small (max 0.3%), however strains of between one and three percent are required to induce a cellular response and bone remodeling. Activation therefore occurs from the flow of interstitial fluid through the lacunocanalicular system. After load application, strain concentrations are produced at osteocytic lacunae and interstitial fluid is expressed out through thin layers of non-mineralised matrix surrounding cell bodies and cell processes (202, 203). Fluid flow is generated in the form of electric streaming potentials and fluid sheer stresses are created by annular porosities resulting from the canalicular and osteocyte diameter. These sheer forces primarily influence osteocyte cell membrane proteins called integrins, and function as adhesion receptors that transduce mechanical stimulation from the ECM to the cell's cytoskeleton. As a result, there is integrin activation and down streaming of the intracellular biochemical cascade, causing a change in the bone cell metabolic activity. The cumulative result is a macroscopic bone reorganization and adaptation (201).

Burger and Klein-Nulend found that basal fluid sheer stresses from normal physical activity provided sufficient mechanical stimulation to induce osteocyte haemostasis that increases above normal sheer stresses resulting in the recruitment of osteoblasts. If physical activity is decreased, a reduction in fluid stresses occurs, resulting in osteocyte apoptosis and recruitment of osteoclasts (201).

2.5 HFLMV, Tooth Movement and Hypofunction

Bone marrow is responsible for the differentiation of Osteoprogenitor cells from self-renewing pluripotent mesenchymal stem cells which then become osteoblasts, osteocytes and bone lining cells and the mononuclear monocyte-macrophage precursor cells give rise to osteoclasts (197, 198). HFLMV, hypofunction and tooth movement modify the mechanisms

that influence the recruitment and production of these cells and may modulate bone remodeling and deposition in the MPS and CBS, however no studies have evaluated this.

3 Midpalatal suture

3.1 Introduction and Definitions

The MPS divides the palate into right and left halves in an anteroposterior direction and is continuous with the intermaxillary suture between the maxillary central incisor teeth. It extends from the incisive canal to the transverse maxillopalatine suture and runs across the palate between the maxillae and palatine bones (13).

At birth, the suture forms the unison between the maxillary bones. Growth occurs through bone deposition at the margins of the sutures from the adjacent cell layers. This eventually leads to ossification and fusion of the sutures between the bony segments.

3.2 Development

The initial primary palate forms the roof of the oral cavity following formation of the nasal cavities. Caudally to the primary palate, the internal aspect of the maxilla produces the palatal processes that bulge into the oral cavity, eventually become the secondary palate. At 47 days *in utero*, the secondary palate is rudimentary, and contains the maxillary and palatine parts of the MPS. Between 56-57 days *in utero*, the related shelves elevate, acquiring a horizontal position above the concurrently descending tongue. The hard palate grows in height, breadth and length and becomes an arched roof in the mouth. This fetal palate increases faster in length than in width between 7-18 weeks *in utero* (13).

At 10 weeks *in utero*, the superior uniting layer of fiber bundles develop across the midline close to the Vomer's periosteum creating the first signs of the suture. By 12 weeks *in utero*, the MPS is definitively established (13, 14). At this stage, sutural cells and fibers run along the suture, parallel to the bone margins. The vomer lies across the superior portion of the

MPS at the point where its periosteum and the upper transverse sutural fiber bundles blend together. At this point, the palate is relatively long, however after the 18th week *in utero*, its width increases faster than the length, resulting in growth at the MPS and appositional bone deposition at the lateral alveolar margins. Starting from the 20th week *in utero*, the vomer progressively grows into the midpalatal joint, reaching a maximum when the cross-section of the suture is Y-shaped. This results in greater articulation between the maxillary bones and vomer than between the two maxillary bones (15).

3.3 Growth

3.3.1 From Birth to the Age of three

At birth, the vomer reaches its greatest relative size, and has a similar width and length to the MPS. After birth, the cancellous bone of the palate remodels, to form a cortex and medullary spaces. Further, the medial ends of the palatal processes become increasingly thickened (13). Increase in length is accomplished by appositional growth at the maxillary tuberosity region and the transverse maxillopalatine suture. The inferior cortical layer remains cancellous in nature for two years and the rapid deposition of bone results in a significant increase in height of the MPS. Between the ages of one and two, growth of the MPS ceases, leading to a loss of the synostosis which results in parallel margins of cortical bone (15). At three years and older, clear medullary spaces and compact cortical bone become confined to the thickened medial area. In order to facilitate a larger nasal cavity in the posterior portion, the anterior portion of the MPS is smaller (15). The intervening sutural tissue consists mainly of fiber bundles arranged parallel to the bone margins (13).

3.3.2 After the Age of 3

The maxilla develops entirely intramembranously through surface remodeling or apposition

that is proportional to the displacement of the maxillary bones. During this process, the MPS acts as a regional growth site, and bone deposits proportionally to the displacement of the maxillary bones. Growth of the soft tissues translates the maxillary complex downwards and forwards as bone fills the space in the frontomaxillary, zygomaticotemporal, zygomaticomaxillary and pterygopalatine sutures followed by surface resorption on the anterior portion of the maxilla (11). During the infantile period, the MPS takes on a broad Y-shape and the vomer is situated in the center which is within a V-shaped groove between the two halves of the maxilla. The suture undergoes several changes, becoming a wavy pattern in the juvenile period then progressively becoming more interlocked, closed and interdigitated in adolescence. Obliteration begins in adolescence; however the rate of bone deposition varies greatly between individuals. By the third decade of adulthood, the suture is usually fused (16, 17).

3.3.3 Remodeling

At 16 weeks *in utero*, remodeling begins to contribute towards growth. Osteoclasts appear on the free nasal aspect of the maxillary palatal bone and osteoblasts proliferate on the oral aspect. This arrangement results in remodeling and inferior relocation of the entire palatal bone (13). During this time, sutural bone deposition continues simultaneously alongside vomer intrusion. Growth at the maxillovomeral portion occurs slower than the intermaxillary portion. The resulting bone deposition tends to form trabeculae parallel to the slower growing surface. After this concludes, there is remodeling inferomedially by resorption on the nasal surface and deposition on the intermaxillary and vomer surface. After birth, remodeling results in inferior relocations of the palate, peaking in activity at six months. The cross-sectional form of the suture is T-shaped and the maxillovomeral portion becomes more aligned to the nasal floor and transitions into a resorptive surface. Separational growth of the

suture slows down and the rate of remodeling and downward displacement increases significantly (15). Remodeling only occurs after the slowing and cessation of sutural growth, thinning of the vomer and increase in vertical height of the suture. This usually occurs between one and two years after birth and is indicated by a change in the growth pattern. The resulting pattern dictates active bone deposition on the sutural margin and corresponding resorption on the adjacent medullary surface. Following the creation of endosteal bone lamellae on the sutural plate, the medullary resorptive surface becomes depository. Endosteal bone is initially localized to small trabeculae or ridges protruding into medullary spaces at around 14 months. At 26 months, endosteal bone extends more evenly across the sutural area and the number of osteoblasts significantly decrease resulting in limited growth from this point onwards (13).

3.4 Maxillary expansion

A ‘narrow maxilla’ can be corrected through maxillary expansion. This process opens the MPS and indirectly influences the circummaxillary sutural system (18). It is used to facilitate correction of transverse maxillary hypoplasia, unilateral or bilateral crossbite, or a narrow palatal vault, and as an adjunctive procedure in the treatment of patients with unilateral or bilateral dark buccal corridors. It is further utilized in some anteroposterior discrepancies and some crowding cases and patients with airway issues (18, 19). The two main methods for achieving skeletal expansion are rapid maxillary expansion (RME) and surgically assisted RME (SARME) which induce a process of bone remodeling and deposition into the bony defect created between the two maxillary bones. It is important to assess the patient’s age and degree of ossification between the maxillary bones when choosing between these two appliances (20). Current methods for maxillary expansion apply a static expansive force to the maxillary bones, however studies investigating the effects of mechanical oscillatory

forces on growth of craniofacial sutures have demonstrated that cyclic strains have a greater degree of bone remodeling response compared to static strains of equal magnitude. As a consequence, application of controlled exogenous oscillatory forces during orthodontic treatment may be considered in the future (21).

3.5 History of Rapid Maxillary Expansion

3.5.1 Early Use

The idea of maxillary expansion involves the placement of force on alveolar ridges of teeth, placing opening pressure on the maxillary suture, leading to deposition of bone on the margins of the suture. RME has undergone many modifications and a variety of techniques have been tested. The first documented case was published in 1860 by Angell in the Dental Cosmos claiming to have demonstrated successful palatal expansion by creating space for crowded maxillary canines on a fourteen-year-old female patient. Expansion was achieved by turning a nut connecting two contra-rotating jackscrews placed against the necks of the posterior maxillary teeth every day for two weeks. In the same year, White attempted to force the first premolars back into the arch on a thirteen-year-old girl. An appliance was designed to fasten onto the premolars with a spiral spring joining the two sides behind the anterior teeth (22). Unfortunately, editors responsible for established dental journals at the time questioned the safety and validity of this method and considered it exceedingly adventurous with regard to accepted science of the time, discouraging further study.

In 1893, Goddard revisited the idea and demonstrated an appliance attached to the maxillary first premolars and molars that improved dental irregularities by separating the maxillary halves (23). Similar criticism and resistance was experienced as its effects on neighbouring hard and soft tissues were not well understood. McQuillen noted in an editorial in the “Dent

Cosmos" that the decreased irregularity of teeth after such treatment would likely cause more problems (24).

Fortunately, research into expansion of the nasal cavity in order to alleviate respiratory problems through midpalatal expansion was being conducted at a similar time. The first in depth study was conducted in by Schroeder-Benseler et al who used a non-spring-loaded jackscrew to separate the maxilla. His appliance was a modification of Angell's, consisting of a jackscrew attached to stainless steel crowns on the maxillary first molars (25).

Unfortunately, just as this method was gaining momentum, it was almost abandoned again in the late 1920s due to the increased popularity of the 'functional concept of development'. This idea proposed that if teeth were moved into a desired position, bone would grow to support them, resulting in an increase in width of the nasal passage. Since it was thought that tooth movement was all that was necessary, orthopedic expansion of the maxilla was deemed unnecessary.

3.5.2 Invention of Radiography

3.5.2.a Cases in Europe

The invention of radiographs proved to be vital, as it allowed for the safety of maxillary expansion to be examined by quantifying the secondary effects on other areas of the maxilla. This reignited interest in the field, attracting the attention of Derichsweiler who was a European orthodontist that became interested in maxillary expansion in 1953. He treated patients with maxillary transverse deficiency and posterior crossbite. An appliance was manufactured that had metal bands around the first premolars and first molars and an attached plate separating the appliance into two halves. A screw acted as the expander. Patients were instructed to turn the screw three times daily for 14 days and to keep the

appliance in place at all times to act as a retainer and stabilise the result (26).

3.5.2.b Animal Experiments in the United States of America

As maxillary expansion was gaining traction in Europe, the idea was brought to the United States by Korkhaus, who was visiting the Department of Orthodontics at the University of Illinois. He presented a series of radiographic images that demonstrated successful maxillary expansion cases. At these meetings, A.J Haas saw great potential and popularised the fixed maxillary expander through a series of influential studies (27). His first study was conducted on eight experimental pigs in 1959, making slight adjustments to Derichsweiler's appliance to optimise usability. He was able to increase the upper arch by up to 15mm, resulting in subsequent compensation from the mandibular teeth and an increased internasal width. Little or no indication of pain and discomfort was experienced by the specimens and minimal resistance towards expansion found. On top of this, the specimens seemed to demonstrate rapid bone resorption and apposition as well. In one case, this happened within five weeks. Haas also commented that a portion of the arch expansion may have been achieved by bending and tipping of the alveolar process, and compensatory lowering of the palatal vault and nasal floor (27).

3.5.3 Haas Appliance

After encouraging results from his animal study, a clinical study was conducted in 1961 on 45 human patients with maxillary deficiency or nasal insufficiency (28). Ten cases were chosen in the situation that orthodontic correction was insufficient and maxillary expansion may have been more effective. The appliance was similar to the ones used in the animal studies, however had an acrylic plate instead of a metal wire appliance in an attempt to achieve a greater degree of orthodontic expansion. The appliance was anchored to the premolars and molars and the screw was activated a quarter turn twice per day until sufficient

over-expansion was achieved. After expansion, the appliance was secured for three months to act as a retainer and replaced with an acrylic plate during retention. The findings demonstrated changes in the transverse dimension which resulted in an increase in nasal width between 2.5-4mm and increase in the maxillary intermolar width by a maximum of 5.5mm and minimum of 1.5mm. In the vertical dimension there was a downward sliding of the maxillary bones whilst resulted in an increased in the anteroposterior dimension through anterior sliding of the maxillary bones dimensions. Further, a significant MPS opening was found, creating a bony defect that initiated subsequent bone deposition in the area afterward. This resulted in a permanent increase in the maxillary apical base that caused spontaneous and permanent compensatory changes in the mandibular arch width. Whether a permanent change in alveolar bending and tooth tipping had occurred was difficult to determine, however this study provided substantial evidence for orthodontists at the time to consider maxillary expansion using the Haas appliance. This appliance remains one of the two main choices for maxillary expansion today.

3.6 Age of Maxillary Suture Closure

In two separate studies from 1964 and 1965, the ideal timing for maxillary expansion was proposed by Isaacson et al to be related to the age of maxillary suture closure (29, 30). Melsen studied the timing of suture closure using microradiology on deceased humans and determined that growth in length occurred until about 13-15 years old, followed by continued bone deposition and eventual closure at about 18 years old. This study provided some insight regarding optimal timing of maxillary expansion, however subsequent studies containing larger sample sizes demonstrated contradictory findings (7). Persson and Thilander also conducted research on deceased persons (16), finding a larger range of results using histological analysis. Some individuals exhibited evidence of bony unison in late adolescence

and others in their mid-twenties still had open sections, leading to the conclusion that the age of MPS closure was highly variable and based on each individual's growth.

3.7 Ideal Timing of Maxillary Expansion

After careful consideration into the timing of MPS closure, Haas proposed that the ideal age for maxillary expansion was either before or during the peak pubertal growth spurt (31). Several studies have found if expansion is conducted after this time, there is alveolar bending, tooth displacement, extrusion, relapse, pain, and periodontal compression resulting in an increased proportion of orthodontic, rather than orthopaedic expansion (32-42). This was challenged by Timms and Vero (43) and Mossaz et al (44), suggesting that expansion is not possible after the age of 25 years old and by Mommaerts (45) who found limited orthodontic expansion in persons older than 12 years old, however little high quality literature can be found to support these findings.

3.7 Orthodontic vs Orthopaedic Expansion

The Hyrax expander is another commonly used device which does not have the acrylic plate from the Haas appliance, aiming to achieve a higher proportion of orthopaedic change by transferring the force entirely through the teeth onto the maxilla. In modern day treatment, either the Haas or Hyrax expander is generally used, with slight modifications on an individual basis as necessary (46). Expansion is achieved through a combination of dental and skeletal movement. If buccal tipping and movement of teeth occurs, it is usually considered an undesirable side effect. Use of skeletal anchorage devices, such as palatal distractors, implant-supported hyrax screws and bone anchors are proposed to result in a higher proportion of skeletal change. Despite differences in design, a limited amount of studies directly compares the efficacy and stability of these tooth born expansion device

techniques (50). Long term follow up has demonstrated that in all cases, a degree of relapse will occur. In order to minimise this, the degree of orthopaedic and orthodontic expansion in each patient must be assessed in order to create an individualised retention program.

3.8 Anatomical Changes During Maxillary Expansion

During maxillary expansion, the two palatal bones rotate laterally to form a triangular shaped opening (28, 31, 47, 48). The greatest degree of expansion occurs in the anterior region from the occlusal perspective, and in the apical region from the frontal perspective. The axis of expansion is within the frontonasal suture (47). Subsequent to maxillary expansion, there is a forward and downward rotation of the maxilla that causes a secondary downward and backward rotation of the mandible and a temporary opening of the mandibular plane angle (47, 49). RME also results in a significant gain in arch length, attributed to buccal movement of both the posterior teeth and the alveolar processes (49).

3.9 Human Studies

3.9.1 Proportions of skeletal or dental expansion

Although true orthopaedic expansion is usually the goal of orthodontic treatment, a certain degree of orthodontic expansion occurs as well. The proportions of each which occur following different expansion techniques have been researched extensively. However there are difficulties in comparing studies due to the large variations in age, amount of expansion accomplished, size, and retention protocol. Chung and Font studied the Haas expander on 20 children and found that crown tipping accounted for 4.3% of expansion in the first molars and 9.7% in the first premolars (50). Podessor et al analysed the effects of a Hyrax expander using CT scans and found that skeletal expansion varied from 25% - 53% of the total expansion (49). Following the invention of CBCTs, more accurate measurements of skeletal

and dental effects were possible. Garrett et al used this method and found the effects of a Hyrax expander on the maxilla are shown in table 1 (51):

Kartalian et al also studied the Hyrax expander using similar methods, but found no statistically significant amount of dental tipping (52). In the RME group alveolar tipping increased by five degrees, however decreased in the control group by 2.84˚, resulting in a statistically significant difference of 2.16˚. In contrast to Kartalian's study, a study conducted on the Hyrax expander by Ghoneima et al found that dental tipping was responsible for most of the expansion (53). Weissheimer et al compared Hyrax and Haas expander directly and found no statistically significant difference in amount and proportions of maxillary expansion (40).

3.9.2 Amount of expansion

Larger meta-analysis studies were conducted, however they often fail to consider the specific appliance used. Instead of studying the Hyrax and Haas appliances separately, these studies grouped them together as "rapid maxillary expanders'. This was the case when Schiffman and Tuncay conducted a meta-analysis of available literature in an attempt to determine the changes resulting from maxillary expansion (54). Their review included literature between 1978-1999 and found an average immediate expansion of 6.0mm, that was then reduced to 4.71mm after short term retainer wear. After the retention period, this was further reduced to 3.88mm. Analysis of studies with long-term results, demonstrated that only 2.4mm of expansion was sustained. This amount of expansion was found to be no greater than normal physiological growth.

Another meta-analysis of literature by Lagravere et al looked at the immediate changes after RME in both the dental and skeletal perspectives (55). Orthopaedically, the nasal cavity increased in width by 2.14mm, and the left and right jugale width increased by 2.73mm.

Further, maxillary inter-alveolar width measured between the buccal plates increased by between two and three mm. Dentally, maxillary inter-molar width increased by 6.0-6.7mm and an increase in inter-canine width by 5.31mm and inter-molar angulation by 3.1˚ was also found. Further, 6.7mm of expansion occurred between the maxillary molar crowns, however only 4.5mm of expansion occurred at the root apices, resulting in an increase in angulation by approximately 3˚.

It is difficult to accurately predict and measure the proportions of skeletal expansion, alveolar tipping and dental tipping resulting from RME. This is because each study differs in the methods, duration of treatment, patients' age, compliance, activation protocol and quantification of outcomes. Further, accurately assessing the degree of maturation and timing of closure is difficult due to the high variance in timing of suture closure between individuals. Despite these difficulties, studies generally agree that RME is beneficial for transverse maxillary deficiencies if used compliantly and at the right time.

4 High-Frequency, Low-Magnitude Mechanical Stimulation

4.1 Introduction and Definitions

Since the 1980s, HFLMV vibration has been used to treat various bone diseases. It was initially researched in the treatment of osteoporosis and has more recently been applied in the orthodontic field. Bone is a living tissue and undergoes reactionary deposition and resorption as a means of homeostasis in response to stimuli. These stimuli may be static or dynamic, axial or torsional, and variable in duration, magnitude and frequency. The reactionary changes in bone can be in length, width, density and angulation depending on the initial stimuli.

Research into bone remodeling has suggested that HFLMV activates mechanotransduction in bone, resulting in stimulation of osteogenesis and alteration of bone metabolism. An example is the induction of HFLMV through a whole body vibration platform in children with cerebral palsy (CP) (2). HFLMV activates mechanotransduction in bone, resulting in stimulation of osteogenesis which counteracts the deficiency in skeletal development present in most patients with CP (3, 4, 6, 7).

In the field of orthodontics, intra-oral vibration devices have been proposed as a means of accelerating orthodontic treatment. Products such as AcceleDent™ are already on the market despite studies since 2013 demonstrating no statistically significant effect on the rate of orthodontic tooth movement (76-80). The effect of HFLMV on cartilage is poorly understood, and is thought to maintain chondrocyte activity despite an advancing age (81).

HFLMV induced through a whole body vibration platform may be experienced in the MPS or CBS and may have an influence on the remodeling and growth of bone and cartilage in this

area, however this has not been investigated.

4.2 The Daily Stress Stimulus Theory (DSST)

The DSST was initially proposed to explain the physiologic response of bone to the external environment. Initial testing of bone during functional activities suggested that bone deposition was proportional to the degree of strain experienced. A synthesis of the peak stresses from each loading event determines the mechanical stimulus and cellular response (82). Natural factors that influenced this were gravitational forces, muscle forces, weight training, running, or high impact exercise and other environmental forces. However, if a large static strain was induced on bone, no cellular response and bone remodeling was found in areas where there was no benefit. From this information, the DSST was deemed invalid, as the degree of stress was not always proportional to the amount of bone remodeling (83, 84).

4.3 Strains During Normal Function

Rubin and Lanyon used Rosette gauges to investigate the physiologic strains produced on the radius and tibia of two horses and dogs when moving at different speeds on a treadmill. From their results, they proposed that high-frequency strains (20-30Hz) remain in a narrow range shared amongst all animals, regardless of species, animal size, speed of travel or gait. Lower frequencies (1-10Hz), have a much larger spectrum of distribution which changes significantly depending on the speed and gait of the animal. This demonstrates that high frequency strains are always present and likely to be responsible for the organisation of bone tissue and that lower range of frequencies and amplitudes may play a significant, but more individual role (85).

4.4 Avian Ulna Model of Testing

Studies until this point were limited as they were unable to eliminate the superimposed load from functional movements. Further, trauma and vascular disturbances from the surgical procedures had a direct effect on bone. These factors provided an inherent mechanical strain on top of the experimental strain induced, complicating the origin of any remodeling that occurred. A major breakthrough in research methodology came in 1984 through the creation of the Avian Ulna model. Unlike previous methods, this model allowed complete control of the strain applied on bone by isolating the ulna shaft from function. This was achieved via an osteotomy at the epiphyseal-metaphyseal junction and placement of a stainless-steel cap on the ends. Strain and load were applied via Steinmann pins onto the caps at the ends of the bone shafts without impeding the bird's movement. The pin emerged out of the skin on the ventral and dorsal surfaces of the wing and the untreated contralateral side served as control. In order to minimise surgical influences, the site of operation was distant from the area for assessment (86, 87).

4.5 Parameters of Mechanical Loading

After the DSST was proven inadequate to explain the process of bone remodeling, the avian ulna model was used to study several different mechanical environments and the resulting cellular response in bone.

4.5.1 Static or Dynamic Loading

Rubin and Lanyon used the avian ulna model to test whether a continuous (static) load regime would be more influential than an intermittent (dynamic) load in osteoregulation. Three male turkeys were included in an eight-week experiment that attached bone pins onto the ulna shaft and helical springs on either end. These pins were dynamically loaded using an

Instron machine at a magnitude of 525N and frequency of 1Hz and a strain rate of 0.01s or statically loaded at 528N. The dynamic and static loading generated the same peak strain of -0.002 that was within physiological range and a third turkey was subjected to complete disuse of the bone. In the disuse group, no remodeling activity was found on the periosteal surface, however the endosteal surface showed evidence of resorption as there was an increase of 11% in the endosteal enclosed area. Intracortical remodeling also increased, and the percentage porosity rose from a mean of 0.52 to 2.1%. These changes combined to induce a reduction in total bone area by 13%. The statically loaded group responded in a similar way to the disuse group, demonstrating a total mean decrease by 13%. However, in the dynamically loaded group, cross sectional area increased on average by 25% due to new bone formation with most of the increase occurring in the periosteal region (87). Results from this study suggest that static loads within the functional dynamic strain range have no effect on remodeling, however a similar load applied intermittently for a short time resulted in a substantially increased bone mass.

5.5.2 Duration of Strain

Whether the duration of mechanical stimuli has an effect on cellular response was studied by Lanyon and Rubin. Roosters were analysed in a 6-week study and the ulna was loaded daily using either 4, 36, 360, or 1800 consecutive load cycles at 0.5 Hz for two seconds and compared to a non-loaded group. The control group demonstrated no change for two weeks, followed by a gradual decrease of 11.8% in bone mineralization. Roosters experiencing four consecutive load cycles had no disuse osteoporosis. Loading 36, 360, and 1800 times exhibited rapid endosteal and periosteal bone apposition between two and three weeks that peaked at four weeks, however there was no difference in size or character of bone between groups (86).

4.5.3 Magnitude of Strain

The effects of the strain magnitude on bone remodeling was examined by Rubin and Lanyon (1985). The avian ulna model was used, and different groups were subjected to peak strain magnitudes of 500, 1000, 1500, 2000, 3000, and 4000 micro-strains whilst the rate of strain (10000 ue/s), frequency (1Hz), and number of loading cycles (100) remained the same as previous experiments. All bones were subjected to external fixation to prevent natural loading. Strains of 1000 ue resulted in bone maintenance, however strains below 1000 ue resulted in bone loss. Strains above 1000ue demonstrated bone deposition on the periosteal and endosteal surface only. Bone formation did not necessarily occur in the areas that had the highest strain, thus there was a clear but non-proportional relationship between the strain magnitude and cellular response. As expected, strain distribution and character were found to have more effect than peak magnitude. (88).

4.5.4 Axial or Torsial Loading

Rubin et al tested whether axial or torsional loads generated different cellular responses (89). Twenty-one adult male turkeys were exposed to the same avian ulna model as previous experiments. Five ulnae exposed to 5000 cycles per day of axial loading equivalent to 1000 microstrain normal to the long axis of bone. Additionally, five ulnae were exposed to 5000 cycles per day of torsional loading sufficient to cause 1000 microstrain of shear strain, and six ulna experienced disuse. Torsional loading did not create area loss, changes in pore sizes, and intracortical porosis, however their findings indicated an overall inhibition of bone resorption. Axial loading also demonstrated no changes in pore size, however cell activity was promoted. This created an increase in intra-cortical turnover that was subsequently expressed as increases in area lost and intracortical porosis. From these results, this study demonstrated that axial and torsional loading influenced bone remodeling in dissimilar, but

positive ways. It also demonstrated that cells are able to discern between different types of loading, and the influence of cellular response on bone architecture depended on the mechanical environment present.

4.5.5 Strain Frequency

Mcleod and Rubin again used the avian ulna model to study whether frequency had an influence on strain and subsequent cellular response. Different frequencies between 1-60 Hz for 10 minutes per day were induced, and the degree of strain required at each frequency to maintain density measured. At 1 Hz (600 load cycles) a strain of greater than 700 microstrain (uE) was required, at 30 Hz (18000 load cycles) a strain of 400 uE was required, and at 60 Hz (36000 load cycles) a strain of 270 uE was required (78). The authors concluded that a higher frequency, required a dramatically reduced strain to maintain bone. This explains how very small strains occurring many times a day are able to maintain bone structure however greater strains that occur only a few times a day have a lesser effect.

4.6 Disproving The DSST

Fritton et al studied the strain history of different animals in an attempt to understand how bone strength was maintained. At that time, the mechanism of bone remodeling was unclear, as animals that had limited peak strains still managed to maintain bone morphology and strength, seemingly going against the DSST. Strain gauges were placed in vivo on one adult male turkey, one adult male dog, one adult female sheep and the ulna of three adult male turkeys. Strain signals were collected over 12-24 hours and subsequent digital analysis was conducted to measure the frequency and degree of each. This allowed for a calculation of the average of the spectral characteristics of bone strain signals. The results demonstrated that peak strains occurred relatively few times per day and small strains occurred much more

regularly (84). A strong relationship between frequency of strain and cellular response was found, demonstrating that bone does not respond greater to strains of higher cumulative magnitude. No proportional relationship was found between strain and resultant bony deposition and the linear mechanical formula proposed to explain the relationship between a mechanical stimulus and subsequent bony adaptation was proved to be non-existant. Fritton proposed a new idea stating that the characteristics of the applied load had the greatest determinant of bone adaptation and that dynamic loading had the greatest influence on mechanostimulation within bone structure.

4.7 Change in Paradigm

Qin et al further disproved the DSST by demonstrating that dramatic increases of peak daily cycles did not successfully predict the minimum threshold strain required to maintain bone mass. Accordingly, either the equation relating to the DSST must not be a linear function, or cellular activity must depend on frequency, strain rates or duration instead. Further investigation demonstrated that an increase in frequency resulted in a dramatically increased cellular activity, requiring smaller strains to maintain skeletal mass. This directed consideration of Wolff's law in a different perspective, moving away from the idea that maximizing strain was the main determinant of bone remodeling. The new theory proposed that the goal of skeletal morphological adaptation to an external environment was to engineer a constant environment within each particular site of bone (90). Accordingly, smaller strains many thousands of times per day have a much greater significance on bone remodeling compared to impact loads that happen only a few times per day (91).

4.7.1 Strains Induced by Muscular Activity

With this changed paradigm, it was found that small strains felt continuously throughout the day have a greater influence than peak impact strains in maintaining bone mass, however the

origin of the strain remained unknown (84). Considering this, Rubin et al conducted a study and found that an increased age resulted in a reduced level of muscular activity that correlated closely to an overall decrease in bone mass. From this information, the authors proposed that these small strains are likely to come from the thousands of muscular contractions occuring in the body every day (92).

Huang et al examined the firing rates of motor neurons in forty human subjects ranging from 20-83 years of age by monitoring acoustic vibrations. Recordings were documented as 1-50Hz, 1-25Hz or 25-50 Hz. With increasing age, high frequency band vibrations (25-50 Hz) decreased 1.2% per year, resulting in a reduction in the mechanical stimuli and strain inputs. As a result of this reduction in high frequency output, a decrease in bone mass is likely which cannot be counteracted by physical activity or any pharmalogical interventions (93). In order to prevent age associated bone loss, therapeutically induced high frequency strains through mechanical vibrations have been proposed.

4.8 Therapeutic Use of High-Frequency, Low-Magnitude Vibration

Robling et al (94) hypothesised that mechanical stimulation in the form of high-frequency, low-magnitude vibration can induce positive effects on bone remodeling. This is utilised in orthopaedic treatment of children with disabling conditions as it activates mechanotransduction in bone and stimulates osteogenesis (95). An applied application has been considered in the orthodontic field. Intra-oral vibration devices have been proposed to accelerate orthodontic tooth movement by increasing bone remodeling. Products such as AcceleDent™ are currently used by many orthodontists despite a Cochrane review in 2013 finding little evidence to support the use of HFLMV. The review suggested that a greater amount of well-designed randomised clinical trials are required to determine if there is a clinically significant improvement in the rate of tooth movement (76-80, 96).

4.8.1 Definition

HFLMV is a mechanical stimulus characterised by an oscillatory motion. The intensity is defined by the frequency (the rate the load is applied measured in Hz) and amplitude (extent of oscillatory motion, measured in mm). The magnitude can also expressed as a function of the earth's gravitational pull (9.8 m.s^-2 = 1g) measured in grams.

4.8.2 Transferring Whole Body Vibration to Sites of Osteogenesis

An initial complication towards the therapeutic use of HFLMV was the feasibility of transferring whole body vibration to sites of osteogenesis. Rubin et al tested this by placing pins parallel to the floor and perpendicular to the spinal column under local anaesthesia into the spinous process of the fourth lumbar vertebra and the greater trochanter of the femur in six health human subjects. Whole body vibration was induced onto these pins using a device developed by Fritton et al (97), transferring vibration between 15Hz and 35Hz at 2Hz intervals. Vibrations were measured at the hip and spine, patients were either relaxed and had their knees straight, knees flexed at 20˚, or knees locked and extended. If the patient was standing erect, vibrations below 20Hz were transferred completely to the hip and spine, however at 25Hz, only 80% was transferred. In a relaxed stance, transmission dropped to 60%, and dropped further to 30% when knees were bent. Rubin's study demonstrated that transmission of whole body HFLMV to the hip and spine does occur, however stance and posture has a large influence on the degree of transfer (98).

4.9 Cerebral Palsy in Children

4.9.1 Introduction

Cerebral palsy (CP) refers to a group of neuromuscular disorders resulting from an injury to the motor cortex of the brain (99). The resulting disability is characterised by diminished motor control and limitations of movement, balance and posture. In Australia, it is one of the

most common causes of disability in children and has a prevalence rate of 2.1 per 1000 live births (100-102). In 94.4% of these cases, the injury that results in CP occurs within 28 days of birth during the prenatal and perinatal period of development.

All children diagnosed with CP suffer from some decreased force generating capacity and control of muscles causing an overall limitation of physical activity (103, 104). The severity of this limitation is measured on a scale based on the five levels of the Gross Motor Classification System (GMCS). For a level I classification there is only a slight hindrance of speed, balance and co-ordination, however in a level V classification, the child must be transported in a manual wheelchair in all settings (101, 105).

4.9.2 Consequences on bone

Carlon et al conducted a review measuring the levels of physical activity in ambulatory children and adolescents who had CP. The results demonstrated that these patients conducted significantly lower levels of physical activity compared to matched controls to a level which was also below the recommended exercise guidelines (6, 106). Adequate physiologic mechanical loading during growth is critical towards developing healthy bone quality. As a consequence, these children demonstrate a lifelong decrease in bone mass and suffer from higher rates of fracture, especially in the lower extremities (107). In moderate to severe cases there is also a decreased trabecular bone micro-architecture (108). Interestingly, there is no statistically significant difference in areal bone mineral density (aBMD) in milder forms of CP compared to children who do not suffer from the disability (106). aBMD is considered the primary evaluation of fracture risk, however this measurement cannot be used in children as it is strongly influenced by bone size (109). During growth, a healthy level of mechanical loading is important in achieving optimal bone quality throughout life. As a consequence,

persons with cerebral palsy demonstrate poor bone quality and have higher rates of low-energy fractures throughout life (6, 7).

About 80% of children with CP are diagnosed with spasticity. Spasticity is a velocity dependent flexor and extensor excitation resulting from hyper-excitability of the stretch reflex (101). Spasticity leads to rigidity of the limb, leading to gait deviations and decreased selective motor control. For children who suffer from violent and repeated spasms, bone fractures and permanent deformities are common and can lead to a decreased quality of life (110).

Currently, there is no cure for CP. However, HFLMV is proposed to counteract the deficiency in bone development by inducing a positive reflex response in muscles resulting in an anabolic effect on bone.

4.9.3 Assessment of bone status

An editorial by Bax in drew attention to the need for further research into the musculoskeletal consequences of CP. It also highlighted the lack of effective treatment options for those suffering from the disease (111). A year prior to Bax's editorial (1995), Roberts et al conducted a study measuring the effects of hemiplegia on skeletal growth and maturation in the upper extremities. The main aim of their study was to determine whether the decreased skeletal maturation was a result of the underlying pathology or associated malnutrition. By using the less affected side as control, a skeletal index (SI) reflecting bone quality was calculated by dividing skeletal age with chronological age. The affected side demonstrated a significantly lower SI; however, the difference became smaller as age increased. As the bones of the upper extremities have limited weight bearing function, it is difficult to understand whether the decreased SI resulted directly from the CP or indirectly from the lack of physical activity (112).

Lin and Henderson conducted a study investigating the effect of spastic CP on bone mineralisation in the lower extremities (n=19, 3-15 years). Dual-energy X-ray absorptiometry (DEXA) was used to compare the affected and unaffected sides, and a 5.6% lower aBMD and 21% lower bone mineral content (BMC) was found. The bones of the lower extremities have a greater weight bearing function compared to the upper extremities, allowing the influence of mechanical loading to be measured. From the findings, it was proposed that if weight bearing occurred in this area, it would partially negate the neurological involvement on bone. Neurotrophic factors were not examined in this study, however the authors postulated that these and the reduced mechanical factors worked in combination to decrease aBMD (113).

A significantly larger sample size was used by Henderson et al in the investigation of bone density and its correlation to fracture rates at the distal femur of non-ambulant children. aBMD was significantly lower, resulting in a 15% increase in fractures for children under the age of 10 and a 28% increase in children over the age of 10. As the severity of CP increased in ambulant children, there was a significantly lower aBMD. This seems likely considering that a higher GMCS would mean greater physical disability and decreased load bearing. However, comparison between non-ambulant children that had a similar capacity of mechanical movements did not follow this rule. Although both level four and five GMCS scores require wheelchair assistance in all settings, a level five score demonstrated a significantly lower aBMD. Accordingly, although it is clear that mechanical loading and severity of CP have an influence on the poor mechanical properties in bone, the proportions of each are not fully understood (106).

Tasdemir et al conducted a study using computed tomography (CT) to compare the volumetric BMD (vBMD) of L1-L3 lumbar vertebrae in ambulant and non-ambulant children who had CP to each other and also typically developing children. Their results found that the

non-ambulant group had a lower vBMD compared to the control group. On top of this, ambulant children who had CP did not have statistically significant differences to typically developing children. Investigations into the relationship between levels of physical activity and vBMD confirmed that mechanical loading is crucial for optimal bone deposition during childhood. As expected, ambulatory children demonstrated a lower vBMD compared to controls, and a higher vBMD than non-ambulatory children, however the differences were not statistically significant. Although the results from this study were promising, the sample size of 24 was too small to draw any strong conclusions. In the future, studies containing larger sample sizes across a range of ages are required in order to confirm this relationship (114).

Wilmshurst et al compared the spinal bone mineral density of twenty-seven pre-pubertal children with CP between 5 and 14 years old to matched controls. Their results found no statistically significant difference between the children despite a range of mobilities and physical activity. Although this may seem contradictory to the proposed relationship between weight bearing and load, the authors suggested that the weight bearing properties of the spine during support of the upper body were relatively similar across all severities of CP and was sufficient to counteract the negative consequences of CP. On the other hand, a statistically significant difference was found in bone quality following Broadband Ultrasound Attenuation (BUA). BUA is a measurement of the structural properties of bone, and a higher score correlates to superior physical properties in bone. The lowest score was found in the group that had the highest level of physical disability.

The score was inversely proportional to the level of disability and as expected, the control group demonstrated the highest BUA score. This is because the calcaneum is a bone of the lower limb and has little weight bearing function in patients suffering from CP (115).

A peripheral quantitative computer tomography (CT) was used by Binkley et al to investigate differences in bone morphology and strength of the distal femur. Thirteen Children between the ages of 2 and 20 were selected for the study, and all except one subject was non-ambulatory. Compared to matched controls, the cortical thickness, cortical BMC, cortical area and periosteal and endosteal circumference were all significantly lower. On top of this, the matched controls demonstrated a greater cortical volumetric BMD and cortical thickness compared to the experimental group. From this information, we can conclude that children with CP have smaller and thinner bones. This study also investigated the effects of mechanical strain on the physical properties of bone. As most cases were non-ambulatory, the level of physical activity had no influences on bone quality. However, heavier children were found to have a higher vBMD, providing further evidence regarding the importance of mechanical loading in achieving optimal bone properties (116).

An increasingly accurate measurement of the physical properties of bone is critical in order to gain an increased understanding regarding the effects of CP and improving treatment modalities. For this reason, magnetic resonance imaging (MRI) has gained popularity as it increases the validity and reliability of the measurements without exposing the child to ionizing radiation. Although the financial cost per scan is usually higher, MRI provides measurements of trabecular bone microarchitecture that were previously impossible, this generates a greater understanding of bone geometry and strength. (117).

Modlesky et al conducted a series of studies using MRI, the first compared the bone quality of the distal femur to matched controls. The results demonstrated a significantly underdeveloped trabecular bone microarchitecture on the lateral half of the non-dominant distal femur. This was reflected in a lower bone volume/total volume (30%), apparent

trabecular number (21%), apparent trabecular thickness (12%) and significantly higher trabecular separation (48%).

In children with CP, the distal femur is the most commonly fractured bone, and rates of fracture are significantly higher compared to controls. In cases of repeated low energy fractures in children, a permanent deformity can result, further compounding their physical disability. The authors of this study proposed that this decreased trabecular bone microarchitecture and bone quality was in part responsible for the high risks of fracture among these children (108).

A second study was conducted in 2009 and compared the femoral midshaft of children who had quadriplegic cerebral palsy to a control group matched by gender, age and BMI. A lower total bone volume (54%), medullary volume (51%), antero-posterior bone width (29%), medial-lateral width (28%), cortical volume (55%), and a thinner cortical wall in the lateral (43%), posterior (32%) and anterior (28%) directions was found. On top of this, resistance to torsion and bending was estimated by measuring the polar moment of inertia, section modulus, and cross-sectional moment of inertia. All these measurements were significantly lower in children who had CP. The authors found that children who had quadriplegic CP demonstrated a significantly deteriorated bone strength and structure of the midfemur, and like previous studies he attributed this in part to the decreased levels of physical activity (118).

Modlesky et al recently published an article that examined the pattern of underdevelopment in the distal femur of non-ambulatory children who had quadriplegic CP. Site specific investigations were conducted to determine the areas within the distal femur that had the highest risk of fracture. The information gathered was used as a diagnostic tool to evaluate the efficacy of a rehabilitation protocol. For children who had CP, bone quality was

significantly lower than the control group in all regions, however App BV/TV was 17% and 24% lower in the region closer to the growth plate and gradually decreased in quality to 27% and 34% for regions further from the growth plate. These results demonstrated that the growth plate of bones in children with CP produced bone of decreased quality. On top of this, as the bone expands towards the periphery, the lack of physiological mechanical loading further decreases the quality of bone. From this information, the authors concluded that the risk of fracture in the distal femur has a proportional relationship to the distance from the growth site(119).

4.9.4 Applications of whole body vibration

HFLMV is proposed to have an anabolic effect on bone by generating a mechanical load on the skeleton. As a mechanism to counteract the acceleration created during vibration, the body induces a spinal reflex that is believed to cause muscle contractions, a muscle tuning response to counteract the vibration, and an excitatory response induced by muscle spindles detecting a change in length. Further, it is proposed to initiate storage and release of mechanical energy from neighbouring tendons (3, 4, 6, 7). As a direct consequence of these responses, a mechanical strain is generated that induces bone deposition in the surrounding bones and counteracts the negative effects of CP. Xie et al concluded that as little as 10 minutes of floor based whole body vibration per day could inhibit trabecular bone resorption in children (2).

Ward et al conducted a study that applied HFLMV on bone to 20 children who had disabling conditions. The children were randomised to stand on active (0.3g, 90Hz) or placebo devices for 10 minutes per day, five days per week, over six months. The tibial and spinal volumetric trabecular BMD was measured using 3-D quantitative CT, and there was a statistically significant benefit in the tibia despite poor compliance levels (44%) (4).

The first study on children with CP was conducted by Wren et al. Thirty-one children who had CP between the ages of 6-12 were allocated to stand on a floor-based vibrating platform (0.3g, 30Hz) for 10 minutes per day for five months and a floor-based placebo platform for 6 months, in a randomly allocated order. Computer tomography measurements of proximal tibia cancellous bone density (CBD), geometric properties of the tibia, vertebral CBD, cross-sectional area, and dynamometer measurements of plantarflex strength were taken at 0, 6 and 12 months. No differences were found in cancellous bone or muscle, however there were significant increases in cortical bone properties during the vibration period(3).

Katušić and Mejaški-Bošnjak measured the outcomes on the musculoskeletal system of vibration induced at 40Hz from a bed pad. Thirteen children who had spastic cerebral palsy between the ages of three and four were included in the study, and placed in a supine position for 20 minutes, once a week, for twelve weeks. A significant improvement in muscle activity was found, characterised by enhancements in motor performance, stability and selectivity of movements. These children developed improved head control, postural trunk stability and increased rotational movements, demonstrating the potential that HFLMV can have in aiding musculoskeletal health and quality of life (5).

4.9.4.a Site specific high-frequency, low-magnitude vibration

Different vibration frequencies applied directly to the bone were examined by Reyes et al. Instead of 30-40Hz as per previous studies, sixty-five children who had CP between the ages of 6-9 were randomised into either placebo, 60Hz, or 90Hz groups. The vibration was applied at 0.3g and was delivered to the radii and femurs for five minutes each day, for six months. Bone mineral density (BMD) and bone mineral content (BMC) was measured at the radii and femoral neck. The 60Hz group demonstrated a statistically significant increase in BMD at the upper distal radius by 31.88% +/- 28.3%, and the 90Hz group experienced a statistically

significant difference of 6.42% +/- 14.32%. On top of demonstrating that HFLMV can improve BMD and muscle strength, this study introduced a means of delivering mechanical vibration directly to an area as oppose to the whole body (6).

Larger randomised control trials that considered the different severities of CP and presence of spasticity must be conducted in order to gain a better insight into the role of vibration, however these initial studies have been promising.

4.10 Whole body HFLMV in Other Medical Treatments

4.10.1 Low BMD in Young Women

Based on the hypothesis that the incidence of osteoporosis can be reduced by increasing bone mineral density of young adolescents and children with CP, Gilsanz et al investigated the effects of HFLMV on muscle and bone mass in young women who had low bone mineral density (BMD) (120). 48 subjects between 15-20 years old were chosen on the basis of having low BMD and at least one skeletal fracture in the past. Half of the subjects received vibration at 0.3g and a frequency of 30Hz for 10 minutes per day for six months. Quantitative CT demonstrated a positive influence on BMD in the vibration group, resulting in an increased muscle and bone mass of the weight bearing skeleton. As a consequence, the authors proposed that if this increase in bone mineral quality is maintained throughout life, there would likely be a reduction in the rates of osteoporosis.

4.10.2 Simulated Postmenopausal Conditions in Rats

Flieger et al (76) and Oxlund et al (121) studied the effects of whole body HFLMV on simulated p was conducted in 1961 postmenopausal conditions on rats. In order to induce post-menopausal conditions, the rats were ovariectomised and vibration was induced between

the parameters of 0.5-3.0g, and 17-50Hz. In both studies, the control group demonstrated an overall decrease in BMD and the vibration group either maintained or increased BMD for the duration of the experimental period. This showed the potentially beneficial effect that passive physical loading may have on ovariectomised rats.

4.10.3 Postmenopausal Women

Seventy women 3-8 years post-menopause were studied by Rubin et al (79). Half were subjected to HFLMV (30Hz, 0.2g) for 10-minute sessions, twice per day when standing. BMD was measured at the spine, hip and distal radius at the start of the experiment, at 6 months, and at 12 months. The results demonstrated that HFLMV can effectively prevent a loss in BMD in the spine and femur. Interestingly, the therapeutic effect was greatest in patients who were compliant and had a lower bone mass.

Verschueren et al also investigated the effects of HFLMV on the BMD of the hip in post-menopausal women through a randomised control trial. Seventy subjects were randomly divided into three groups (122). One group carried out resistance training, the second received whole body vibration (30-40Hz, 2-5g) during dynamic knee extensor exercises, and the final group served as control. No changes were found in the control, and resistance training groups, however for the vibration group BMD of the hip and dynamic and isometric bone strength significantly improved. The authors proposed that vibration was an effective way to decrease risk factors associated with falls and fractures in post-menopausal women by improving balance, muscle strength and BMD, however further studies containing a greater sample size are required.

4.10.4 Bone Healing in Mice

In a similar way that vibration improves bone quality in post-menopausal women, it is proposed to improve the healing capacity of bones following fractures or surgical wounds. Omar et al investigated the effects of vibration on the healing of surgical defects within cranial bones. Twenty 12-week-old rats underwent surgery to induce bony lesions in the parietal bone (123). Half of these rats had vibration induced at 30Hz for 20 minutes per day, 5 days a week, for a 4-week experimental period. The rats were sacrificed at 0, 14, and 28 days, and micro-CT analysis demonstrated that healing was significantly more pronounced in the vibration group.

4.10.5 Bone Healing in Sheep

Goodship et al investigated the influence of HFLMV on fracture healing in the bones of sheep. Osteotomies of 3mm were performed in the tibia of sheep, and vibration was applied at 30Hz for 17 mins per day for 10 weeks. At the end of the experimental period, the callus in the experimental group was 2.5x stronger, 3.6x stiffer, and 29% larger than controls (124). Bone mineral density was 52% greater, and there was a 2.6x increase in bone mineral content in the periosteum. Both Goodship and Omar's studies demonstrated that the presence of HFLMV following a traumatic injury significantly improves the healing capacity of bones.

4.10.6 Inhibition of Osteoclast Formation

Within bone, there is a complex network of cells which work together to facilitate bone remodeling. Osteocytes form a crucial portion of this pathway as these cells send signals to stimulate generation and function of osteoblasts and osteoclasts. Lau et al tested the effect of HFLMV on osteocytes by applying vibration at 0.3g, and 30, 60, or 90 Hz for one hour to osteocyte like MLO-Y4 cells. Osteocytes were found to be sensitive to this stimulus, as

COX-2 increased by 344% at 90Hz and RANKL decreased by 55% at 60Hz. The formation of large osteoclasts was attenuated by 36%, resulting in a 20% decrease in the amount of osteoclastic bone resorption. RANKL decreased by 53% in the vibration group and PGE2 release decreased by 61%. Lau concluded that HFLMV had an inhibitory effect on the signaling pathway between osteocytes and osteoclasts, leading to a decreased production of osteoclasts and a reduced amount of bone resorption (125).

4.10.7 Therapeutic Effect of HFLMV

From the literature, it is evident that vibration can play a significant role as an adjunct orthopaedic treatment methodology. Studies have demonstrated positive responses in BMD and bone quality in children with disabling conditions, young women diagnosed with a low BMD, fracture healing, repair of bone defects and prevention of pathological bone conditions such as osteoporosis.

4.11 Mechanical Vibration and Cartilage

Several studies have demonstrated a therapeutic effect of HFLMV on bone, prompting researchers to investigate whether there is an effect on cartilages within the body. It was initially proposed that if cartilage responded in a similar way to bone, treatment using HFLMV would prevent deterioration of articular cartilages in patients who had osteoarthritis (81).

4.11.1 In Vitro Studies

4.11.1.a Cultured Rabbit Articular Chondrocytes

An in vitro study conducted by Liu et al studied the effects of mechanical vibration on proteoglycan and DNA synthesis in cultured rabbit articular chondrocytes (126). Vibrations

were induced at 200, 300, 400, 800 or 1600 Hz and 1.4g in a sinusoidal waveform. It was found that at 300Hz, the DNA and proteoglycan synthesis in chondrocytes was upregulated, reaching a maximum when vibration was applied for right hours per day. Peak proteoglycan synthesis occurred at 15 days, however peak DNA synthesis occurred at ukytt s days. Down regulation was noted at all other frequencies. Different frequencies either enhanced or depressed incorporation of 3H-thymidine into DNA and $H_2{}^{35}SO_4$ into proteoglycans. The authors proposed from their results that vibration had a positive effect on metabolism on the articular cartilage, especially during the regeneration process.

4.11.1.b Cultured Chondrocytes of Pig Joints

The effects of vibration (100Hz) and hyaluraonic acid on cultured chondrocytes from joints of six month old pigs were investigated by Takeuchi et al (127). Measurements were taken at 3, 7, 10 and 14 days and production of chondroitin six sulfate and chondroitin four sulfate was recorded as indicators of proteoglycan synthesis. After histological, immunohistochemical and electron microscope analysis, it was found that chondrocytes subjected to vibration and hyaluraonic acid had thicker stratified structures of collagen, stronger chromatic features, and long, slender prominences that were associated with extracellular substance..

The authors suggested that hyaluraonic acid and vibration activate the production of proteoglycan in 3D cultured chondrocytes, suggesting that mechanoreceptors on the surface of the chondrocytes respond to vibration positively by activating intracellular pathways.

4.11.2 In Vivo Studies

Sriram et al conducted an in vivo study that studies the mandibular condylar cartilage and its endochondral bone in 40, 12-week-old mice. Half of the animals were subjected to vibration

of 30Hz at 0.3g for 20 minutes per day, five days per week, for four weeks and the other half served as controls (128). Micro CT analysis of the experimental group showed that the condylar cartilage volume decreased and the trabecular bone demonstrated an associated increase. The decrease in condylar cartilage volume was concluded to be due to endochondral bone replacing hypertrophic cartilage faster than cartilage could be replaced. As a consequence of this observed response, vibration was proposed to have a potentially therapeutic effect on the adaptive orthopaedic growth of the mandibular condyle.

4.12 High-frequency, Low-magnitude Vibration in Orthodontics

The use of HFLMV in the orthodontic field is an adjunctive treatment methodology based on the concept that PDL and bone cells surrounding teeth should respond in the same way as bone cells in the medical treatment of osteoporosis. Vibration is proposed to activate mechanotransduction, leading to an increased rate of cell differentiation and maturation. This would alter bone metabolism and stimulate osteogenesis, resulting in an increased rate of bone remodeling and an accelerated rate of tooth movement. If successful, concomitant use HFLMV during orthodontic treatment would lead to a decrease in orthodontic treatment time. Unfortunately, whilst initial studies were positive, a 2015 Cochrane review found little evidence to support the use of HFLMV. The review suggested that a greater amount of well-designed randomised clinical trials are required to determine if there is a clinically significant improvement in the rate of tooth movement (96, 129-131).

In cases of decreased orthodontic treatment duration, incidence of caries, root resorption and periodontal disease declines, minimising the psychological and physical burden on the patient (129-131). Unfortunately, studies in this field remain controversial and a 2013 Cochrane review found little evidence to support the use of HFLMV. The review suggested that a

greater amount of well-designed randomised clinical trials are required to determine if there is a clinically significant improvement in the rate of tooth movement (96).

4.12.1 Animal Studies

Initial studies induced the mechanical stimulus using a pulsed electromagnetic field (PEMF). Stark and Sinclair conducted a study on Hartley guinea pigs and found that applying 25Hz of PEMF with simultaneous 12cN of orthodontic force increased the rate of tooth movement. Darendeliler conducted two separate studies however found similar improvements in the rate of tooth movement. The first was conducted in 1995 and studied guinea pigs at a frequency of 15Hz and 15cN, followed by another in 2007 that studied the effects of HFLMV on tooth movement at a frequency of 30Hz on 44 wistar rats. Nd-Fe-B magnets and coil springs were bonded onto the molar teeth to generate tooth movement, however an electromagnetic field (EMF) interacted with the magnets to generate a mesiodistal vibration stimulus. The rats were subjected to different stimuli on either side and divided into four groups that had either vibration alone, vibration compared with vibration and coil spring, PEMF alone compared with PEMF and coil spring, and vibration and coil spring compared with PEMF and coil spring and sacrifice of the specimens occurred after 14 days. The results demonstrated that coil springs with sham or active magnets moved the molar teeth more than magnets with or without vibration and no statistically significant difference was found between magnets and sham magnets without PEMF. The coil-magnet combination moved the teeth less than the coil spring under PEMF and the sham magnets under PEMF moved the teeth less than the magnets. As a consequence from these findings, the authors concluded that PEMF induced vibration may enhance coil and magnet induced orthodontic tooth movement (132).

A second method to induce vibration tested more recently involved the intermittent stimulation through resonance vibration. Nishimura et al studied the effects of vibration on

the rate of tooth movement and root resorption and the underlying molecular and cellular mechanisms responsible in 11 Wistar male rats. Half of the rats were subjected to tooth movement and vibration at 1g and 60Hz +/- for eight minutes once per week, and the other half were only exposed to tooth movement. The tooth movement was induced by placing a buccal force on the molar for 21 days. Immunohistochemical analysis at the end of the experimental period demonstrated an increased activation of the RANK-RANKL signaling pathway in the vibration group. There was an enhanced RANKL expression from osteoclasts and fibroblasts of the PDL that resulted in an increase in orthodontic tooth movement. After analysis using haematoxylin and eosin, no significant difference in root resorption was found. The enhanced expression of RANKL in the PDL was concluded to be responsible for the increased rate of orthodontic tooth movement. On top of this, the authors determined that this enhanced bone turnover rate did not result in additional damage to the tissues (133).

HFLMV may also influence tooth movement through inhibition of osteoclast generation. Kalajzic et al investigated this by measuring the rate of tooth movement, number of osteoclasts and the subsequent consequence on the bone volume fraction in the periodontium. Twenty-six seven-week-old rats were randomly assigned to either movement (Mvt), vibration (Vib) or movement + vibration (Mvt + Vib) groups. Vibration was induced onto the occlusal surface of the first maxillary molar using an electromechanical actuator that applied unilateral cyclic forces at 30Hz, between 0.1-0.4N for 10 minutes, twice per week. Tooth movement was conducted using a 9-mm NiTi closed-coil spring and delivered 25g of orthodontic force for 14 days. It was found that the Mvt group had a statistically significant increase in tooth movement compared to the Mvt + Vib group. Almost no osteoclasts were detected in the Vib and Mvt + Vib group, however there was an increase in the Mvt group. Bone volume fraction was significantly lower in the Mvt group andcollagen fibers on the tension area of the PDL were significantly thicker in the Vib group and Mvt group. Further, the Mvt + Vib group

demonstrated a disturbed morphology of bone volume fraction. The authors concluded that vibration induced at 30Hz may have a negative effect on the overall number of osteoclasts, resulting in a decreased rate of tooth movement and a greater bone volume fraction (134).

Yadav et al conducted a similar study on sixty-four male CD1 mice, using a force of 10g on the maxillary first molars, and vibration applied for 15 minutes at five, ten or 20Hz in 3-day intervals for 14 days. At the end of the experimental period, the results demonstrated no statistically significant difference in rate of orthodontic tooth movement and quality of collagen fibers, however vibration had a positive effect on bone volume (135).

4.12.2 Human Studies

In 2012, the Tooth Masseuse device was tested on patients. This device was initially proposed to decrease the pain of orthodontic treatments. The tooth masseuse device induces vibration at a frequency of 111 Hz and magnitude of 6.1g for 20 minutes per day. 66 patients were randomly assigned to either a control or experiment group, with all patients fitted with a 0.014 inch thermal NiTi wire for a 10 week experimental period. Impressions of the mandible were taken, and Little's Irregularity index was used to assess the six anterior teeth at the start of treatment, five weeks, eight weeks, and 10 weeks into treatment. Patients were asked to complete a discomfort score chart at each time interval. No significant differences were found in arch irregularity or pain levels, suggesting no clinical advantage for this device (131).

A 2016 study investigated whether interleukin-1 beta secretion was increased as a result of vibration produced from an electric toothbrush. This study was conducted on 15 patients that previously had bilateral maxillary first premolar extraction and required subsequent canine distalisation. Orthodontic tooth movement was induced using a power arm fabricated from a

0.021 x 0.025-inch stainless steel archwire. A split mouth model was used, and patients were randomly assigned to place the electric toothbrush on either the left or right maxillary canine at the mesio-labial surface. No definitive amount of time for vibration was set, as patients were instructed to place the toothbrush on the tooth for a minimum of five minutes, three times a day for 2 months. The results demonstrated that the canine on the non-vibration side had an average of 1.77mm of movement, compared to 2.85mm on the vibration side, equating to a statistically significant difference of 1.08mm. A statistically significant increase in IL-1B secretion in the GCF was also found at the pressure sites in bone during tooth movement. Although these results seem promising, the validity of such a study design must be questioned due to the varying amount of vibration experienced, poor compliance levels and unpredictable transmission of vibration from an electric toothbrush to teeth and surrounding bone (136).

4.12.3 AcceleDent™

As the case in Animal studies, the initial human study was promising, as Kau conducted a retrospective study which used an early activator device consisting of a mouthpiece that generated vibration at a frequency of 30Hz, and 20g for 20 minutes per day. The device was placed for a period of 6-month consecutive months and the rate of tooth movement was significantly improved for cases of Class I lower incisal crowding. However, research published more recently has been less promising.

Bowman investigated the effects of AcceleDent™ on upper-molar distalisation in sixty-five adolescent Class II patients. Each patient underwent non-extraction treatment and a mini-screw supported upper molar distalisation device was inserted. The mini-screw implants were inserted in between the upper first and second premolars in the palatal alveoli. 240g open-coil springs were then attached to this apparatus and recompressed every fpir weeks. Lateral

cephalometric analysis demonstrated no significant differences when comparing upper first molar tipping, intrusion, or crown distalisation, however for the molar root apex the vibration group demonstrated a statistically significant increase of 71% compared to control (137).

In 2015, the effects of AcceleDent™ on the rate of movement of maxillary canines into the upper first premolar extraction space was investigated. 45 patients were included in the study who fit the inclusion criteria of having extracted maxillary first premolars, three mm of extraction space after initial alignment, and maximum maxillary anchorage. 23 of the patients were randomly assigned to the acceledent group and the other 22 were given placebo devices. Comparing the rate of movement, the average monthly rate of the vibration group was measured at 1.16mm, compared to 0.79mm in the control group. This difference of 0.37mm was statistically significant, and the authors concluded that simultaneous HFLMV during orthodontic treatment accelerates tooth movement (138). Unfortunately, this study was funded by "Orthoaccel", and a clear conflict of interest was found. Further, a large range of ages (between 12-40 years of age) with a small sample size was used allowing for the influence of many co-variates. Little information was provided about the method of assessing tooth movement apart from it being conducted directly in the mouth with digital calipers. Accordingly, this study was not considered when determining the effectiveness of HFLMV on accelerating orthodontic tooth movement.

In a randomised clinical trial conducted by Woodhouse et al on 81 orthodontic subjects requiring extraction of lower first premolars and treated with fixed appliances were randomised to three groups (139). The first were given a fully functional acceledent device, the second given a sham device and the third given no device. A standard protocol was used and analysis using Little's irregularity index demonstrated no difference in the number of days required to achieve initial and final alignment per each arch wire. As was the case with

animal studies, earlier human studies showed greater promise, however more recent higher quality studies have failed to find a statistically significant difference in the rate of tooth movement. As emphasized by the 2015 Cochrane review, further higher quality studies testing a range of vibration stimuli and orthodontic tooth movements are required to determine conclusively if vibration may improve the rate of tooth movement (140).

Katchooi (2017) investigated the effect of AcceleDent™ on Invisalign treatment. 26 adult patients were randomly allocated to equal groups of 13 and were either given an active or placebo AcceleDent™ device. They were instructed to change their aligners once a week and the aligner fit was reviewed every three weeks. If the aligners were not sitting, the patient was not included in the results. No statistically significant differences were found when comparing the regularity of anterior teeth at the end of treatment and the completion rate of the initial series of aligners (141).

5.13 Influence on the Midpalatal Suture

There have been no high quality studies to support the use of HFLMV in orthodontics (96). Despite this, HFLMV as an adjunct to orthodontic treatment is readily available on the market for orthodontic patients. Although appliances for orthodontic treatment aim to apply local vibration, anatomically close areas of the cranium may also be susceptible to this vibration. Further, the treatment of mechanical disabilities such as cerebral palsy using whole body HFLMV may be experienced by the MPS. As a result, there may be an effect on bone deposition during growth of the MPS or in the bone defect created following maxillary expansion. Currently, there are no studies investigating this.

HFLMV is routinely used in the medical field to improve bone quality in children with motor disabilities such as cerebral palsy (CP). For this treatment, vibration is induced during a

period when growth of the craniofacial sutures is heavily influenced by the external environment. In natural growth, sutures of the head rely on mechanical strains to modify and promote growth and lie dormant until an external signal such as the pressure of soft tissue growth is experienced. An example is the MPS, which acts as a growth site responding to external signals stimulating deposition of bone on the sutural edges that facilitate expansive growth of the maxilla (10-12). Any additional mechanical strains such as whole body HFLMV are likely to modify the rate of bone remodeling at the interface of the two maxillary bones and affect natural growth (8-12). Other examples are the CBS, which rely on the external environment for growth. However, unlike the MPS, undergo endochondral ossification, where bone replaces cartilage.

5 Oscillating Mechanical Stimulation

5.1 Introduction

Growth of the craniofacial sutures is heavily influenced by the external environment. In natural growth, sutures of the cranium rely on mechanical strains to modify and promote growth and lie dormant until an external signal such as the pressure of soft tissue growth is experienced. Accordingly, any additional external mechanical strains are likely to modify the rate of bone remodeling at the interface of the two maxillary bones and affect natural growth (8-12).

Sutures are unique to skull bones and are made up of several cell types such as osteogenic cells, fibroblast-like cells, and mesenchymal cells. There are two main mechanisms associated with active sutural growth (8, 10, 142). Firstly, sutural osteoblasts deposit bone matrix whilst sutural fibroblasts synthesise matrices that are non-mineralised and function to maintain the presence of sutures (8, 10, 142). Sutural growth is characterised by increases in differentiation, proliferation and matrix synthesis of mesenchymal, fibroblastic and osteoblastic cell lineages (9).

5.2 Strain absorption in the Craniofacial Sutures

Herring and Mucci were the first to investigate whether externally induced strains were experienced within cranial sutures and found that craniofacials sutures of miniature pigs experienced large strains during normal function. Specific investigation of the zygomatic sutures demonstrated that these regions experienced 1000-2000 microstrain, comparable in magnitude to those measured in the postcranial skeleton during vigorous locomotion (143). In a study on goat crania, Jaslow found through mechanical tests that areas of bone with sutures absorbed more energy under impact loading than without sutures, and the amount absorbed

was proportional to suture interdigitation (144). Herring and Teng studied the cranial sutures of miniature pigs during natural masticatory function, and stimulation of various cranial muscles in anaesthetized animals. It was found that upon jaw closing only the coronal suture experienced tension, while all other sutures experienced compression. Whether compression or tension was experienced depended on muscle usage. It was also found that maximum tensile strengths were greater than compressive strengths (145).

Herring and Mucci's study also suggested that adjacent sutures may experience strains of opposite polarity, and the morphology of the sutures dictated the direction and degree of energy absorbed (143). For example, well developed contacts of zygomatic and squamousal bone are connected with compression resisting fibers in a vertical direction, but in a horizontal direction have much simpler tension resisting fibers. This may be due to the anatomical interdigitating projections present, allowing for compression resisting fibers to attach.

5.3 Importance of Mechanical Loading in the Growth of the Craniofacial Sutures

Persson (1995) found that cranial sutures do form in the absence of muscle activity and a functional environment (146). However, finer sutural morphologies were modeled and formed as a response to secondary extrinsic forces. Further, Markens and Oudhof found that the coronal and sagittal suture can become transposed and inverted if anatomical morphology is changed (147). Kokich found that masticatory resection and mechanical immobilsation of sutures resulted in decreased sutural complexity, demonstrating the importance of the external environment in determining sutural morphology.

It can therefore be concluded that in natural growth, sutures of the cranium rely on mechanical strains to modify and promote growth and lie dormant until an external signal such as the pressure of soft tissue growth is experienced.

According to a study investigating the mechanobiology of craniofacial sutures, sutural osteogenesis is likely modulated by microscale shear stresses induced by the tension or compression forces (8). This study demonstrated that fibroblastic cells in sutures increase proliferation and matrix synthesis following induction of mechanical stresses with cyclic strains having the greatest effect (8). It has been proposed that the fluid flow in bone which is modified and induced by the strain rate and oscillatory bone strain is responsible for triggering mechanotransductive responses, whereas constant forces have no ability to induce fluid movement (95, 148-150). As a consequence, amplitude of bone strain above a certain strain likely has no influence upon the rate of bone deposition compared to strain rate and energy (84, 151-153).

5.4 Effect of Externally Induced Dynamic Loading on Craniofacial Sutures

HFLMV and their effects on growth of the cranial sutures have not been studied. This is important as HFLMV is used routinely in the medical field during a period when growth of the craniofacial sutures is heavily influenced by the external environment, especially in children with CP. In natural growth, sutures of the head rely on mechanical strains to modify and promote growth. Any additional mechanical strains such as whole body HFLMV are likely to modify the rate of bone remodeling at the interface of the bones adjacent to the suture (8-12). An example is the manipulation of the MPS where static forces have been used for over a century to modulate osteogenesis of craniofacial sutures in both laboratory research and clinical practice. They induce their effects through mechanical strain which is applied in a static waveform to craniofacial sutures through devices such as headgear, facemask and functional appliances.

Until 2003, only static forces were used in the modification of sutural growth as the characteristics of dynamic force transmission between bone and sutural tissue were unknown.

HFLMV and their effects on growth of the cranial sutures have not been studied. This is important as HFLMV is used routinely in the medical field during a period when growth of the craniofacial sutures is heavily influenced by the external environment, especially in children with CP. In natural growth, sutures of the head rely on mechanical strains to modify and promote growth. Any additional mechanical strains such as whole body HFLMV are likely to modify the rate of bone remodeling at the interface of the bones adjacent to the suture (8-12). An example is the manipulation of the MPS. Static forces have been used for over a century to modulate osteogenesis of craniofacial sutures in both laboratory research and clinical practice. They induce their effects through mechanical strain which is applied in a static waveform to craniofacial sutures through devices such as headgear, facemask and functional appliances.

Previous studies have investigated the effects of mechanical oscillating strains on the growth and dimensions of the PMS and NFS (8, 21, 154-156). For these studies, vibration was induced at a frequency of less than 10Hz at a magnitude of between 0.3 – 5N for between 5 and 12 days. In the first of these studies, Kopher demonstrated that static, sine or square wave forms between one N and five N at with one N increments were transferred and expressed as compressive strain in the PMS and tensile in the NFS. Further, PMS demonstrated compressive forces of 10-fold greater magnitude compared to the tensile force experienced in the NFS. Herring and Mucci explained this by suggesting that adjacent sutures may experience strains of opposite polarity, and the morphology of the sutures dictated the direction and degree of energy absorbed (143).

Subsequent studies have demonstrated an escalation in cellular activity and bone remodeling, producing physical changes in the form of an increase in width within the suture. In Mao et al's study, a tensile sinusoidal vibration stimulation at frequencies of between 0.2 Hz and

1Hz in 0.2Hz increments and magnitude of two N for ten minutes per day over 12 days were placed on the maxillary incisor. A significant increase in average sutural cell count was demonstrated for the vibration group (156). In addition, Kopher and Mao (2003) also observed an increase in sutural width when vibration was induced at a magnitude of 5N and all other parameters kept the same (142). This seems contradictory to the expected results considering osteoclasts, osteoblasts and fibroblasts increase in number and activity. However, the early stages of sutural response are predominated by osteoclastic and fibroblastic, rather than osteoblastic action (142, 155).

Mao and Kopher utilised histologic staining and photomicrography and demonstrated that the dynamic group had a statistically significantly increase in the sutural cell count. The left side was dehydrated, trimmed and demineralised with 20% sodium citrate and 50% formic acid and embedded in paraffin. H&E staining was conducted following cutting of eight um sequential sections in the parasagittal plane. Construction of circles within the photomicrographs with diameters equal to the width of the suture demonstrated the average sutural width. Further, total sutural cells regardless of cell type were manually tagged in grids that were 110 x 110um^2 at 10x objective in six randomly selected grids per specimen (142). Fluorescent microscope labelling was conducted to identify new bone formation and the rate of osteogenesis. The right side of the PMS and NFS was dehydrated and trimmed in ethanol and acetone and prepared for uncalcified embedding with 15% dibutyl phthalate and 85% methyl methacrylate (MMA). 15-um sections were cut in the same plane using a microtome and used to calculate newly mineralised bone along the sutural edges with calcein labelling in undemineralised sections using a fluorescent microscope (142). These measurements also demonstrated an increase in the sutural width compared to the statically loaded and control groups (142).

Vij and Mao induced cyclic compressive forces at 300Mn and 5Hz onto the maxilla for five consecutive days on rats, starting at either 7, 23 or 32 days old over 20 minutes per day and discovered an increase in the average number of osteoblasts and osteoclasts. This study found that mesenchymal cells and fibro-blast like cells decrease apoptosis and increase the rates of proliferation following cyclic loading (155). This study also demonstrated an increase in multinucleated osteoclast cells demonstrating that net rapid growth is preceded by bone resorption. The method used was similar to Kopher and Mao, however measured the total number of nucleated cells excluding those lining blood vessels and sutural surface bone. On top of this, the average surface osteoblast was calculated by recording the percentage of the sutural surface resided by the cuboidal, mononuculated cells using computerised analysis. Further, osteoclast-like cells were quantified by recording all cells with three distinct nuclei (155). A similar result was found in Peptan et al when cyclic tensile and compressive forces of magnitude 1N and frequency eight Hz were placed on the maxilla for 20 minutes per day for 12 days using a computerised servohydraulic system. Distinct sutural bone formation and resorption surfaces were found. However, a decrease was found in the average osteoclast surface cells (154).

These experiments demonstrate that by 12 days, osteoclasts had decreased in number whilst proliferation and activity of osteoblasts and fibroblasts continued to increase (154). If the experiments were conducted for a period greater than 12 days, the expected results would have been a decreased amount of bone resorption and increased bone deposition onto the newly deposited sutural matrix. As a result, the bone remodeling equilibrium would have shifted towards net bone deposition and decreased sutural width.

Research has demonstrated that mechanical strains have an influence on cellular activity, bone remodeling, and matrix deposition within the suture. As a consequence, it seems likely

that present day treatment regimens for the improvement of bone quality in children with CP using HFLMV also have the ability to modify bone remodeling and bone deposition within the midpalatal suture. Although previous studies have investigated frequencies below 10Hz, there have been no studies investigating the influence of vibration at higher frequencies on the cranial sutures. If a statistically significant difference is found, there may be implications towards the growth and development of the maxillary complex.

6 Hypofunction and Tooth movement

6.1 Hypofunction

Everyday activities result in physiologic forces that are transferred from teeth to the periodontium an infinite number of times each day. These strains act to maintain normal alveolar and dental morphology by influencing the action of osteocytes and modifying osteoclast and osteoblast action. Hypofunction refers to the situation following removal of teeth from ordinary occlusal function, usually resulting from an open bite occlusion or extraction of opposing teeth. This results in a poorly function periodontium, that causes a decreased bone density, enlarged marrow spaces and thinning of the outer shells of alveolar bone (94, 170). Hypofunctional teeth encounter less resistance to movement from the alveolar bone during orthodontic treatment. On top of this, the PDL of these teeth demonstrate atrophic changes such as disorientation of collagen fibers, vascular constriction, and narrowing of the periodontal space. This results in a physiological remodeling of teeth that causes pathologic resorption and abnormal root shapes (171-173).

6.2 Surrounding Alveolar Bone

6.2.1 Studies on Mice

Previous studies have shown that RANKL is a crucial local molecule that acts to promote osteogenesis. On top of this, TRAP-positive cells are considered the hallmark of osteoclastic activity. To determine whether these mechanisms were responsible for changes in bone morphology in hypofunction, Enokida et al used histomorphometric analysis and immunohistochemical detection of RANKL and TRAP-positive cells. In this study, 40 seven-week-old male Wistar rats were divided into two groups. Half were exposed to a device

composed of a stainless steel anterior bite plane and cap. Feeding was not a problem as all specimens gained weight during the experimental period. The specimens were either sacrifised at three or seve days. tnree 3 days, the hypofunction group demonstrated bone marrow space widening and elongation vertically parallel to the concurrent supraeruption. Measurement of osteoclastic activity demonstrated that TRAP-positive cells decreased at the mesial aspect of the interradicular alveolar bone surface and increased at the margin of the marrow spaces. There was also an increase in the number of RANKL-positive osteoblasts and RANKL-positive multinucleate cells in bone. At seven days, marrow spaces elongated further in the vertical direction to include the superior part of the interradicular alveolar bone. On top of this, periodontal spaces narrowed further, and blood vessels increased in number and volume as well. This increased amount of blood vessels and a further decrease in TRAP-positive cells was also found on the mesial aspects. Finally, a significant decrease in bone to tissue volume ratio and an increase in RANKL-positive osteoblasts and multinucleate cells was found indicating an increase in osteoclast action. These results demonstrated that occlusal stimuli play an important role in the maintenance of alveolar bone and periodontal structures in rats (174).

In a similar 2005 study also on Wistar rats, CT scans were used to determine bone mineral density of the mandible around hypofunctional teeth. Hypofunction was induced on the molars by inserting a metal cap between the maxillary and mandibular incisors on twenty 6-week-old rats. Another 20 rats served as controls and had a metal band around the cervical area, ensuring molar occlusion. The rats were sacrifised at either two, four or six weeks. At 4 weeks, cancellous bone mineral density of the mandible started declining in the hypofunctional group, and at 6 weeks, a decrease of 11.6% on the buccal side, 12.3% at the bifurcation of the root, 16.7% on the lingual side, and 39.1% at the root apex was found. There was a decrease in cortical bone density by 8-12% on the lingual side however no

difference was observed on the buccal side. From these results, the authors proposed that the different areas of the mandible have responses depending on the levels of function and mechanical strain(175).

In 2006, the effects of hypofunction on the macroscopic dimensions of the maxilla were studied on rats. Hypofunction was induced through unilateral extraction of mandibular molars and the specimens were sacrificed after four weeks. Histological analysis of the extracted maxillary bones showed an increase in the bone marrow space surrounding the hypofunctional molar. On top of this, elongation by 1.92mm from the top of the alveolar bone to the bottom of the orbitale was found, that was 0.63mm greater than in controls. Trabecular-like structures were found, and the compact alveolar bone underneath demonstrated long, narrow tubular spaces. On the marrow surface, there was an increased number of osteoclasts that lead to a decrease in bone volume by 8.32%. Finally, the hypofunction group demonstrated a bone density of 68.86% compared to 77.18% for the teeth in occlusion (176).

Diet as the main cause of hypofunction was investigated in 2009. Twelve 4-month-old female Wistar mice underwent trimming of the incisors to the gingiva. Half were fed a soft diet, and this group demonstrated a bone mineral density in the mandible of 0.1942 g/cm^{-3}, compared to 0.2108 g/cm^{-3} in the control group. The authors concluded that mechanical loading during mastication had a significant effect in maintaining bone mineral density, and removal of this stimulus adversely affected bone quality (177).

In 2011, previous studies on rats were increased in scale. A metal cap was inserted between the maxillary and mandibular incisors in sixty 6-week-old male Wistar rats. These rats were randomly divided into equal hypofunction or recovery groups and another 20 served as controls. Cancellous and cortical bone mineral density was measured in the first molar region

at 2, 4, 6, and 8 weeks after induction of hypofunction. In order to measure the potential for reestablishment of bone mineral density, the recovery group had the metal cap removed under anaesthesia at 4 weeks. BMD of the mandibular cancellous and cortical bone at the first molar region was measured using quantitative CT. At four weeks, the density of the cancellous bone around the root apex and bifurcation of molars had decreased and was unable to improve to the levels of the control in the recovery group. The density of the buccal and lingual sides also demonstrated a decrease by the fourth week although bone density in the recovery group was found to be the same as the control group by week six. In the cortical bone, a decrease in bone density was only found in the lingual central and basal area, this recovered to the levels of control by week six as well. These results demonstrated that occlusal hypofunction causes a decrease in BMD for both cancellous and cortical bone. Following restoration of occlusal function, this decreased density was able to recover to control levels except in root apex and root bifurcation in cancellous bone (178).

6.3 Growth

In a 2007 study, the effect of hypofunction on growing alveolar bone was investigated on 10 five-week-old Wistar rats. Half had molar hypofunction induced using a similar bite plane and cap to previous experiments. After two weeks, histologic analysis demonstrated significant suppression of mandibular bone in the hypofunction group, especially in the alveolar bone surrounding the second mandibular molar. Interestingly, removal of the appliance allowed growth to recover to a level that was greater than controls (179). Wada et al used a similar study design and focused on the changes in elasticity of alveolar bone following hypofunction. Right maxillary first molars were extracted in five-week-old Sprague-Dawley rats and bone histomorphometric analysis was conducted after the two-week experimental period. In the bucco-lingual direction, the hypofunction group demonstrated a

decreased elasticity of alveolar bone. On top of this, there was an increased rate of bone deposition at the alveolar crest, however the opposite occurred at the root apex. This study confirmed that hypofunction in a growing rat altered regular bone formation, resulting in a change in physical and mechanical properties (180).

6.3.1 Studies on Larger Animals

Small scale rat studies demonstrated that hypofunction had a negative effect on alveolar bone morphology and density. However, in order to relate this information more accurately to humans, subsequent studies were based on larger animals.

Koizumi et al used Japanese white rabbits to investigate the effects of hypofunction on bone quality. Hypofunction was induced through trimming of the left maxillary and mandibular molars every two weeks to the level of the gingiva in ten, five-week old Japanese white rabbits. At 17 weeks old, the rabbits were euthanized, and micro-CT measurements were taken of the extracted mandible. Comparison of the opposing sides demonstrated significant differences in total volume. Table 2 provides a summary of the results:

No significant differences were found in trabecular separation, spacing, number or total volume. Accordingly, the authors concluded that masticatory dysfunction or parafunction during growth affects the morphology and internal structure of the mandible (181).

6.4 The Macroscopic Dimensions of the Mandible

6.4.1 Studies on rats

A study to measure the macroscopic mandibular dimensional changes following hypofunction was conducted by Guerreiro et al. Twelve 21-day-old Wistar rats were fed a powder diet for 50 days. After the experimental period, extraction and histomorphometric

analysis demonstrated that the mandible of the hypofunction group was smaller in all dimensions (182). The results are summarised in the table 3

A similar study conducted in the same year, demonstrated a 5.8% decrease in thickness of the alveolar process and a 28.2% decrease on the buccal side compared to controls. No statistically significant difference was found on the palatal aspect (183). This was investigated further by a study on rats in 2015 following induction of hypofunction through a bite plane. At two weeks, the hypofunction group demonstrated a lighter masseter, shorter mandibular incisor crown, and a higher mandibular alveolar process and first molar fossae compared to the control group (184). These three studies provided evidence that mandibular morphology and associated muscles are negatively affected if the optimal occlusal function is not present. In order to have a greater understanding of this process and how it relates to humans, further studies on larger animals containing a greater sample size are required.

6.5 Biological Basis of Microscopic and Macroscopic Changes

In order to understand the biological basis for these changes in bone morphology, the sympathetic nervous system was hypothesised to play a role in the decreased bone density around hypofunctional teeth. Similar to previous studies, rats were studied by attaching appliances onto the anterior teeth to create molar hypofunction. In order to study the proposed theory, an additional group was included that underwent both hypofunction and suppression of the sympathetic nervous system through ingestion of a non-selective B adrenergic receptor antagonist (propranolol) in drinking water. Marrow spaces increased in the hypofunction group, stayed the same in the occlusion group, however decreased in the group exposed to hypofunction and propranolol. On top of this, the interradicular bone of the hypofunction group demonstrated significantly lower bone volume/tissue ratio, trabecular thickness, and trabecular number compared to control; however, for the group subjected to

hypofunction and propranolol, these were significantly greater. Finally, TRAP-positive cells were significantly increased in the hypofunction group, stayed similar in the occlusion groups and decreased in the hypofunction and propranolol group. These results seemed to provide evidence that the sympathetic nervous system plays a role in decreasing bone quality in the bone surrounding hypofunctional teeth (185).

Another proposed mechanism was the upregulation of sclerostin. This pathway acts to inhibit the activity of the Wnt/B-catenin signaling pathway. A study in China conducted by Xu et al investigated this pathway and hypofunction was induced through unilateral maxillary molar extraction on 14 male Sprague-Dawley rats. The opposing side served as controls and the rats were studied using a split mouth method. At eight weeks following the extraction, the rats were sacrifised and histological analysis of the mandibular alveolar bone was conducted. Protein expression levels of sclerostin and receptor activator of nuclear factor-xB ligand was increased and B-catenin was decreased at hypofunctional side.

Although these studies indicated that the sympathetic nervous system, sclerostin and the Wnt/B-catenin signaling pathway are responsible for the changes in bone morphology following hypofunction, further research into these chemical pathways are required to make any conclusive statements (186).

6.6 Midpalatal Suture

Areas surrounding hypofunctional teeth have demonstrated accelerated bone resorption, reduced cancellous bone mass and density, reduced trabecular bone volume and thickness, and a decreased elasticity of bone (170, 171, 174-177, 179, 181). Macroscopically, elongation of the maxilla was found in rats from the alveolar crest to the orbitale around a hypofunctional maxillary molar (175). In growing rats, hypofunction was found to reduce

bone formation and limit the growth potential of the mandible (178, 182, 184). However, if hypofunction was removed, growth was found to rebound to the levels of matched controls in most cases. This was found to be due to a cascade of cellular processes that modified recruitment of osteoclasts and osteoblasts (178, 185).

Due to the close proximity, hypofunctional teeth may have some effects on bone deposition during growth in the MPS and CBS, and remodeling in the defect created during maxillary expansion, however studies in this area have not been conducted.

6.7 Tooth Movement

If an orthodontic force is applied to a tooth, a cascade of cellular events is triggered resulting in the recruitment of leukocytes, osteoclasts and osteoblasts to the area. This results in bone deposition in the regions under tension and resorption in the regions of compression, combining to cause a decreased bone density surrounding the tooth (187).

6.8 Animal Studies

The first study measuring the effect of tooth movement on surrounding bone density was conducted in 1988 following induction of a tipping force on the maxillary molars in both young (21-28 days old) and adult (90-100 days old) rats. The bone volume to tissue volume ratio (BV/TV) was measured through frozen sample ash weight per cubic centimetre. Initially, a slight but insignificant increase in BV/TV was found, followed by a drastic decrease until day five in young rats, and day seven for adult rats. Gradual normalisation occurred afterwards, reaching levels similar to controls by day sevem in the young rat group and day 14 in the adult group (188). A similar study was conducted on 54 adult male Wistar rats in 1999, following induction of a mesial tipping of the left maxillary first molar. Analysis

using the Zeiss Videoplan device found a significant decrease in bone density between days 7 and 14 compared to the control side (189).

Zhuang et al conducted a study on twenty-two 11-week-old Sprague Dawley rats which were randomly assigned to receive either a 30g or 100g force on the right maxillary molar for two weeks whilst the left side served as control. Micro-CT analysis demonstrated that both groups had a statistically significant increase in bone volume fraction and root resorption volume. Trabecular separation increased significantly in the 100g group and volume of the upper mesial root surface in the 30g group increased compared to the 100g and control group. On top of this, the volume of the lower distal surface in the 100g group increased significantly compared to controls. The authors concluded that bone density increased after an orthodontic force was applied for 14 days, however the effects of 100g and 30g forces were different in the mesial and distal surfaces of the maxillary molars. Unfortunately this study did not provide information about the bone density between days 1-14 (190).

Another Micro-CT study was conducted in 2013 on twenty 10-week-old rats, and a 10g mesial force was applied on the first maxillary molars for 2 weeks. Scans were taken at days 0, 3, 7 and 14. Between days 3 and 7, the compression side demonstrated decreased structure model index, trabecular thickness, trabecular separation, and BV/TV that continued to decrease until day 14. The same parameters increased on the opposite side between 7 and 14 days (191).

These animal studies generally agreed that there was a decrease in bone density on the compression side that gradually recovered towards the levels of controls. Other areas demonstrated site specific changes in bone density dependent on the degree and location of the orthodontic force applied.

6.9 Human Studies

Following the general consensus that orthodontic tooth movement resulted in modifications of bone densities in rats, researchers focused their attention on the potential effects on humans. 30 untreated individuals were compared to 15 orthodontically treated patients who had finished treatment at least one year prior. CBCT analysis of the periapical region of the maxillary incisors demonstrated lower bone mineral densities shown by a 630.28 Hounsfield Unit measurement, compared to 674.84 in the control group (192).

A 2015 micro-CT study focused on an area encompassing the entire height to crest of the alveolar bone in the coronal, middle and apical area of interalveolar septum. 41 adult patients were observed for the duration of orthodontic treatment, and bone density decreased from 148.93 to 135.97 Hounsfield Units and had no influence on the alveolar bone height (193). In 2016, eight patients had CBCT analysis of six teeth (maxillary central and lateral incisors, and canines) and their surrounding bony structures (cervical, apical and intermediate) at the beginning of orthodontic treatment, and seven months into treatment (T1). The findings demonstrated a decreased bone density of between 20-29% adjacent to each tooth and a mean decrease of between 22-26%. At 20-22 months post-retention (T2), gradual normalisation of bone density had occurred. Compared to the scans at T1, an average increase of 31.81% in bone density was found, and compared to pre-treatment scans (T0), an overall increase of 0.75% had occurred. Interestingly, 11% of sites were unable to recover to 80% of original density, demonstrating great variance in response between individuals (194).

6.10 Midpalatal Suture

In humans, hypofunction generally resulted in a decreased bone density surrounding teeth that does not recover to the original bone density in 11% of patients (188-195). Interestingly,

some cases demonstrated a recovery of bone density to a level greater than controls. Similar to hypofunction, this is due to a cascade of cellular processes, causing a modified recruitment of osteoclasts and osteoblasts to the surrounding areas of the tooth. The MPS and CBS potentially lie within the affected area, however, there have been no studies investigating this.

7 Simultaneous Tooth Movement and Hypofunction

7.1 Introduction and Definitions

By 2013, multiple studies had demonstrated that bone volume thickness, BMD, and BV/TV ratio decreased surrounding orthodontic tooth movement and hypofunctional teeth. Hypofunction was additionally found to cause macroscopic morphological changes encompassing the entire mandibular and maxillary bones. Although hypofunction and tooth movement resulted in similar consequences on bone, no studies examined whether the effects were amplified if both were present simultaneously.

7.2 Human studies

A micro-CT study was conducted by Shitano et al investigating the effects on bone if tooth movement and hypofunction were present simultaneously. 32 twelve-week-old male Sprague-Dawley rats were divided randomly into normal occlusion (C), normal occlusion with tooth movement (M), hypofunction (H), and hypofunction with tooth movement (HM). Hypofunction was applied by bonding composite resin to the incisors and the maxillary molars had a palatally directed force of 10g from a nickel titanium wire placed on the buccal surface. Tooth movement was the greatest in the HM group, however tissue volume increased in the H group and was the smallest in the HM group. Bone volume was much smaller in the H group compared to normal occlusion, these two groups were both similar to the M group and the smallest in the HM group. Bone mineral density of the M group was smaller compared to the C and H groups, however was the lowest in the HM group. Trabecular thickness was smaller in the HM group compared to H and was again smaller than the normal C groups. Finally, the trabecular number was reduced in the H group compared to the C group and was the smallest in the HM group. Their most significant finding was that

orthodontic tooth movement and occlusal hypofunction acted synergistically on bone volume, mineral density and trabecular thickness, resulting in severe bone loss in the surrounding alveolar bone (196).

7.3 Midpalatal Suture

Bone surrounding teeth in hypofunction or undergoing orthodontic movement experience a process of remodeling that results in an altered morphology and decreased bone density (170, 171). This is due to a cascade of similar cellular processes, causing a modified recruitment of osteoclasts and osteoblasts to the surrounding areas of the tooth (178, 185). If both hypofunction and orthodontic tooth movement are present together, there is a synergistic amplification of these effects on the surrounding bone. The MPS and CBS potentially lie within the affected region and bone turnover during natural growth or midpalatal expansion may be modified. Neither the effects of hypofunction and orthodontic tooth movement nor any combinations of these two with high-frequency, low-magnitude vibration on the MPS and CBS have been studied.

8 Micro-CT

8.1 History

To date, there have been no studies measuring the 3D volume of the MPS and CBS. However, studies which measured the volume of root resorption craters resulting from orthodontic tooth movement have been considered as a starting point. Methods previously used in measuring root resorption craters include radiography, serial sectioning, light microscopy, transmission electron microscopy and micro-computered tomography (micro-CT) analysis.

8.2 Radiography

Radiographs are inaccurate due to magnification, angulation and repositioning errors. This is especially true regarding interspecimen reliability. Further, radiographs are a 2D representation of a 3D area and are therefore unable to provide an accurate representation of volume. Dudic et al discovered that root resorption was underestimated if using radiographic analysis compared to micro-CT analysis (204).

8.3 Serial Sectioning and Light Microscopy

This method has been used to study resorption and the subsequent repair that occurs following orthodontic tooth movement. An extracted tooth is sectioned parallel to the long axis of a tooth and embedded using haematoxylin and eosin histological staining. A light microscope is used to quantify resorption craters using a micrometer that is fitted within the eyepiece (205). Due to the technical sensitivity and nature of the technique, complete areas can be destroyed or left out following sectioning, and as such this method was deemed inaccurate for root craters (206).

8.4 Scanning Electron Microscopy (SEM)

Reitan proposed that SEM may provide an enhanced visual assessment of root resorption surfaces compared to previously available methods (207). By having stereo pairs of recordings, it provided resolution and detail that cannot be obtained from histological staining. Kvam used this method to measure root resorption craters produced from tooth movement (208), however if the area to be measured was curved, parallax errors would occur. Further, sections must be pieced together to allow for a volumetric measurement digitally, increasing the possibility for human errors and problems regarding inter-researcher reliability (206).

8.5 SEM Stereo Imaging and 3D Measurements

Chan et al used 3D scans to measure the area of root resorption, as using 2D imaging did not provide an accurate description of the volume to be examined (209). To combat previous problems, they converted the paired stereo images into an 8-bit greyscale depth map and volumetric analysis was conducted using a specially written software. The planar area was multiplied by the average depth of the nominated crater to give the volume. This technique was found to be highly accurate and easily reproducible (210).

8.6 Micro-Computed Tomography

This is a method that is useful for measuring and assessing hard tissues. It utilises computerised axial tomography and has a high spacial resolution in the order of a few micrometers. The method is based on a theory by Hounsfield (1973) that a 3D view can be generated by taking a series of X-ray projections from various angulations at an axis perpendicular to the slice. It has the ability to map, measure and quantify structures in three dimensions on a microscopic scale. A 3D map is created by computing the number of slices

together through an understanding of attenuation coefficients. With the development of micro-tomography, conventional medical scanners were scaled down and image resolution was enhanced. Current micro-scanners are able to produce an image resolution between five and ten μm, down to a minimum of oneμm in certain situations (211). In order to achieve this, the specimen moves instead of the x-ray source (212).

To date, no published studies have attempted to measure the 3D volume of the MPS. However, a study conducted by Korbmacher successfully used micro-CT scanning to quantify the degree of obliteration of the 3D MPS in the frontal and axial planes (213). In this study, the hard palate of 29 deceased humans between 14-71 years of age were resected and fixed in formalin solution, followed by scanning using a Scanco Micro-CT machine. Scanning was conducted at 114uA and 70kV, with Isotropic voxel size at 37um. Each specimen was scanned for approximately 200 minutes and 3D reconstruction of the datasets was done using AMIRA 3.00 software. The osseous architecture was determined in the sagittal dimension by aligning the raw dataset with the suture's midline and subsequent analysis was conducted with the Image Tool 3.00 software was used to compute bone volume and quantify morphology of the suture (213). The ability of Korbmacher's study to accurately differentiate between hard and soft tissue of the midpalatal suture using micro-CT imaging provided the basis for our method which used the SkyScan machine to quantify the 3D volume of the midpalatal suture.

Micro-CT analysis uses computerized axial tomography and has a high spatial resolution in the order of a few micrometers. It is highly accurate in determining hard tissue boundaries and has the ability to map, measure and quantify structures in three dimensions on a microscopic scale. Unfortunately, micro-CT analysis can only be used in deceased

specimens, as the technology requires the specimens to be completely still and exposes the specimen to excessive doses of radiation.

8.7 SkyScan Micro-CT

This study uses the Skyscan 1172 micro-tomograph (Skyscan, Aartselaar, Belgium) to examine the MPS and CBS due to the high spacial resolution and ease of use. This is the fourth generation of the compact desktop machine and has been used in multiple studies at the University of Sydney. It consists of an x-ray microscope machine and a paired tomographic reconstruction software. The recommended image field is 70mm high and 68mm wide and the cone beam X-ray source has a spatial resolution between 2 and 5µm. The magnification of the sample depends on the distance from the x-ray detector and consists of a high resolution 1024 x 1024 pixel charged couple device using output images that are 16-bit Tagged Image File (TIFF) file format.

9 Conclusion

9.1 Relevance of Research

Whole body HFLMV activates mechanotransduction in bone, resulting in stimulation of osteogenesis. As a result, this idea was formulated into a treatment methodology which is currently used to counteract the deficiency in skeletal development in patients with cerebral palsy (3, 4, 6, 7). On a different front, bone surrounding teeth in hypofunction or undergoing orthodontic movement experience a process of remodeling, resulting in altered morphology and decreased bone density (170, 171). This is due to a cascade of cellular processes, causing a modified recruitment of osteoclasts and osteoblasts to the surrounding areas of the tooth (178, 185). The MPS and CBS potentially lie within the affected areas, and bone deposition during natural growth or orthodontic treatment may be modified.

13.2 Need for further investigation

The MPS and CBS potentially lie within the area affected by HFLMV, tooth movement, and hypofunction and bone turnover during natural growth or MPS may be modified. This literature review identifies that neither the effects of hypofunction and orthodontic tooth movement nor any combinations of these two with high-frequency, low-magnitude vibration on the MPS and CBS have been studied.

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List of Tables

Table 1: (Chung and Font) Proportions of Skeletal Expansion, Alveolar Tipping and Dental Tipping

	Skeletal expansion	Alveolar tipping	Dental tipping
First premolar	55%	6%	39%
Second premolar	45%	9%	46%
First molar	38%	13%	49%

Table 2 (Koizumi et al): Proportions of Skeletal Expansion, Alveolar Tipping and Dental Tipping

	Hypofunctional side	Control side
Total volume	696.1mm^3	890.8mm^3
Cancellous bone volume	83.3mm^3	165.1mm^3
Cancellous bone volume density	12%	20.9%
Trabecular thickness	153.2µm	202.7µm
Trabecular number	0.8mm	1.0mm
Trabecular separation	1136.8µm	773.2µm
Spacing	975.8µm	1290µm

Table 3 (Guerreiro et al): Macroscopic changes in the mandible

	Hypofunctional group	Control group
Mandibular ramus	10.77mm	11.11mm
Mandibular body length	21.67mm	22.36mm
Mandibular height	4.24mm	4.54mm
Mandibular base depth	1.24mm	1.47mm

11 Manuscript

11.1 Abstract

Introduction

Whole body high-frequency low-magnitude vibration (HFLMV) is routinely used in the medical field to improve bone quality in children. For this treatment, vibration is induced during a period when growth of the midpalatal suture is heavily influenced by the external environment. The midpalatal suture acts as a growth site responding to external signals stimulating deposition of bone on the sutural edges, facilitating transverse maxillary growth. Any additional mechanical strain such as HFLMV could modify the rate of bone remodelling at the interface of the two maxillary bones and affect natural growth.

Objectives

To evaluate the effects of HFLMV on the volume of the midpalatal suture (MPS) of rats.

Materials and methods

This study consisted of forty-two, five-week-old Fisher Strain male rats which were previously used in a study investigating the effects of vibration on hypofunctional teeth. The rats were randomly allocated into Vibration and Non-vibration groups.

In the vibration groups, HFLMV was induced through whole body vibration platforms. The rats were kept in their cages and placed two at a time on the vibrating platform set at a magnitude of 0.3g and frequency of 30Hz. This stimulus was applied for 20 minutes per day, five days per week for a total of 30 days.

The MPS was analysed as two separate volumes of interest in order to avoid the regions complicated by the pre-maxillomaxillary and palatomaxillary sutures. Three-dimensional

micro-computed tomography (micro-CT) was used to quantify the volumes of the MPS. The measured volumes of the mid-palatal suture were adjusted by the length of the rat heads and statistical analysis was conducted.

Results

There was no statistically significant difference in the volumes of the MPS in any region between the Vibration and Non-vibration groups.

Conclusion

The findings of this 30-day animal study indicate that HFLMV applied through whole body vibration platform does not affect the volume of the MPS of rats.

11.2 Introduction

The concept 'form follows function' was proposed by Julius Wolff in the 19th century, to explain the relationship between mechanical loading and skeletal morphology (1). Although Wolff's law could not predict the exact reactions of bone to a mechanical load, it demonstrated that daily activities and their interactions with gravity or ground reaction forces created mechanical strains that maintain or improve bone quality (1-3). This is achieved through a process of bone remodeling, defined as the ratio of bone formation to bone resorption (1).

The use of High-frequency, low-magnitude vibration (HFLMV) as a medical intervention emerged from a series of studies investigating the relationship between strain and osteogenic activity. Initially, it was thought that the magnitude of the externally induced strain was proportional to the amount of bone deposition (4). However, Fritton disproved this and proposed a new idea that frequency of the applied load has greater importance in determining bone remodeling (5). Further investigations demonstrated that as the frequency of the load

increases, the strain required to induce bone formation decreases dramatically (3). Extremely small magnitude strains at a frequency of 20-40Hz applied continuously on the skeleton were responsible for bone tissue organization (5, 6). Whole-body vibration devices capable of transferring HFLMV stimuli onto the skeleton are currently used effectively in the treatment of many medical conditions including women suffering from postmenopausal osteoporosis (7-12), children with disabilities (13-16), and in individuals with prolonged bed rest (17-20).

Sutures are unique to skull bones and are made up of several cell types such as osteogenic cells, fibroblast-like cells, and mesenchymal cells. There are two main mechanisms associated with active sutural growth (21-23). Firstly, sutural osteoblasts deposit bone matrix whilst sutural fibroblasts synthesise matrices that are non-mineralised and function to maintain the presence of sutures (21-23). Sutural growth is characterised by increases in differentiation, proliferation and matrix synthesis of mesenchymal, fibroblastic and osteoblastic cell lineages (24).

Cyclic forces with a sine wave characteristic have induced endocortical and periosteal appositions in long bones and postcranial skeletons of vertebrate species (3, 25, 26). More recently, exogenously applied oscillatory mechanical stresses have demonstrated bone apposition in the cranial sutures as well (23, 27-30). According to a study investigating the mechanobiology of craniofacial sutures, sutural osteogenesis is likely modulated by microscale shear stresses induced by the tension or compression forces (22). This study demonstrated that fibroblastic cells in sutures increase proliferation and matrix synthesis following induction of mechanical stresses with cyclic strains having the greatest effect (22). It has been proposed that the fluid flow in bone which is modified and induced by the strain rate and oscillatory bone strain is responsible for triggering mechanotransductive responses, whereas constant forces have no ability to induce fluid movement (31-34). As a consequence,

amplitude of bone strain has no influence upon the rate of bone deposition compared to strain rate and energy (5, 35-37).

The introduction of HFLMV to the orthodontic field as an adjunctive treatment methodology was based on the concept that increased bone remodeling could subsequently increase the rate of bone healing and orthodontic tooth movement.

Products inducing HFLMV marketed to improve the rate of tooth movement are readily available and used by orthodontists around the world despite contradictory results (29, 44, 45). Later, two higher quality prospective randomised clinical control trials found no improvement in the rate of tooth movement with mechanical vibration (46), (47). HFLMV has also been proposed to reduce pain and decrease root resorption, however, no high-quality studies have demonstrated any significant positive results in either (7, 47-50).

Although no high-quality evidence supports the use of HFLMV in orthodontics, it is routinely used in the medical field to improve bone quality in children with motor disabilities such as cerebral palsy (CP). For this treatment, vibration is induced during a period when growth of the craniofacial sutures is heavily influenced by the external environment. In natural growth, sutures of the cranium rely on mechanical strains to modify and promote growth and lie dormant until an external signal such as the pressure of soft tissue growth is experienced. An example is the midpalatal suture (MPS), which acts as a growth site responding to external signals stimulating deposition of bone on the sutural edges that facilitate expansive growth of the maxilla (21, 51, 52). Any additional mechanical strain such as whole body HFLMV could modify the rate of bone remodeling at the interface of the two maxillary bones and affect natural growth (21, 22, 24, 51, 52).

Prior to a study by Kopher and Mao, only static forces were used in the modification of sutural growth as the characteristics of dynamic force transmission between bone and sutural tissue were unknown. Their study was the first to demonstrate that cyclic forces placed on the maxillary bone create tissue-borne mechanical strains of corresponding wave forms that are experienced by premaxillomaxillary (PMS) and nasofrontal (NFS) sutural cells. In the second part of their study, Kopher and Mao investigated the relationship on 19 male six-week old rabbits that were randomly allocated to either static, dynamic or control groups. Both loading groups were exposed to the same peak strain, whilst the dynamically loaded group received vibration at a magnitude of five N and frequency of one Hz for 10 minutes per day over a 12-day experimental period. The PMS experienced compressive strains and the NFS tensile strains. Bone strain recordings demonstrated that waveforms of dynamic and static loading were communicated as corresponding wave forms in the sutural tissue, however, the mean peak strains experienced in the PMS were 10-fold higher than in the NFS. In the dynamic group, there was a statistically significantly increase in the sutural cell count and rate of osteogenesis. On top of this, there was also an increase in the sutural width compared to the statically loaded and control groups (23).

Subsequent studies conducted by Mao et al (2003), Peptan et al (2008) and Vij and Mao (2006) investigated the effects of mechanical oscillating strains at a frequency less than 10Hz and magnitude of 0.3 – 5N for between 5 to 12 days on the dimensions of the PMS and NFS (22, 27-30). On top of the unanimous increase in sutural width, an increase in the number of fibroblasts, osteoblasts and osteoclasts by day five was found. This overall increase in cell number combined with the increased production of sutural matrix and minimal initial bone resorption was proposed to have resulted in an increased sutural width (29, 30, 53). By 12 days, osteoclasts had decreased in number whilst proliferation and activity of osteoblasts and

fibroblasts continued to increase, likely to result in deposition into the suture and decreased sutural width if the experimental time was increased (30).

Although no previous studies have investigated the effect of HFLMV on the midpalatal suture, craniofacial sutures that have experienced mechanical stimuli with parameters similar to this experiment have demonstrated increased cellular number and activity resulting in increased bone remodeling. These were quantified by comparing the sutural width, with the groups experiencing vibration having a greater sutural width when compared to control groups (21, 22, 24, 51, 52). The volume of the midpalatal suture represents a three-dimensional area as oppose to the linear measurement of sutural width, however alterations to the volume of the midpalatal suture found during the induction of HFLMV also represent changes in the physical proportions of the midpalatal suture occurring as a consequence of modified bone remodeling.

Present day treatment regimens for the improvement of bone quality in children with CP usually use HFLMV between 12-30 Hz (15, 54-58). Although previous studies have investigated frequencies below 10Hz, there have been no studies investigating the influence of vibration at higher frequencies on the cranial sutures, specifically the MPS. Furthermore despite no evidence, devices such as AcceleDent™ are widely available and used by orthodontists in an effort to improve the rate of tooth movement.

Therefore the aim of this study was to evaluate the effects of high-frequency low-magnitude vibration on the volume of the MPS in a rat model. Based on previous experiments, it can be hypothesized that the volume of the midpalatal suture would decrease as a result of continued osteoblast activity and bone deposition following the initial periods of osteoclastic action and extracellular matrix deposition.

11.3 Materials and Methods

All experimental procedures followed the ethics approval (A-15—116) provided by the Committee for animal research of Hiroshima University.

This study consisted of forty-two, Fisher Strain male rats. The rats were previously used in a study investigating the effects of vibration on hypofunctional teeth by Kohan et al (59). The rats were obtained at five weeks old and were held in approved housing at Hiroshima University comprising of a 12 hour day and 12 hour night cycle maintained at a constant temperature of 23^0 C. Water was made available at all times and the animals were fed a powder diet (Rodent Diet CE-2; Japan CLEA Inc, Tokyo, Japan) (59).

For this study the animals were randomly allocated into seven groups of six rats: Control (C), Tooth movement (M), Vibration (V), Vibration and tooth movement (VM), Hypofunction and tooth movement (HM), Hypofunction and vibration (HV), Hypofunction, vibration and tooth movement (HVM) groups.

11.3.1 Experimental protocol

In the hypofunctional group, an anterior bite-raising appliance was placed for a period of seven weeks before the experimental period to induce occlusal hypofunction at the molar region (60). It consisted of an anterior bite plate on the mandibular incisors made of NEW ST LOCK base (Dentsply-Sankin, Tokyo, Japan) and a metal cap made of band material on the maxillary incisors (3M Unitek Co, Tokyo, Japan) bonded with composite resin (Clearfil Majesty LV; Kuraray Co Ltd, Okyamam Japan) (Figure 1) (59).

In the tooth movement groups, orthodontic tooth movement was induced on the maxillary first molars via closed coils extending between incisors and first molars, applying 50g of force (Figure 2) (61).

In the vibration groups, HFLMV was induced through whole body vibration platforms (Solofex, Soloflex Inc. Hillsboro, OR). The rats were kept in their cages and placed two at a time on the vibrating platform set at a magnitude of 0.3g and frequency of 30Hz. At the Earth's surface where g=9.8m.s-1, the peak strains experienced were approximately 5ue (62). This stimulus was applied for 20 minutes per day, five days per week for a total of 30 days based off previous studies (25, 62-68).

11.3.2 Animal weight

All rats included in the study remained healthy and gained weight significantly before and during the entire experimental period. However, rats in the occlusal hypofunction group gained less weight due to difficulties in eating. At the beginning of the experimental period, the rats experiencing hypofunction had an average weight of 84.6 g, whilst before sacrifice weighed 231.67g. This equated to an average weight gain of 147.1g. In comparison, the average weight of all rats at the beginning of the experimental period was 85.26g and weight before sacrifice was 268.89g. This equated to an average weight gain of 183.63g.

11.3.3 Specimen imaging

After the 30-day experimental period, the rats were euthanised through carbon dioxide inhalation. The heads were decapitated, degloved and superfluous tissue was removed and perfused with 10% buffered formalin solution for storage.

Specimen scanning was performed using the SkyScan 1172 desktop X-ray microtomography (SkyScan, Aartselaar, Belgium). A purpose built radiolucent positioning jig was made, and specimens were secured with parafilm. In order to ensure consistency, the midpalatal suture of each specimen was aligned to a marking on the jig which was also perpendicular to the straight edge of the jig. The rats were scanned individually with each scan lasting approximately six hours.

The vertical dimensions of the region of interest was larger than the maximum field of view of the SkyScan. As a result, the oversize function was used and several scans in the vertical dimension were taken and interpolated. Preliminary viewing of each scan was conducted at rotation steps of 45, 90, 135, 180, 270 and 360 degrees to ensure the region of interest was contained in the field of view.

The specimen was rotated 360 degrees around the vertical axis, and 3D microstructural information was captured. The following parameters were used.

- Pixel Size (μm) = 7.41823
- Camera Pixel Size (μm) = 11.75
- Filter = Al 0.5 mm
- Exposure (ms) = 590
- Rotation Step (°) = 0.110
- Frame Averaging = OFF
- Random Movement = OFF

Preliminary scans at a range of resolutions were conducted, and subsequent reconstructions demonstrated that 7.41μm was sufficient for the analysis of the midpalatal suture. Digital recordings were taken at angular increments of 0.11 degrees, creating 1855 projections for each specimen.

Skyscan's volumetric reconstruction software "NRecon" (SkyScan, Aartselaar, Belgium) was used to reconstruct 3D data sets from the raw data of acquired 3D projections through application of a modified Feldkamp algorithm. Interactive thresholding was conducted to differentiate between bone and sutural tissue. The reconstructed data sets were saved in a

bitemap data format. Angular projections were used to orientate the reconstructions and cross sections of the midpalatal suture were created in the frontal section.

The "CT analyser" (CTan) software was used to calculate the volume of the midpalatal suture. As CTan performs binarised analyses, a threshold was interactively selected by one operator to define between sutural tissue and bone. The sutural tissue was defined as the radiolucent areas between the maxillary bones. Each frontal slice was individually analysed and the area representing the midpalatal suture was quantified. Addition of the areas of each individual slice produced the volume of the midpalatal suture.

This particular method of quantifying the boundaries between bone and sutural tissue is based on a method used by Korbmacher. In this study, the hard palate of 29 deceased humans between 14-71 years of age were resected and fixed in formalin solution, followed by scanning using a Scanco Micro-CT machine. Scanning was conducted at 114μA and 70kV, with Isotropic voxel size at 37μm. Each specimen was scanned for approximately 200 minutes and 3D reconstruction of the datasets was done using AMIRA 3.00 software. The osseous architecture was determined in the sagittal dimension by aligning the raw dataset with the suture's midline and subsequent analysis was conducted with the Image Tool 3.00 software was used to compute bone volume and quantify morphology of the suture.

11.3.4 Sample analysis

11.3.4.a Quantitative analysis of the volume of the midpalatal suture

Anatomically, the premaxillary bone is proportionally longer than the equivalent incisive bone in humans. Further, the anatomy of the premaxillary and palatomaxillary sutures are complex and interferes with the borders of the midpalatal suture, making identification of the midpalatal suture in this region difficult (69-71). Accordingly, the areas between the palatine

and maxillary bones and the entire pre-maxilla were not included in the volume of interest in this study and reconstructions were utilized to measure the volume of the midpalatal suture in two separate volumes of interest in the frontal plane.

The anterior volume of interest was measured from a line perpendicular to the coronal plane joining the first appearances of the palatine fossae to a line parallel to that joining the first appearance of the interpalatine suture within the maxillary bones (Figure 3).

The posterior volume of interest was measured from a line perpendicular to the frontal plane joining the first appearance of the interpalatine suture within the palatine bones to a line parallel to that joining the termination of the midpalatal suture (Figure 4).

To increase accuracy, each measurement was taken twice and the mean of the two measurements were taken as the final measurement. The data for the volumetric analysis of the midpalatal suture was collected by a single operator (AL).

Previous studies have demonstrated food deficiencies greater than 25% of normal intake cause growth deficiencies that lead to macroscopic changes in the dimensions of the craniofacial skeleton (72, 73). In our sample, since the animals in the Hypofunction groups had less weight gain, digital calipers were used to measure the length of the head from the bony tip of the nose to the posterior cranial synchondrosis and all volumetric measurements of the midpalatal suture were adjusted to reflect the relationship with this baseline covariate. This adjustment was performed by dividing the measured volume of midpalatal suture with the length of the head.

11.3.4.b Statistical analysis

Statistical analysis was processed using IBM SPSS Statistics program. The first analysis was conducted to compare the influence of vibration only. Therefore the groups were combined

such that all rats that experienced vibration were in one group, whilst all rats that did not in another.

One-way analyses of variance were conducted to determine if vibration had an impact on the volume of the midpalatal suture when groups were analyzed all together or seperately.

For all of these analyses, three separate analyses were conducted, comparing the anterior, posterior and total volume of the midpalatal suture separately. A p-value of less than 0.05 was considered to be statistically significant.

11.4 Results

11.4.1 Analysis 1: Comparing Vibration and Non-Vibration groups

For Analysis 1 all the sub-groups were re-grouped into two main groups in regards to their vibration status:

All the groups that had vibration were named Vibration and all the groups that did not have any vibration were re-grouped under Non-vibr.

As the volumes were adjusted by the length of the rat head, the adjusted volume used in statistical analyses were no longer an exact measurement of the volume of the midpalatal suture. As a consequence, Z-scores were used in the figures as they provide an accurate value for comparison. Each figure demonstrates the mean, as well as standard deviations.

Vibration (n=24):

- Vibration (V)
- Vibration and tooth movement (VM)
- Hypofunction and vibration (HV)

- Hypofunction, vibration and tooth movement (HVM)

Non-vibr (n=18):

- Control (C)
- Tooth movement (M)
- Hypofunction and tooth movement (HM)

There was no statistically significant difference in the volumes of the midpalatal suture in any region between the Vibration and Non-vibr groups. The results are represented graphically for the anterior portion in Figure 5, the posterior portion in Figure 6, and the combined volume in Figure 7.

11.4.2 Analysis 2: Comparison of sub-groups

- Control (C)
- Tooth movement (M)
- Vibration (V)
- Vibration and tooth movement (VM)
- Hypofunction and tooth movement (HM)
- Hypofunction and vibration (HV)
- Hypofunction, vibration and tooth movement (HVM)

There was no statistically significant difference in the volume of the midpalatal suture in any region between any of the groups, when all seven groups (Vibration (V), Vibration and tooth movement (VM), Hypofunction and vibration (HV), Hypofunction, vibration and tooth movement (HVM), Control (C), Tooth movement (M) and Hypofunction and tooth movement

(HM)) are compared individually. The results are represented graphically for the anterior portion in Figure 8, the posterior portion in Figure 9, and the combined volume in Figure 10.

11.5 Discussion

This was the first study to investigate whether mechanical strains at a frequency of 30Hz has an effect on the volume of the midpalatal suture in rats. It was also the first to evaluate the effects of oscillatory mechanical strains on maxillary sutures using micro-CT imaging.

The results demonstrate that HFLMV has no statistically significant effect on the volume and growth of the midpalatal suture in developing rats when induced over a 30-day experimental period.

In vivo studies have demonstrated that a chamber in the center of craniofacial sutures contains cells that respond to external mechanical stimuli (24, 53, 74-77). Any additional oscillatory stimulus outside of daily activities stimulates proliferation and decreased apoptosis of mesenchymal, fibroblast, osteoclast and osteoblast cells, resulting in increased matrix deposition and bone remodeling within cranial sutures (23, 24, 28-30, 53).

Previous studies have investigated the effects of mechanical oscillating strains at a frequency less than 10Hz and magnitude of 0.3 – 5N for between 5 to 12 days on the dimensions of the PMS and NFS (22, 27-30, 78). In these experiments a unanimous increase in bone demineralization and resorption was found, resulting in an increased sutural width. This seems contradictory to the expected results considering osteoclasts, osteoblasts and fibroblasts increase in number and activity. However, early stages of sutural response to mechanical vibration are predominated by osteoclastic and fibroblastic action (22, 23, 28, 29). At five days following induction of vibration, the production of sutural matrix by fibroblasts, bone resorption from osteoclasts and overall proliferation in cell number resulted in an increased sutural width whilst limited osteoblastic bone deposition occurred (29, 30, 53). By 12 days, osteoclasts had decreased in number whilst proliferation and activity of osteoblasts and fibroblasts continued to increase (30). For the experimental period of 30 days

used in this experiment, it is likely that sutural response has transitioned through the initial stages of bone resorption and matrix deposition to being predominated with bone deposition into the suture (29, 30, 53). Logically, this should equate to a decreased sutural volume as bone is deposited into the sutural tissue, however this was not found.

A possible explanation of the lack of result compared to previous experiments examining the effects of vibration and craniofacial sutures relates to the difference in parameters of vibration induced and experimental duration. Previous experiments have focused on lower frequency vibration of higher magnitude, for a maximum of 12 days. Despite this, several studies have shown that mechanical stimulation at or around the parameters of 30Hz and 0.3g resulted in a decreased amount of bone resorption and increased bone deposition in affected regions capable of bone remodeling (64, 66, 79-81). Rubin et al conducted an experiment on sheep in which vibration was induced at the same parameters (30Hz, 0.3g, 20 min/day, five days per week) as this experiment for an experimental period of one year. It was found that bone deposition on the regions which experienced vibration was significantly greater (68). Lau discovered that HFLMV at 0.3g and 30Hz had an inhibitory effect on the signaling pathway between osteocytes and osteoclasts, leading to a decreased production of osteoclasts and a reduced amount of bone resorption with effects occurring in as little as 30 minutes after induction of vibration (81). Further studies using either a frequency of 30Hz or magnitude of 0.3g have also found that vibration enhances bone deposition resulting in replacement of hypertrophic cartilage in experimental durations as short as 10 weeks (64, 66, 79, 80).

These studies demonstrate the potential that vibration at 30Hz and 0.3g has the ability to alter bone remodeling in affected regions. Combining this with the previous studies on vibration and craniofacial sutures, it can be hypothesized the experimental protocol used in this

experiment should result in bone deposition that subsequently modifies the volume of the midpalatal suture of rats. However, it is clear that between 12 days and 10 weeks there is a transition from net bone resorption to bone deposition. As no studies have been conducted between these time frames it is difficult to know exactly when this transition is expected to occur. It is likely that this experimental period of 30 days was in the transitional period between net bone resorption and bone deposition, and no statistically significant difference in bone volume was visible when measuring sutural volume. If the experiment was conducted for over 10 weeks, the bone remodeling equilibrium in this experiment may have already shifted towards net bone deposition into the craniofacial suture, leading to a decreased volume of the midpalatal suture.

Another consideration regarding the result relates to the lack of transverse growth available during the experimental period. Transverse growth occurs between 4 and 30 days of age as a result of cartilage and connective tissue proliferation. It is likely that our experimental period was conducted at a time when most of the ossification of the midpalatal suture had already occurred, limiting the amount of bone deposition into the suture and any volumetric changes within the measured area (82).

Although no statistically significant change was found in the volume of the midpalatal suture, additional examination of cellular activity, sutural width, degree of bone mineralization and matrix deposition would have provided supplementary information regarding the status of bone remodeling in the midpalatal suture.

Previous studies have used histological staining and photomicrography to measure cellular activity and morphological changes. In these studies, the PMS and NFS were dissected with five mm of surrounding bone and analysed following H&E staining, allowing the average sutural width and total sutural cells to be quantified (23). The average surface osteoblast was

calculated through quantifying the cuboidal, mononuculated cells whilst the osteoclast-like cells were distinguished through counting the cells with three distinct nuclei (29, 30). If histological examination was conducted in this experiment, there would have been information provided regarding the activity and number of mesenchymal cells, fibroblasts, osteoclasts and osteoblasts.. The results may have provided information regarding presence or absence of sutural growth and bone remodeling following induction of vibration (83-85).

Calcein labelling of undermineralised sections was also utilized in previous studies which allowed determination of new bone formation following investigation with a fluorescent microscope (23). This method in particular if conducted in the current experiment would have provided a significantly greater understanding of morphological changes and new bone formations that were not detected by measuring the volume of the midpalatal suture alone.

No measurements were taken to evaluate the properties of the mechanical strain experienced by midpalatal sutural cells. Previous studies have demonstrated that the degree of vibration experienced within a suture is related to the distance from the source of vibration. Adjacent sutures may experience strains of opposite polarity, with the morphology of the sutures dictating the direction and degree of energy absorbed (86). Kopher and Mao placed strain gauges and rosettes across the PMS and NFS and stimulation was placed onto the maxillary incisor and expressed as compressive strain in the PMS at 10-fold greater magnitude compared to the tensile force experienced in the NFS and tensile in the NFS. This demonstrates a loss of strain amplitude following transmission between maxilla to adjacent calvarial bones as well as reversing of polarity. The authors stated that the PMS, which is close to the source of the strain is directly being influenced whilst the NFS has some distant effects of bending and tensing. As this study relied on whole body vibration, quantification of the mechanical strain within the midpalatal suture would have provided important

information regarding the type and degree of strain experienced and subsequent effect on the sutural cells.

In the present study, apart from vibration, some of the rats were subjected to hypofunction and/or orthodontic tooth movement. Although this is unlikely to have an effect on the volume of the midpalatal suture, several studies on rats have demonstrated that occlusal hypofunction results in decreased bone density, enlarged marrow spaces and thinning of the outer shells of alveolar bone (87, 88). Macroscopically, molar hypofunction in rats resulted in elongation of the maxilla from the alveolar crest to the orbitale around a hypofunctional maxillary molar (89). Katsaros et al found that the reduced masticatory function resulting from an experimentally induced soft diet resulted in less bone deposition in the internasal, naso-premaxillary and inter-premaxillary sutures of growing rats when compared to a group fed with a hard diet. It was proposed in this study that the reduced rates of bone apposition were related to the decreased width of sutural space following decreased functional demands (90).

Studies investigating the effects of OTM on bone deposition in rats have demonstrated that a tipping force results in a drastic decrease in the bone volume to tissue volume ratio until day five for young rats, and day seven for adult rats. Gradual normalisation occurred afterwards, reaching levels similar to controls by day seven in the young rat group and day 14 in the adult group (91). Shitano et al demonstrated that simultaneous OTM and occlusal hypofunction resulted in a synergistic effect on bone volume, mineral density and trabecular thickness, causing severe bone loss in the surrounding alveolar bone (92). The groups were also individually analysed to investigate whether hypofunction and/or tooth movement affected the sutural volume. There were no significant differences between the groups when compared individually. OTM and occlusal hypofunction have demonstrated an effect on surrounding alveolar bone, with limited effects on the macroscopic dimensions of the maxilla or

mandible. These factors may have had an influence on the volume of the midpalatal suture, as bone deposition occurs into the suture in normal growth; possibly contributing to the lack of statistically significant result.

11.5.1 Future directions

The sample size used in this experiment was small, which may have contributed to the lack of any statistically significant difference between various examination groups. Variations in anatomy and growth have a greater impact on the overall results when the sample size is small (93-95). In the future, a larger sample size could provide results with greater reliability in results. Compared to other studies, this study demonstrated no significant differences in the rate of deposition of bone growth, which may have been also attributed to the insufficient duration of the project, previous studies have found that bone deposition occurs if vibration is induced for more than 10 weeks. Furthermore, histological analysis of bone deposition, cellular activity and number, as well as quantification of the strain experienced in the midpalatal suture would have provided significant information about bone remodeling and cellular level activity despite the lack of significant difference in the volume of the midpalatal suture. Also bone density or the degree of bone mineralization could be assessed in future studies. It can also be considered to conduct the experiment between the age of 4 and 30 days, as most of the growth, bone deposition and remodeling of the midpalatal suture occurs during this period of time. Although unlikely to influence the midpalatal suture, hypofunction and OTM should ideally be removed from future experiments in the field to prevent supplementary factors from influencing the result.

Rat studies are a preliminary basis for further studies on larger animals and humans. This is because they cannot be directly associated as there are significant anatomical differences between rats and humans. Rats have denser bone compared to humans, the bones lack

osteons and have less abundant osteoid tissue (96-98). Despite this, rats provide a good starting point for pilot studies considering cost and time effectiveness of this model.

11.6 Conclusion

The findings of this 30 day animal study indicate that high frequency low magnitude mechanical vibration applied through whole body vibration platform does not affect the volume of the midpalatal suture of rats.

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Figure 1: Anterior bite block appliance

Figure 2: Tooth movement appliance

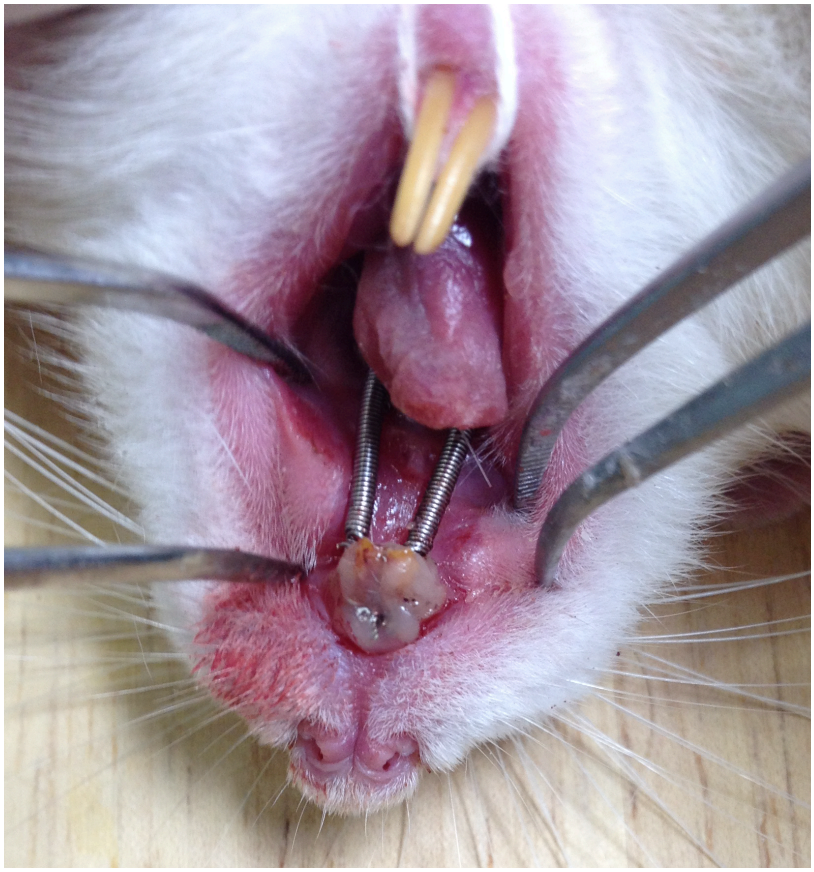


Figure 3: Boundaries of the anterior volume of interest

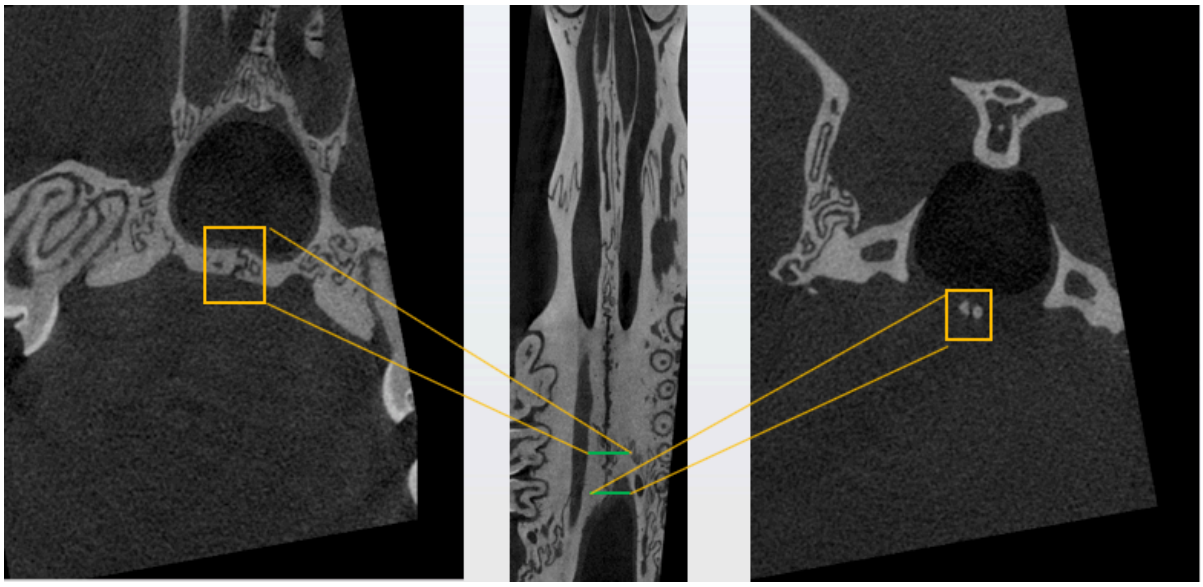


Figure 4: Boundaries of the posterior volume of interest

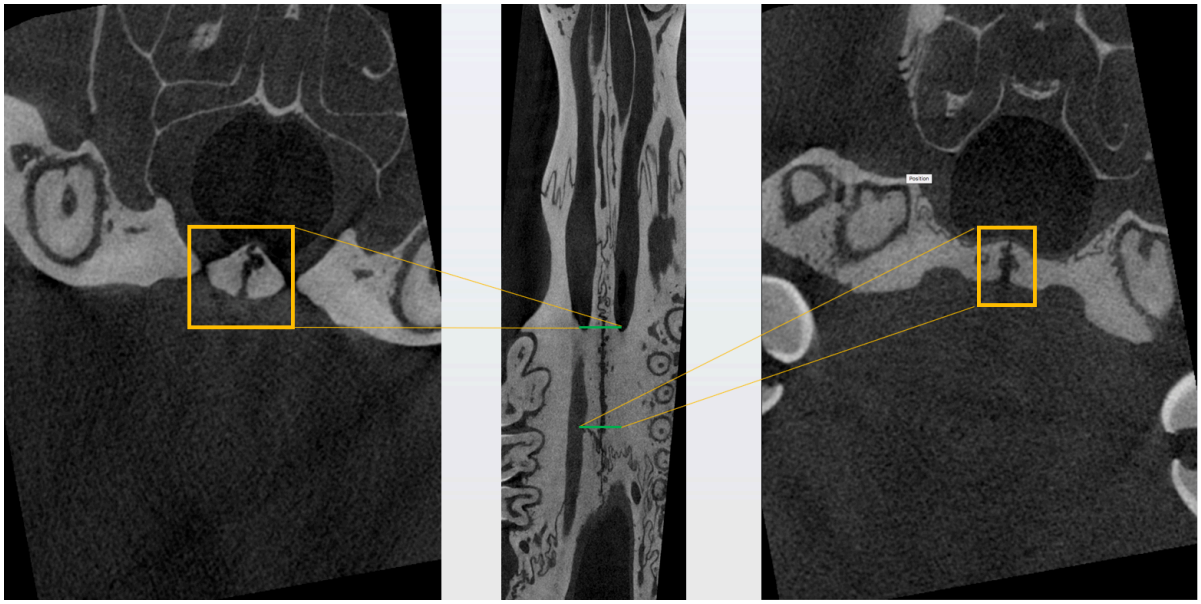


Figure 5: Anterior portion of the midpalatal suture comparing vibration and non-vibration

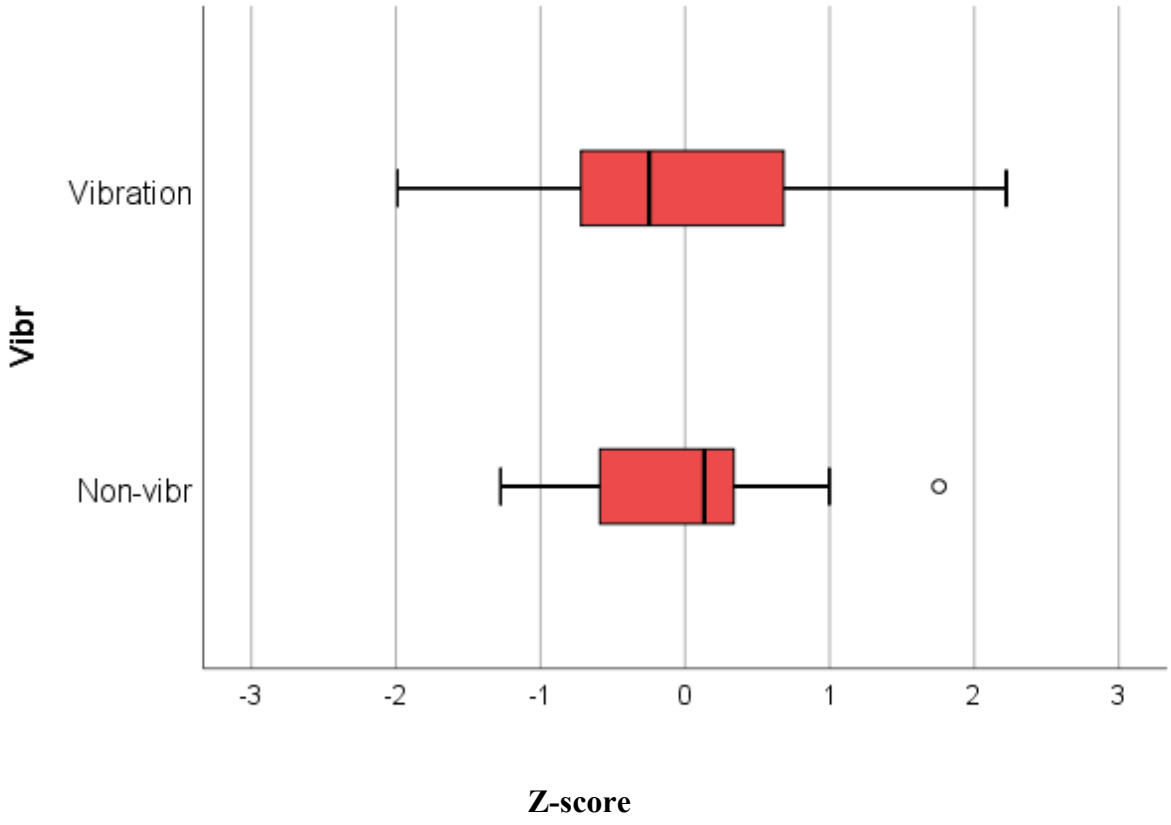


Figure 6: Posterior portion of the midpalatal suture comparing vibration and non-vibration groups

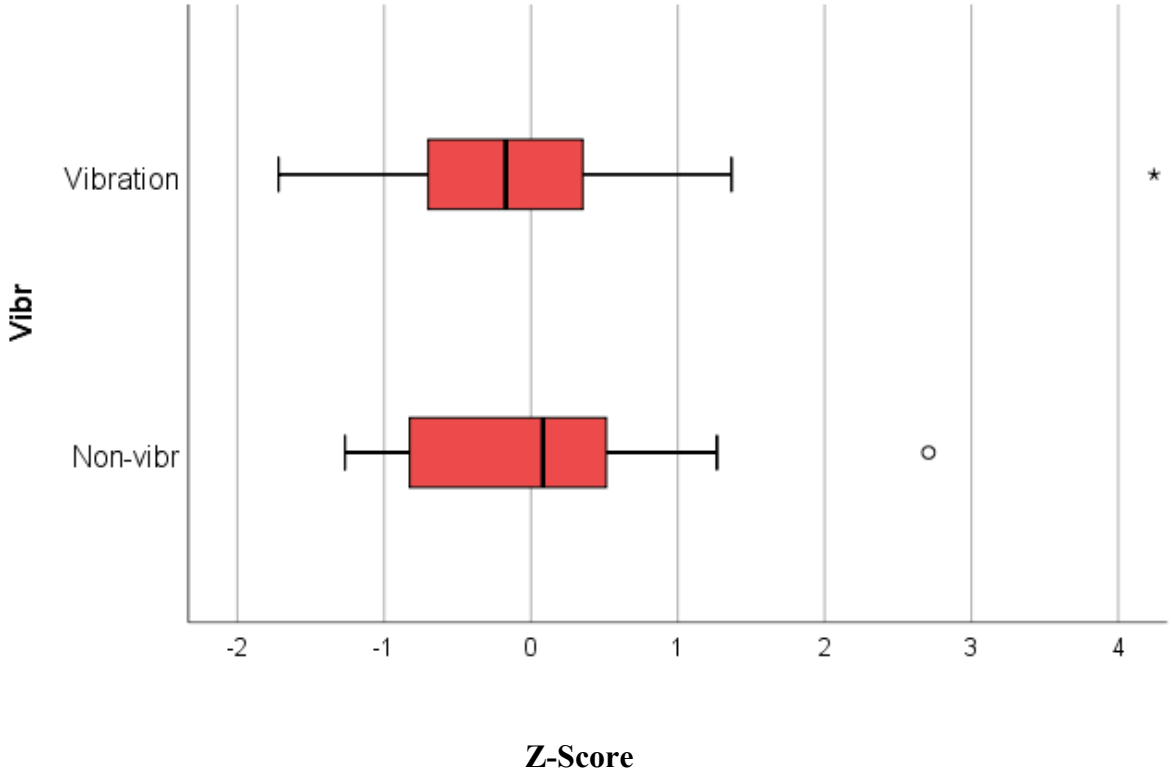


Figure 7: Total volume of the anterior and posterior portions of the midpalatal suture comparing vibration and non-vibration groups

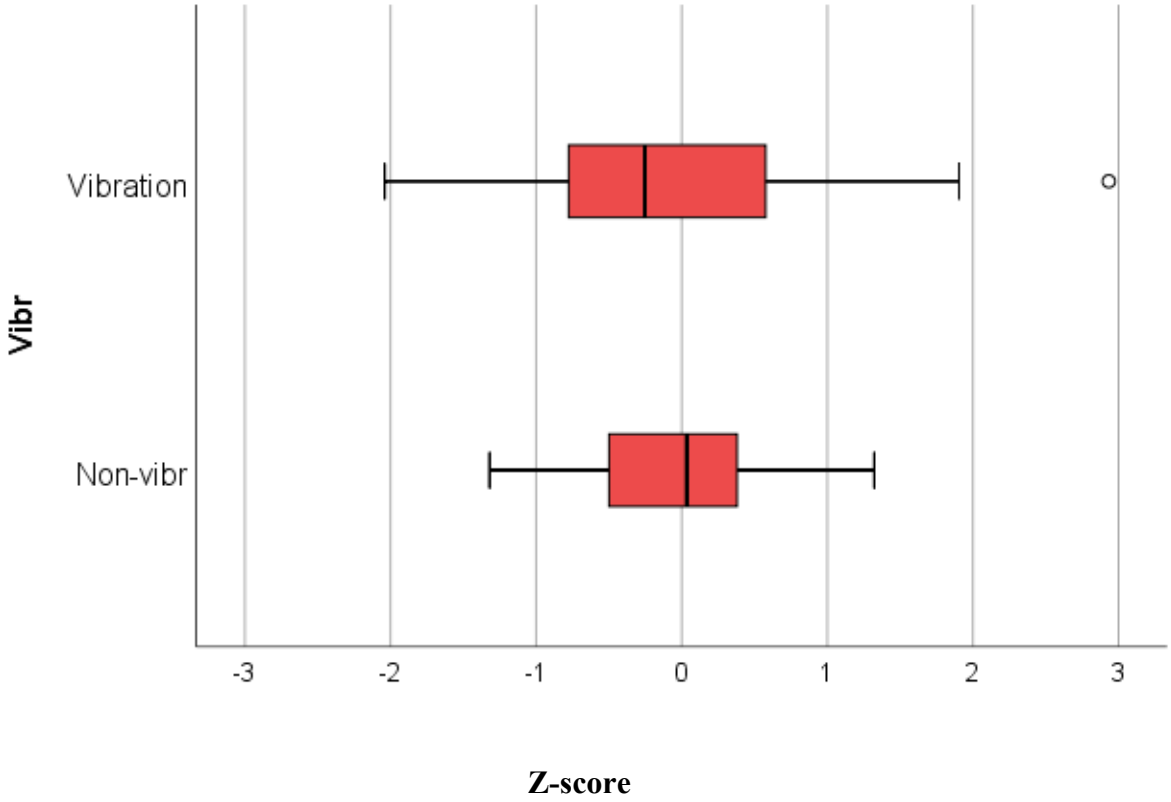


Figure 8: Anterior portion of the midpalatal suture comparing different experimental groups

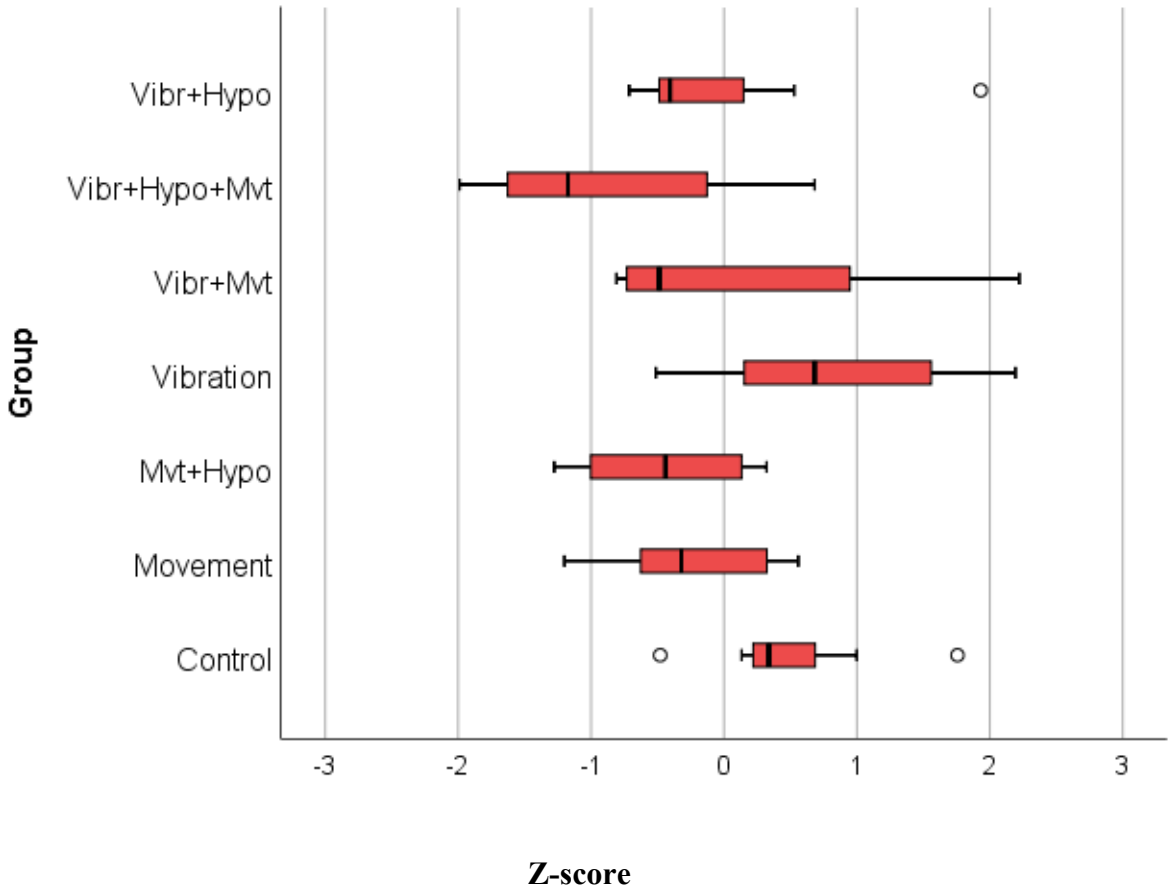


Figure 9: Posterior portion of the midpalatal suture comparing different experimental groups

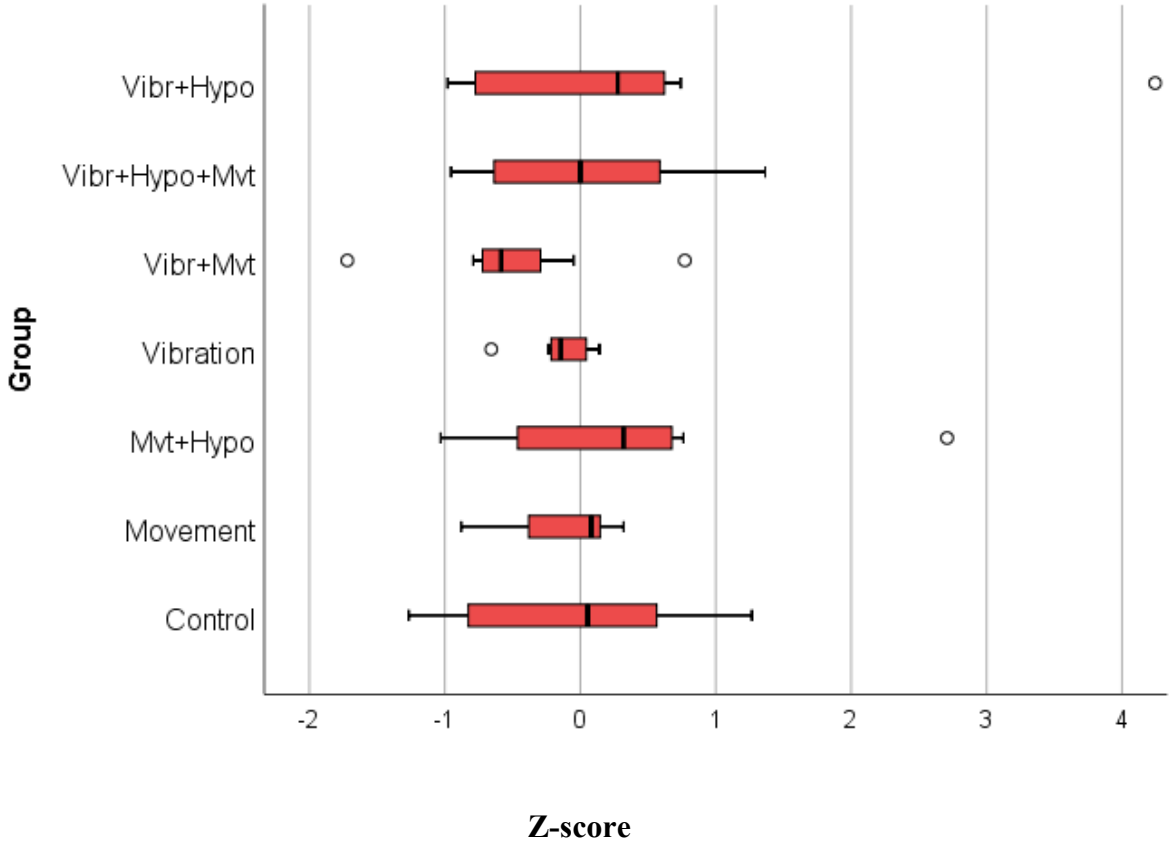
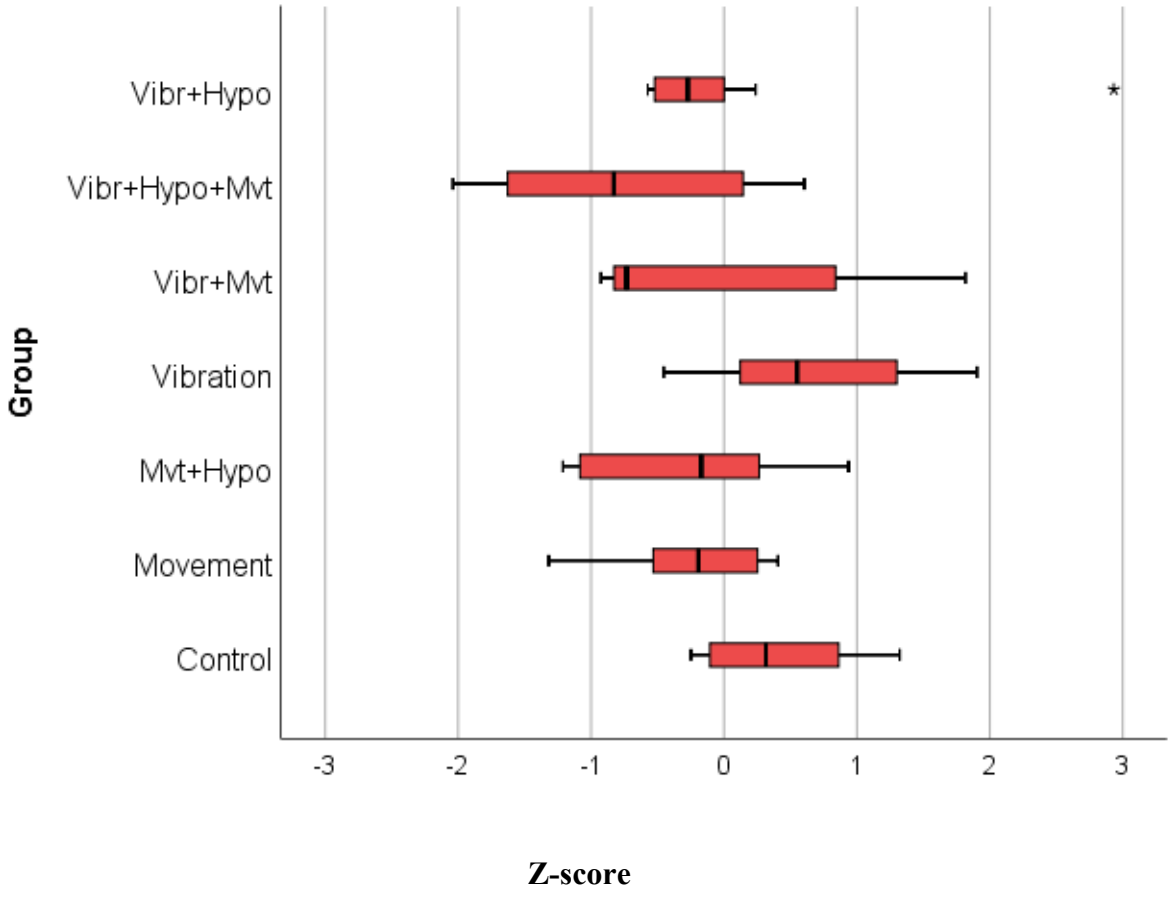


Figure 10: Total volume of the anterior and posterior portions of the midpalatal suture comparing experimental groups



List of Tables

Table 1: One-way ANOVA analysis of the anterior portion of the mid-palatal suture

		Sum of Squares	df	Mean Square	F	Sig.
Volume of the anterior portion of the midpalatal suture	Between Groups	.030	1	.030	.140	.796
	Within Groups	8.520	40	.213		
	Total	8.550	41			

Table 2: One-way ANOVA analysis of the posterior portion of the mid-palatal suture

		Sum of Squares	df	Mean Square	F	Sig.
Volume of the posterior portion of the midpalatal suture	Between Groups	.002	1	.002	.068	.710
	Within Groups	.957	40	.024		
	Total	.958	41			