MORPHOLOGY AND FUNCTION:

ASPECTS OF MANDIBULAR DEVELOPMENT AND GROWTH AT DIFFERENT STAGES ACROSS THE LIFESPAN

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Original published work submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy

Johannesburg, South Africa, 2017

DECLARATION

I Erin Frances Hutchinson declare that this Thesis Report is my own work. It is being submitted for the Degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



Erin Frances Hutchinson

5th Day of April 2017 in Johannesburg

This Thesis is dedicated to

Professor Jules Kieser

(1950 – 2014)

and

My family

Presentations arising from this research project:

2016 **Faculty of Health Sciences Research Day, Faculty of Health Sciences, University of the Witwatersrand**

Oral Presentation

Variations in bone density in the human mandible during the early stages of growth and development

Hutchinson E.F. Hoffmann J. Farella M. Kramer B.

44th Annual Conference of the Anatomical Society of Southern Africa (Bloemfontein, South Africa, 9-11 May)

Oral Presentation

Variation in bone density in the human mandible during the early stages of growth and development

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Poster Presentation:

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American Association of Physical Anthropologists (General Session: Knoxville, Tennesee, USA)

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Poster Presentation:

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The effects of tongue growth on the fetal and paediatric mandible

European Academy of Forensic Sciences (EAFS 2012) (Den Haag, Netherlands)

Oral Presentation:

EF. Hutchinson, B. Kramer, JA Kieser

The effects of tongue growth on the fetal and paediatric mandible

International Association of Dental Research (IADR) (Johannesburg, South Africa)

Oral Presentation:

EF. Hutchinson, B. Kramer, JA Kieser

Changes in the morphology and osteometry of the mental region

Publications arising from this research project:

Hutchinson, E.F., Kieser, J.A. and Kramer, B. (2014). Morphometric growth relationships of the immature human mandible and tongue. *European Journal of Oral Sciences*. 122, 181 – 189.

Hutchinson, E.F., Farella, M. and Kramer, B. (2015). Importance of teeth in maintaining the morphology of the adult mandible in humans. *European Journal of Oral Sciences*. 123, 341 – 349.

Hutchinson, E.F., Farella, M., Hoffmann, J. and Kramer, B. (2017). Variations in bone density across the body of the immature human mandible. *Journal of Anatomy*. Accepted 2016.

AUTHOR CONTRIBUTIONS

Chapter 3:

Hutchinson, E.F., Kieser, J.A. and Kramer, B. (2014). Morphometric growth relationships of the immature human mandible and tongue. *European Journal of Oral Sciences*. 122, 181 – 189.

E.F. Hutchinson contributed to the conception, design, data acquisition, analysis and interpretation as well as drafted the manuscript. In addition E.F. Hutchinson secured funding through the Carnegie Foundation of New York which was subsequently used to fund parts of this project. J. Kieser contributed to the conception, design and validated data interpretation, revised the manuscript and supervised the project. B. Kramer contributed to the conception, design and data interpretation, critically revised the manuscript and co-supervised the project. All authors gave final approval and agree to be accountable for all aspects of the final published paper.

Chapter 4:

Hutchinson, E.F., Farella, M., Hoffmann, J. and Kramer, B. (2017). Variations in bone density across the body of the immature human mandible. *Journal of Anatomy*. Accepted 2016.

E.F. Hutchinson contributed to the conception, design, data acquisition, analysis and interpretation as well as drafted the manuscript. In addition, E. F. Hutchinson secured funding for the project through the Seed Funding Initiative, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg (Grant Number: 00125184211015121105). M. Farella contributed to the design and validated the data interpretation, critically revised the manuscript and co-supervised the project. J. Hoffmann contributed to the data acquisition as a micro-CT specialist and analysis as well as critically revised the manuscript. B. Kramer contributed to the conception, design and data interpretation, critically revised the manuscript and supervised the project. All authors gave final approval and agree to be accountable for all aspects of the final published paper. The article has been accepted for publication during

December and formal license agreement is not included in the thesis. However, formal approval to use this article as part of the thesis has been granted by Wiley & Sons publishing.

Chapter 5:

Hutchinson, E.F., Farella, M. and Kramer, B. (2015). Importance of teeth in maintaining the morphology of the adult mandible in humans. *European Journal of Oral Sciences*. 123, 341 – 349.

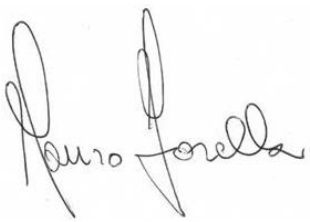
E.F. Hutchinson contributed to the conception, design, data acquisition, analysis and interpretation as well as drafted the manuscript. In addition, E. F. Hutchinson secured funding for the project through the National Research Foundation (South Africa) (Grant Number: UID#91645). M. Farella contributed to the design and validated the data interpretation, critically revised the manuscript and co-supervised the project. B. Kramer contributed to the conception, design and data interpretation, critically revised the manuscript and supervised the project. All authors gave final approval and agree to be accountable for all aspects of the final published paper.

As supervisors of the candidate, we confirm that she has described the roles of her co-authors accurately, and that she functioned as the principal investigator for all three studies.



Professor Beverley Kramer:

Date: 5 April 2017



Professor Mauro Farella:

Date: 5 April 2017

ABSTRACT

During development and growth, the mandible adapts to accommodate increased biomechanical loading associated with the development and growth of the tongue and the dentition. Biomechanical loads play a vital role in the modelling and remodelling of bone, with site-specific effects on bone density. In the functional transition from the prenatal to the postnatal period, biomechanical loading appears to be intensified and may affect the morphology of the mandible. The aim of this study was to analyze the growth and development of the human mandible during the functionally complex perinatal period of growth in order to investigate biomechanical effects. As edentulism modifies the biomechanical landscape of mastication, the effects of tooth loss on the morphology of the adult mandible were also investigated. Seven hundred and seventeen mandibles were sourced from cadaveric and skeletonized remains forming part of the Paediatric Collection, Raymond A. Dart Collection of Human Skeletons and Johannesburg Forensic Paediatric Collection, University of the Witwatersrand. The morphometric growth relationships between the mandible and tongue were initially investigated. A strong correlation between changes in the dimensions of the mandible and tongue was observed between 20 gestational weeks and 2 years. It was hypothesized, that mandibular growth was directed by the growth of the tongue. This would result in areas of increased bone modelling and remodelling, directly associated with the attachment of the tongue to the mandible and would manifest in areas of lower density bone. Subsequently, variations in bone mineral density of the body of the mandible were assessed. The lingual surface had significantly higher bone density values when compared to the external surface across the period of 30 gestational weeks to 5 years. Variations in the bone density across the external surface of the mandible followed the patterns of postnatal dental development. Thus, the effects of edentulism on the morphology of the adult mandible were also considered. The edentulous mandible had a shorter alveolar height and mandibular body length as well as more obtuse gonial and mental angles, when compared to the dentate mandible. Thus, changes in the morphology of the mandible over time appear to be indicative of an altered mandibular morphology prompted by a changing biomechanical landscape.

ACKNOWLEDGEMENTS

I am deeply indebted to many people who have assisted me in this challenging and enlightening journey. Firstly, I would like to thank Professor Beverley Kramer, who has been an immense source of support to me through a number of very challenging periods of this project. Beverley has been a fantastic supervisor, mentor and a very dear friend and has patiently taught and guided me, as well as allowed me the freedom to explore this field of research. I would also like to thank Professor Mauro Farella for taking up the role of supervisor after the untimely passing of Jules Kieser. Mauro has been very supportive as a supervisor and has assisted in strengthening the quality of this research from a dental and orthodontic point of view. I would also like to extend my thanks to Glynny Kieser, who has generously offered her editorial advice and has been very supportive throughout the duration of this project.

I am most grateful to the following colleagues from the Faculty of Health Sciences at the University of the Witwatersrand. I would like to thank Professor Elena Liebhaber from the Faculty of Health Sciences Research Office, for her statistical advice throughout the duration of this project. I would also like to thank Mr. Jason Hemingway from the School of Anatomical Sciences. Jason has been immensely helpful in teaching me the various aspects of geometric morphometrics and has been a great sounding board across a number of stats conversations. Thank you to Dr. Guin Gordon and Professor Jeanine Vellema from the Division of Forensic Medicine and Pathology for granting me access to the Johannesburg Forensic Pathology Services and for granting me permission to use paediatric specimens in my research. I would also like to extend my thanks to Professor TJM Daly (previous Head: School of Anatomical Sciences) as well as Mr. Brendon Billings (Collections Curator) for granting me access to specimens housed within the Raymond A. Dart Collection of Human Skeletons, School of Anatomical Sciences, University of the Witwatersrand.

A vote of thanks is extended to various members of the MIXRAD Facility at the South African Nuclear Energy Corporation (NECSA). Firstly thank you to Mr Jakobus Hoffmann and Mr Lunga Bham, for patiently teaching me the process of micro-CT scanning and data analysis. Your patience with all my crazy and unconventional

suggestions is immensely appreciated. Mr Frikkie De Beer was kind in granting me access to the facilities.

Dr. Chris Carlson and Dr. Tea Jashashvili from the Paleosciences Centre, University of the Witwatersrand were very helpful in granting me micro-CT access time to do additional data analysis. The National Research Foundation, Carnegie Foundation of New York and the Seed Funding Initiative are all duly acknowledged for their funding support of various aspects of this project.

Last but certainly not least, I wish to extend my most heartfelt thanks to my family. There is not a single aspect of this project that would have been possible without your love, support and guidance. You have all been truly incredible and your patience during this time was and is immensely appreciated.

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LIST OF ABBREVIATIONS

3D	-	Three-dimensional
ANOVA	-	Analysis of variance
BI	-	Buccal inferior
BM	-	Buccal middle
BMP	-	Bone morphogenic protein
BWMF	-	mental interforaminal distance
BS	-	Buccal superior
C	-	Canine
CBCT	-	Cone-beam computed tomography
CI	-	Central Incisor
Cos	-	Cosine
CT	-	Computed tomography
DM1	-	Deciduous molar 1
DM2	-	Deciduous molar 2
DXA	-	Dual energy X-ray absorptiometry
F	-	Female
FGF	-	Fibroblastic growth factors
GM	-	Geometric mean
GPA	-	Generalized Procrustes Analysis
HREC	-	Human Research Ethics Committee
HSD	-	Tukey’s honest significant difference
LI	-	Lateral incisor

LI	-	Lingual inferior
LLM	-	Maximum length of the mandible
LS	-	Lingual superior
M	-	Male
M1	-	Molar 1 / First permanent molar
M2	-	Molar 2 / Second permanent molar
MAN_ANG	-	Gonial angle
MANCOVA	-	Multivariate Analysis of Covariance
MANOVA	-	Multivariate Analysis of Variance
MBL	-	Mandibular body length
MBS_BAG	-	Maximum basilar width biantegonial
MBS_BG	-	Maximum basilar width bigonial
MBW	-	Maximum bicondylar width
MEA	-	Mental angle
MF_IB		Distance between the inferior border of the mandible and mental foramen
MF_SB		Distance between the superior border of the mandible and mental foramen
Micro-CT	-	Microfocus computed tomography
MMP	-	Matrix metallo-proteinase
MNTL	-	Minimum tongue length
MW	-	Maximum width of the mandible
MXTL	-	Maximum tongue length
ND	-	Depth of notch

NW	-	Width of the mandibular notch
PAST	-	Paleontological statistics
PC1	-	Principal Component 1
PC2	-	Principal Component 2
PCA	-	Principal Component Analysis
RH	-	Ramus height
SD	-	Standard Deviation
Shh	-	Sonic hedgehog
TB	-	Tongue breadth
TGF- β	-	Transforming growth factor-β

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measurements. BWMF, mental interforaminal distance; LLM, maximum length of the mandible; MBL, mandibular body length; MBS_BAG, mandibular basilar width bigonial; MBW, maximum bicondylar width; MEA, mental angle; MF_IB, distance between the inferior border of the mandible and the mental foramen; MF_SB, distance between the mental foramina and the superior border of the mandible; MW, maximum width of the mandible; ND, depth of the mandibular notch; NW, width of the mandibular notch; RH, height of the ramus …….108

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CHAPTER 1:

INTRODUCTION

Mechanisms for estimating age in immature and mature individuals have been considered in great depth by many researchers (Liversidge, 1994; Karup *et al*., 2005; Rozylo-Kalinowska *et al*., 2008; Mitchell *et al*., 2009; Sema *et al*., 2009). The accurate and reliable estimation of an individual's age not only assists in the identification process of previously unidentified individuals, but also has significant legal, social and clinical implications (Rozylo-Kalinowska *et al*., 2008; Jacobs *et al*., 2014; du Toit-Prinsloo *et al*., 2016). In a clinical setting, the accurate estimation of age is imperative, as it assists in identifying the associated stage of development and thus forms an integral part of the diagnosis of aberrant growth patterns, craniofacial anomalies and dentofacial deformities (Mao & Nah, 2004; Rozylo-Kalinowska *et al*., 2008). Furthermore age estimations play an integral part in the planning of treatment regimens for paediatric patients (Rozylo-Kalinowska *et al*., 2008).

Over time, various biological indicators *e.g.* skeletal maturity, morphological age (body height and weight) and sexual dimorphism have been suggested as a means of estimating the age of an individual (Rozylo-Kalinowska *et al*., 2008). However, while being highly effective in the adolescent and later stages of growth, the application of these indicators is limited across younger age cohorts. As such, dental development standards have, been established across a variety of population groups as a means of estimating age of immature individuals (Fanning & Moorrees, 1969; Demirjian *et al*., 1973; Alqahtani *et al*., 2010). However, while providing a baseline for estimating the age of immature individuals, these indicators do not provide clarity of the complex nature of development and growth of the craniofacial complex during the earlier stages of life.

A comprehensive understanding of the development and growth of the craniofacial complex is required for diagnosing and interpreting the changes in the morphology and function of structures associated with an aberrant growth pattern, craniofacial anomaly or dentofacial deformity (Mao & Nah, 2004). This knowledge is also of clinical importance when interpreting changes in the morphology of a structure or its functional capacity with a change in the surrounding biomechanical environment. Over time the development and growth of various structures of the craniofacial complex have been studied as a means of understanding the nature of the

associated growth relationships as well as the nature of the interaction between these structures within a functional context (Bjork, 1955; 1969; Tsunori *et al*., 1998; Bishara, 2000). The mandible, due to its position in the craniofacial complex and role in the support and functioning of the masticatory apparatus, has been the central focus of a variety of these studies (Bjork, 1955; 1969; Tsunori *et al*., 1998; Bishara, 2000; Koolstra, 2002; Radlanski *et al*., 2016). Furthermore, the mandible, owing to its high levels of preservation and recovery, has over time, also been the focus of several anthropological studies assessing its value as an indicator of an individual's age (Foti *et al*., 2003; Franklin *et al*., 2007; de Oliviera *et al*., 2008; Oettle *et al*., 2009), sex (Iscan & Kedici, 2003; Balci *et al*., 2005; Franklin *et al*., 2006; Ayoub *et al*., 2009; Oettle *et al*., 2009; Archarya *et al*., 2011; Oettle *et al*., 2016) and ancestry (Iscan & Steyn, 1999; Franklin et al., 2007; Oettle *et al*., 2009). While these studies have provided valuable insights into the changes in the dimensions and morphology of the mandible during adulthood (Foti *et al*., 2003; Iscan & Kedici, 2003; Balci *et al*., 2005; Franklin *et al*., 2007; Ayoub *et al*., 2009; Archarya *et al*., 2011), there is a paucity of information regarding how the morphology of the mandible is altered by the intricate growth relationships of the craniofacial complex in younger individuals. This poses a significant problem when trying to interpret changes in the morphology and dimensions of the mandible relative to age, particularly across the late prenatal and early postnatal period of growth.

In general the development and growth of the mandible has been studied across the embryonic (0 to 9 gestational weeks) and early fetal (9 to 20 gestational weeks) periods of growth (Merida-Velasco, 1990; Lee *et al*., 2001; Radlanski *et al*., 2002; 2003). While these studies described changes in the morphology of the mandible during the early periods of growth (Merida-Velasco, 1990; Lee *et al*., 2001; Radlanski *et al*., 2002; 2003), they did not provide clarity of the complex functional interactions which occur between the mandible and the surrounding oral cavity tissues. Studies investigating the complex functional interactions between the mandible and its associated oral cavity tissues have predominantly made use of animal based models (Mavropoulos *et al*., 2004, 2005; Fleischmannova *et al*., 2010; Alfaqeeh *et al*., 2013). While these latter studies provide valuable insights into the complex functional interactions between the mandible and the surrounding oral cavity environment, direct inferences between these models and humans is difficult owing to the

differences in the length of the gestation periods. Furthermore, studies investigating the changes in the morphology of the human mandible as a result of function have made use of juvenile and adult age cohorts (Cawood & Howell, 1988, 1991; Karup *et al*., 2005; Chrcanovic *et al*., 2011; Ozturk *et al*., 2013). While these studies assist in explaining the changes in the morphology of the mandible as a result of a change in function, they do not clarify the nature of the interactions between the mandible and its associated structures during the functionally complex late prenatal and early postnatal periods of growth.

The late prenatal (20 gestational weeks to birth) and early postnatal (birth to 5 years) periods of growth, in particular, is considered to be a multifaceted period in the overall development and growth of the mandible and craniofacial complex. These periods are significant as they involve the transition from a relatively inactive to a highly active functional environment, which effectively introduces a series of biomechanical influences linked to suckling, dental development and eruption as well mastication (Miller *et al*., 2003; Delaney & Arvedson, 2008). These biomechanical influences are thought to significantly impact the changes in the dimensions and morphology of the mandible. This thesis will address the nature of the influences of the tongue and the developing dentition on the morphology of the mandible during the late prenatal and early postnatal periods of growth. In addition, as the dentition is thought to have a significant effect on the functioning of the mandible during the adult stages of life, this thesis will also clarify the role of the dentition in maintaining the morphology of the mandible. The introductory chapter will review the existing knowledge of the patterns of prenatal and postnatal development and growth of the mandible. In addition, it will also explain the possible mechanisms involved in the adaptation of the mandible to a changing environment.

1.1 Development and growth of the mandible

1.1.1 Introduction

Over the past century, the development and growth of the human mandible has been a central focus in understanding the overall function and evolution of the masticatory system (*e.g.*, Moss & Rankow, 1968; Carlson & Van Gerven, 1977; Lee *et al*., 2001; Radlanski *et al*., 2003; Karup *et al*., 2005; Holmes & Ruff, 2011; Holton *et al*., 2014). Interest in mandibular growth has effectively led to theories such as “the functional matrix model”, proposed by Moss and Salentijn (1969) and the “principle of archial growth” (Ricketts, 1972). These theories have commendably been suggested as a mechanism for explaining changes in the overall structure of the mandible associated with the growth of the oral cavity and the evolution of mandibular function. The theories have also been expanded by several authors to consider that the observed variations in mandibular form may be the result of a possible influence from changing biomechanical demands associated with alterations in the composition of the diet as well as overall survival strategies (Carlson & Van Gerven, 1977; Atchley & Hall, 1991; Hatch *et al*., 2000; Fukase & Suwa, 2008; Holmes & Ruff, 2011; Lepley *et al*., 2011).

1.1.2 Theories of craniofacial growth and patterns of mandibular growth

The morphogenic pattern of the craniofacial complex and the specific cells assigned to the development and growth of this region, have been of significant interest to morphologists and clinicians alike (Brodie, 1941a,b; Sicher, 1947; Scott, 1959; Moss & Rankow, 1968; Enlow & Harris, 1964; Enlow, 1966; Enlow *et al*., 1971; Ricketts, 1972). Much of the interest in the variations associated with the morphogenic pattern of the craniofacial complex has stemmed from the description of a series of gradients *i.e*. age, time, rate and locus (Brodie, 1941a,b). These gradients have assisted in understanding the variation in the sequence of events associated with the development of the craniofacial complex. However, while providing a plausible explanation for the variability in the development of craniofacial structures, these gradients did not identify the specific centres directing its growth (Brodie, 1941a,b). As such, the early theories of growth suggested that the craniofacial complex developed and grew as a single unit with certain sites contributing greater percentages than others to the growth of this region (Brodie, 1941a,b). These

theories further suggested that while certain sites had a greater contribution to the growth of the area, there was no change in the general form of the craniofacial complex *i.e.* growth was proportionate across the entire region (Brodie, 1941a,b). While these theories provided an explanation for the normal development and growth of the craniofacial complex, they could not account for the changes in form associated with developmental abnormalities.

As such, the focus of theories of craniofacial growth shifted from a general concept to identifying and predicting the role of growth centres in the overall development and growth of the craniofacial complex (Brodie, 1941a,b; Sicher, 1947; Scott, 1959). Over time, various growth centres were identified across the facial complex with the condyle of the mandible being identified as the principal growth centre of the mandible (Brodie, 1941a,b; Sicher, 1947). The condylar growth of the mandible was considered to be particularly significant in the prediction of the growth patterns of the face and cranial base, as the mandible is closely associated with both the glenoid fossa and the alveolar process of the maxilla (Brodie, 1941a,b; Sicher, 1947; Scott, 1959). In general, the growth of the craniofacial complex was widely attributed to growth taking place at the cranial sutures (Sicher, 1947; Scott, 1959). However, the increase in the height of the alveolar process of the maxilla was thought to be heavily dependent on the antero-inferior growth and divergence of the condyles of the mandible *i.e.* increases in bicondylar width (Sicher, 1947; Scott, 1959). This concept was well illustrated when assessing the skeletal relationships in a Class II malocclusion (Sicher, 1947). The reduced development of the mandible impaired the growth of the maxillary alveolar process, but had no visible effect on the sutural growth of the maxilla (Sicher, 1947). As such, the maxilla appeared to protrude beyond the under-developed mandible with a visibly shortened alveolar process. While these forgoing theories of craniofacial growth highlighted the importance of genomics and growth centres in analyzing and predicting growth patterns, they did not consider the effects of soft tissue influences or a change in function on the overall morphology of the complex with growth.

Theories related to craniofacial growth have undergone a substantial shift from the popular genomic paradigm, which proposed that craniofacial growth was primarily driven by genetics and heredity, to the functional paradigm which emphasized the flexibility of the development and growth of the craniofacial region (Carlson, 2005). This major shift in the theories of craniofacial growth originated with the proposal of the “functional matrix hypothesis” (Carlson, 2005). This hypothesized that the craniofacial skeleton develops initially due to genetic influences, but grows in direct response to the surrounding extrinsic, non-genetic environment (Moss, 1960, 1962; Carlson, 2005). Thus, the assessment of craniofacial growth shifted from the classical osteological perspective of bones growing independently to a more operational ideal of bone growth being primarily related to functions such as digestion and speech (Moss, 1962).

As the surrounding environment is thought to influence the growth of the craniofacial skeleton, it is therefore necessary to understand the degree of interaction between the various functional and skeletal components proposed in the functional matrix hypothesis. Fundamentally, functional cranial components are categorized as either a functional matrix or a skeletal unit (Moss, 1960, 1962; Carlson, 2005). The functional matrix makes general reference to all soft tissues and associated spaces that may perform a given function and is further categorized as either a periosteal matrix or a capsular matrix (Moss, 1960, 1962; Carlson, 2005; Moss, 2007). The periosteal matrix encompasses structures within the immediate local environment of a skeletal unit, which broadly include muscles and associated neurovascular structures (Moss, 1962; Carlson, 2005; Moss, 2007). The capsular matrix is viewed as the organs and/or spaces that occupy a larger anatomical complex *e.g.* the orofacial capsule (Moss, 1962; Moss & Salentijn, 1969; Carlson, 2005; Moss, 2007). In contrast, the skeletal unit is any bony structure that provides support and at times facilitates the operation of the functional unit *e.g.* the mandible (Moss, 1960, 1962; Carlson, 2005). The skeletal unit is further categorized into a micro-skeletal unit *e.g.* a tuberosity or a bony ridge, and a macro-skeletal unit *e.g.* neurocranium or maxillomandibular complex (Carlson, 2005). Thus, the mandible in the context of craniofacial growth is viewed as a skeletal unit supporting the functional periosteal matrix of the oral cavity and in particular, the masticatory apparatus.

While the mandible is categorized as a single skeletal unit, it is also made up of a series of micro-skeletal functional units. These functional units include: the condylar process, the coronoid process, the angular process, the body, the alveolar process and the chin (Moss, 1962; Moss & Rankow, 1968). The functional units collectively contribute to the overall functional matrix of the mandible within the oral cavity. However, they may also act as independent units. When the soft tissues of one or a combination of these functional units are either absent, abnormally developed or misaligned, only the corresponding functional area of the mandible should be affected (Moss, 1962). This principle was demonstrated with the bilateral removal of the malformed mandibular condyles in a seven year old girl, who had previously presented with a restricted anterior open bite (Moss & Rankow, 1968). The condyles of the mandible appeared to be hypoplastic bilaterally and double forked on the left side of the mandible, with no further malformation reported across the mandible (Moss & Rankow, 1968). Shortly after the bilateral condylectomy, the patient was able to open her mouth normally and the general level of functioning was restored. The return of the mandible to a fully functional state with no further malfunction or abnormal development being reported, illustrates the concept of a functional unit acting independently. However, while the principle of functional unit independence was highlighted with the bilateral condylectomy, there was no consideration given as to how the overall morphology of the mandible would subsequently be affected by the resultant change in the biomechanical environment. This also raises questions as to what proportion of mandibular growth is genetically predetermined and what proportion is predominantly directed by function. These questions are of chief clinical and forensic importance in the prediction of growth patterns across areas of investigation assessing changes in facial form (Bjork, 1969).

To investigate the growth pattern of the human mandible relative to facial form, Bjork (1955, 1969) implanted a series of metal implants into the body of mandibles of individuals aged between 4 and 24 years. The main purpose of the study was to locate possible sites of growth and resorption in the individual mandibles and then to evaluate the variations in growth occurring relative to direction and intensity (Bjork, 1955; 1969). In general, some thickening of the posterior surface and inferior border of the symphysis was observed. This thickening of the symphysis was attributed to bone apposition, while bone resorption was observed below the angle of the

mandible (Bjork, 1969). In addition, the condyles of the mandible were identified as primary sites of mandibular growth. However, the growth of the mandible at the condyle did not follow the direction of the ramus. Instead growth was angled slightly anteriorly *i.e.* along a curve-like trajectory and was thought to occur about a centre of rotation (Bjork, 1969). While the centre of rotation was thought to occur in relation to the temporomandibular joint, other possible locations also included the anterior or posterior ends of the bone or anywhere along the body of the bone (Bjork, 1969).

In general, the concept of the mandible growing in relation to a centre of rotation provided valuable insights into potential areas of bone apposition and resorption (Bjork, 1955; 1969). It also highlighted the growth relationship between the cranial base, facial height and the mandible during the adolescent stages of growth. However, while illustrating a rotational pattern of growth in the adolescent mandible, it was only able to suggest possible sites of growth rotation. As a result, the principle of archial growth was proposed as a means of identifying more definite points of mandibular growth. This principle fundamentally expanded on the observations of the metal implant study and thus proposed that the mandible grows along an arc-like trajectory (Ricketts, 1972). While the metal implant study (Bjork, 1955; 1969) identified various locations for the centre of rotation, the principle of arcial growth was based on an arc-like pattern as determined by using two fixed points *i.e.* the mental protuberance and the termination point of the oblique ridge. Thus, the concept of rotational growth (Bjork, 1955; 1969) and the principle of archial growth (Ricketts, 1972) both provided insights into the intricacies of the growth of the mandible relative to general craniofacial growth.

The complex nature of craniofacial growth was further expanded in an assessment of the postnatal growth of the mandible of individuals aged between 4 and 12 years of age (Enlow & Harris, 1964). In this assessment the distributions of endosteal and periosteal bone were investigated, using transverse sections at serial locations across all areas of the mandible. The technique of mapping the distributions of endosteal and periosteal bone deposits across the mandible provided significant insights into the direction of growth associated with specific areas of the mandible (Enlow & Harris, 1964; Enlow, 1966). Three basic principles or concepts of growth

were identified and described as being the fundamental doctrines of structural remodelling associated with the development and growth of the human mandible. These principles included the concepts of area relocation and activity at surfaces determined by regional differences in the growth of the bone, as well as the principle of the ‘V’ (Enlow & Harris, 1964).

The concept of area relocation broadly suggests that there is a relative change in the location of certain areas of the mandible as a result of its growth (Enlow & Harris, 1964). Thus, over time, a particular area of the mandible may experience a successive series of changes in its location relative to the accompanying principal directions of growth associated with that area. Furthermore as an area undergoes a change in its relative position with growth, this area may either be partially or totally removed as a result of a significant degree of bone remodelling associated with the development and growth of the mandible (Enlow & Harris, 1964). In contrast, when considering how activity at a particular surface is determined by regional differences in the growth of the bone, the surface facing a particular direction of growth is significant. In general, a periosteal or endosteal surface facing a particular direction of growth is observed to receive new deposits of bone, while bone resorption is observed across the surface opposing the direction of growth (Enlow & Harris, 1964). This mechanism of bone growth is pertinent when considering that the growth pattern of cortical bone occurs in an inward direction in response to remodelling changes associated with regional shifts in location. Furthermore, this mechanism of bone growth has significance when considering the principle of ‘V’. The principle of ‘V’ is fundamentally a mechanism of bone growth by which an area of bone may increase in size, while at the same time moving in the direction of the wide end of a ‘V’ *i.e.* the principal direction of growth (Enlow & Harris, 1964). The inner surface of the ‘V’ faces the principal direction of growth and receives new deposits of bone, while the outer surface of the ‘V’ is usually subjected to corresponding amounts of bone resorption. In general these fundamental principles of growth serve as a guide to understanding the contrasting growth processes which contribute to the remodelling movements occurring across the mandible during growth (Enlow & Harris, 1964).

When considering these principles of growth within the context of craniofacial growth and in particular the growth of the mandible, various examples of area relocation and the principal of ‘V’ are observed (Enlow & Harris, 1964; Enlow, 1966). The condyle of the mandible during the early stages of its growth is thought to undergo a significant degree of area relocation). Over time, the bone associated with the early condyle of the mandible is relocated to a position in the condylar neck as a result of the modelling and remodelling of the condyle in a posterior-cephalic direction (Enlow & Harris, 1964; Enlow, 1966). Moreover, a significant degree of bone resorption is observed across the anterior surface of the ramus, while bone is deposited in accordance with the principal direction of growth across its posterior border. When considering the body of the mandible, various principal directions of growth may be applied. In the case of the alveolar region, bone is deposited across the alveolar surface of the body of the mandible, while bone resorption is observed to a lesser degree across the inferior border of the mandible (Enlow & Harris, 1964; Enlow, 1966).

In general, the concept of rotational growth (Bjork, 1955; 1969), the principle of archial growth (Ricketts, 1972) and the basic principles of growth (Enlow & Harris, 1964; Enlow, 1966) all provide insights into the complex nature of the growth of the mandible relative to general craniofacial growth and patterns of bone remodelling. However, these studies were based on observations of growth across juvenile and adolescent age cohorts and commenced after the eruption of the deciduous dentition and onset of mastication. As such, these studies could not explain the complex nature of the growth of the mandible during the late prenatal and early postnatal period. Furthermore these studies were not able to clarify the nature of the growth relationships between the mandible and the functional apparatus of the oral cavity *i.e.* the dentition, tongue and muscles of mastication.

1.1.3 Molecular aspects of the growth of the mandible and masticatory apparatus

Theories such as the functional matrix hypothesis (Moss, 1960; 1962), rotational patterns of mandibular growth (Bjork, 1955; 1969) and principle of arcial growth (Ricketts, 1972), provide a significant platform for describing the complexities of mandibular growth on a macroscopic scale. However, these theories were not able to explain the underlying molecular mechanisms directing the development and growth of the mandible and the masticatory apparatus (Moss, 1997a,b). The underlying molecular mechanisms provide valuable insights into the genomic contribution to mandibular growth. Furthermore they assist in clarifying the complex prenatal growth relationships between the mandible and the associated structures of the oral cavity (Chin & Werb, 1997; Moss, 1997a; Chai *et al*., 2000; Nie *et al*., 2006; Fleischmannova *et al*., 2010; Lungova *et al*., 2011; Martinez-Maza *et al*., 2012; Sun *et al*., 2012; Alfaqeeh *et al*., 2013; Boell, 2013; Boell *et al*., 2013).

The embryonic stages of craniofacial growth and development are highly dependent on the invasion of neural crest cells into the ventral aspects of the craniofacial region (Cordero *et al*., 2011; Hall & Gillis, 2013). This invasion of neural crest cells and their interaction with the early general mesenchyme of the pharyngeal arch is of pivotal importance in the development of the craniofacial region, as the neural crest cells are the precursor cells for structures such as the Meckle's cartilage and the mandible (Cordero *et al*., 2011; Hall & Gillis, 2013). Thus, the formation, migration pattern and differentiation of the neural crest cells during the embryonic stages of development forms the foundation of investigations into the underlying molecular mechanisms directing mandibular growth (Chin & Werb, 1997; Thesleff, 1998; Chai *et al*., 2000; Nie *et al*., 2006).

The formation of the neural crest cells within the crests of the neural folds are dependent on the presence of transcription factors including fibroblast growth factors (FGF) and members of the transforming growth factor-β (TGF- β) superfamilies *i.e.* Dorsalin-1 and BMP-4 (Nie *et al*., 2006; Oka *et al*., 2007; Cordero *et al*., 2011; Hall & Gillis, 2013). In order for these cells to migrate out of the neural folds activation of the

‘slug’ gene must occur, this facilitates the conversion of the neural crest cells from epithelial cells to mesenchymal cells (Cordero *et al*., 2011; Hall & Gillis, 2013). As the neural crest cells are derived from ectoderm, this mesenchyme is referred to as ectomesenchyme. When the neural crest cells migrate to their final destinations within the pharyngeal arches they encounter an environment rich in extracellular matrix molecules *e.g.* matrix metallo-proteinases (MMP) (Chin & Werb, 1997; Cordero *et al*., 2011; Hall & Gillis, 2013). Once embedded within the mesenchyme of the pharyngeal arch, the neural crest cells are exposed to a combination of transcription factors and signalling molecules. These transcription factors *e.g.* Dlx and Msx and signalling molecules *e.g.* BMP and sonic hedgehog (Shh), facilitate and regulate the development of the pharyngeal arch and its associated structures (Thesleff, 1998).

Transcription factors such as Msx, Dlx and MHox have all been implicated in the regulation of the development of the first pharyngeal arch and, in particular, in the development of the mandible and the dentition (Thesleff, 1998; Depew *et al*., 2002; Doshi *et al*., 2016). The expression of the Dlx gene is significant within the mandibular and the maxillary arch ectomesenchyme (Depew *et al*., 2002; Doshi *et al*., 2016). Msx gene expression is prevalent across epithelial-mesenchymal interactions within the pharyngeal arches in areas associated with the future dentition and is also expressed in the formation of the skull and facial primordia (Doshi *et al*., 2016). The role of the Hox gene group, in particular the Homeobox gene group, in the morphogenesis of the jaws and the associated dentition is important and has been detailed in the literature (Couly *et al*., 1998; Trainor & Krumlauf, 2001; Doshi *et al*., 2016).

In contrast to the transcription factors, the signalling molecules mediate the general communication between the epithelium and the ectomesenchymal components involved in bone and tooth development (Thesleff, 1998). These molecules broadly include FGF, Shh, BMPs and the Wnt-family of signalling molecules (Thesleff, 1998). Sonic hedgehog (Shh) plays a crucial role in maintaining the viability of neural crest cells within the first pharyngeal arch (Dworkin *et al*., 2016). Shh also plays a significant role in regulating the migration of neural crest cells and closely interacts

with factors such as BMP-4 and FGF-8 through various paracrine feedback loops (Dworkin *et al*., 2016). The role of Shh across the first pharyngeal arch is significantly mediated by endodermal Shh regulating the expression of FGF-8 within areas such as the pharyngeal ectoderm. As such, a loss of either FGF-8 within the pharyngeal ectoderm or the foregut endoderm would lead to a substantial reduction in the mandible (Dworkin *et al*., 2016).

Fibroblastic growth factor (FGF) signalling also promotes the formation of chondrocyte lineages within the cranial neural crest in addition to its inductive role in neural crest formation. In the advanced stages of development, FGF signalling is prevalent in both the epithelia and the ectomesenchyme and thus mediates the epithelial-mesenchymal interactions associated with nearly all structural development (Nie *et al*., 2006). In craniofacial patterning and growth, FGF-8 is particularly important as ectoderm-expressed FGF-8 induces homeobox gene expression in the ectomesenchyme, which is thought to be critical for the formation of structures within the facial primordia. As such, a loss of FGF-8 function in the ectoderm of the first pharyngeal arch results in the complete absence of the skeletal structures of the first pharyngeal arch as well as dental agenesis (Nie *et al*., 2006).

The FGF signalling molecule is also potentially a mitogen for the rapid embryonic expansion of the tongue in addition to its role in chondrocyte and neural crest cell formation (Nie *et al*., 2006). FGF signalling is widely thought to promote myogenic proliferation, but in fact it depresses myogenic differentiation, with FGF-4 playing a crucial role in mediating the FGF signal in myogenesis. In addition to the myogenic potential of the FGF signalling molecules, matrix metalloproteinases contribute to the migration and invasion of paraxial mesoderm into the first pharyngeal arch (Chin & Werb, 1997). Matrix metalloproteinases are also involved in the morphogenesis of the tongue and Meckle's cartilage during the development of the arch of the mandible (Chin & Werb, 1997). Thus, a sophisticated system of transcription factors, signalling molecules and extracellular matrix enzymes are involved in the development of the mandible, dentition and the tongue.

In general, molecular studies have over time been successful in exposing the underlying mechanisms behind the growth and development of the craniofacial complex, particularly during the embryonic and early periods of fetal growth (Chin & Werb, 1997; Moss, 1997a; Chai *et al*., 2000; Nie *et al*., 2006; Fleischmannova *et al*., 2010; Lungova *et al*., 2011; Martinez-Maza *et al*., 2012; Sun *et al*., 2012; Alfaqeeh *et al*., 2013; Boell, 2013; Boell *et al*., 2013). These studies have also provided a basis for describing irregularities in the general growth and development of the craniofacial complex, through the identification of genes and growth factors (Couly *et al*., 1998; Thesleff, 1998; Trainor & Krumlauf, 2001; Depew *et al*., 2002; Doshi *et al*., 2016; Dworkin *et al*., 2016). The role of distant-acting transcriptional enhancers in mammalian genomes has also gained significance in understanding and describing the sequences that regulate the development of structures associated with the craniofacial complex (Attanasio *et al*., 2013). This complex of enhancers is thought to act on individual genes and as such, provides a mechanism for the autonomous fine-tuning of particular aspects of gene expression associated with various developmental processes (Attanasio *et al*., 2013).

While these studies have clarified the role of genetics in early craniofacial growth, there is still a paucity in understanding the overall effect of a change in the biomechanical environment on the morphology of the mandible.

1.1.4 Spatial versus biomechanical

The development and growth of the mandible and the masticatory apparatus is of interest during the functionally complex late prenatal and early postnatal period of growth. It is at this stage of life that these structures must adapt their growth to accommodate the development of functions such as sucking, suckling and mastication, which are required for successful nutritional intake (Herring, 1985; Herring *et al*., 2001; Miller *et al*., 2003; Delaney & Arvedson, 2008). In addition, during this period the mandible must also develop and grow to accommodate the growth of the associated craniofacial complex and the descent of the tongue in the oral cavity (Humphrey, 1971; Herring, 1985; Herring *et al*., 2001; Liu *et al*., 2008a, b). The mandible is thus subjected to a series of spatial and biomechanical demands

emanating from the surrounding craniofacial and oral cavity complexes. While it is acknowledged that there is a progression in the degree of complexity of oral cavity functioning during this period, the nature of the growth relationships between the structures supporting these functions is not well understood.

During the fetal period, the descent of the tongue in the oral cavity and subsequent elevation of the palatal shelves is viewed as a complex growth relationship within the context of craniofacial growth (Cordero *et al*., 2011; Hall & Gillis, 2013). However, the effect of the tongue descending in the oral cavity on the general development and growth of the mandible is not clear. Humphrey (1971) first investigated the correlation between orofacial activity and morphologic changes in the orofacial region between 8 and 29 gestational weeks using a combination of motion picture negatives and photographs. While evaluating changes in the craniofacial complex at 8 gestational weeks, a small mandible and protrusive maxillary region relative to the mandible were observed. In addition, the mandible was in a depressed position and mild reflex actions were also observed (Humphrey, 1971; Herring, 1985; Herring *et al*., 2001). Shortly after the descent of the tongue and elevation of the palatal shelves during the 9th week of gestation, the size of the mandible increased without a corresponding change in the position of the maxillary region. The mouth was open and the mandible assumed a more depressed position (Humphrey, 1971). The increase in the size of the mandible resulted in a significant reduction in the protrusive position of the maxilla relative to the mandible (Humphrey, 1971). However, it was not clear if the change in the relationship between the maxilla and the mandible at this stage of growth was the result of the tongue descending in the oral cavity or if there were other extrinsic factors involved. Thus, the growth relationship between the mandible and tongue has not been directly assessed during the prenatal period of growth.

Subsequent to the increase in the size of the mandible, there was a change in the complexity of the functioning of the oral cavity from the initial mouth opening reflexes to swallowing between 10 and 12 weeks of gestation (Humphrey, 1971). In the 13th week of gestation, the maxilla protruded anteriorly once again and extended beyond the mandible. As the oral cavity adapted to the process of swallowing, changes were observed in the posture and position of the tongue, as well as in the movement of the

mandible. The tongue assumed an elevated posture and there was an increase in its protrusive and retractive actions. In addition, there was an increase in the depression of the mandible and active mouth closure, which were thought to be part of the swallowing reflex (Humphrey, 1971; Herring, 1985; Herring *et al*., 2001). While the movements and the posture of the tongue were observed to change between 15 and 29 weeks of gestation, there was no further evidence of unequal growth rates between the maxilla and the mandible (Humphrey, 1971). Furthermore the direct effects of a change in the function of the tongue on the growth and development of the mandible remain unclear.

The growth of the oral cavity and in particular the mandible, during the late prenatal (30 gestational weeks and birth) and postnatal periods of growth, is further complicated by the development, emergence and eruption of the dentition (Wise & King, 2008; Alqahtani *et al*., 2010). It is anticipated that during this period of growth, the mandible grows to accommodate the spatial demands of the developing dentition and adapts to the subsequent progression in function from suckling to mastication. As such, the development and growth of the mandible in response to the developing dentition was investigated using a combination of mouse (Radlanski *et al*., 2015) and human samples (Radlanski *et al*., 2016).

In the assessment of the molar region of the mandible between the dental lamina and root formation stages of development, particular attention was paid to the morphological development of the surrounding bony dental crypt (Radlanski *et al*., 2015). The development and growth of the bony edges of the dental crypt were well synchronized with the development of the associated tooth germ. The bone of the lingual surface of the dental crypt appeared to be more advanced in its development when compared to its buccal surface (Radlanski *et al*., 2015). These observations were subsequently observed across the development and growth of the molar region of the mandible in humans (Radlanski *et al*., 2016). These studies also noted that the bone associated with the lingual and buccal surfaces of the mandible appeared to develop by means of appositional growth, while the dental crypts increased in depth as a result of bone resorption (Radlanski *et al*., 2015; 2016). When considering the findings of these studies, it may be concluded that the bone of the mandible develops

in response to the development of the dentition (Wise & King, 2008; Radlanski *et al*., 2015; 2016). In general these studies provided valuable insights into the appositional and resorptive patterns of bone growth during periods of dental development. However, they did not consider the effects of a change in the biomechanical loading conditions on appositional and resorptive patterns during the late prenatal and postnatal stages of growth.

While it is acknowledged that the oral cavity must adapt to a change in function with growth (Miller *et al*., 2003; Delaney & Arvedson, 2008), there is a limited understanding as to how this adaptation and the subsequent change in the accompanying biomechanical environment is influenced by the growth of the associated structures. During the late prenatal and early postnatal period of growth, there is thought to be a change in the loading conditions imposed on the mandible by both the developing dentition (Wise & King, 2008), and the associated oral cavity soft tissues (Van Eijden, 2000). However, the very nature of these loading conditions and their overall impact on the development and growth of the mandible during the late prenatal and early postnatal periods is unclear.

Bone density has been proven to be an effective and reliable means of assessing the effects of a change in the loading conditions on the mineralization of bone (Jager *et al*., 1990; Mavropoulos *et al*., 2004, 2005; Mulder *et al*., 2006; Ruffoni *et al*., 2007; Park *et al*., 2008; Roschger *et al*., 2008; Cioffi *et al*., 2010; Hsu *et al*., 2011; Chang *et al*., 2012; Hichijo *et al*., 2015). Over time studies investigating changes in bone density have directed their focus to changes in either the volume or distribution of mineralized bone tissue across a specific area of bone.

Volumetric changes in the mineralized content of bone *i.e.* volumetric bone density serve as a reliable indicator of the biomechanical potential of bone and have been a valuable source of information clinically (Park *et al*., 2008; Hsu *et al*., 2011; Chang *et al*., 2012). Clinical investigations assessing changes in the volumetric bone density of the mandible have evaluated the effects of orthodontic treatment on bone density (Hsu *et al*., 2011; Chang *et al*., 2012) and have also been used for the purposes of

locating suitable sites for dental implants (Park *et al*., 2008). While those studies assessing changes in the volumetric bone density have made use of dual energy X-ray absorptiometry (DXA) (Mavropoulos *et al*., 2004, 2005), cone-beam computed tomography (CBCT) (Hsu *et al*., 2011; Chang *et al*., 2012) and conventional CT (Computed tomography X-ray) (Park *et al*., 2008), they have been predominantly focused on juvenile and adult age cohorts.

In contrast, the distribution of bone mineral density, assists in identifying specific areas of bone which are subjected to higher levels of biomechanical force and strain across a developing bone (Roschger *et al*. 2008; Cioffi *et al*. 2010; Hichijo *et al*. 2015). This is achieved through the identification of areas of bone presenting with low levels of mineralization and a higher degree of bone modelling and remodelling (Roschger *et al*. 2008; Cioffi *et al*. 2010; Hichijo *et al*. 2015). In addition, bone mineral density distributions provide an indication of the strength, stiffness and toughness of the bone (Ruffoni *et al*. 2007; Hernandez, 2008; Roschger *et al*. 2008; Cioffi *et al*. 2010; Hichijo *et al*. 2015). As such, variations in the distribution of bone mineral density serve as a valuable indicator of changes occurring in the surrounding biomechanical environment.

Studies assessing variations in the distribution of bone mineral density of the developing mandible have thus far focused on the areas of the mandible that are thought to have higher levels of biomechanical loading *e.g.* the condyle (Mulder *et al*., 2006; Cioffi *et al*., 2010). In a micro-CT assessment of the relationship between the architecture and mineralization of bone during the early developmental stages of the mandible, variations in bone mineral density were evaluated across the body and condyle of the fetal and newborn pig mandible (Mulder *et al*., 2006). While a general increase in the bone mineral density of both the condyle and body of the mandible was observed with age, bone mineral density in the body of the mandible was higher than that of the condyle of the mandible (Mulder *et al*., 2006). This was attributed to the condyle of the mandible being subjected to increased bone modelling and remodelling arising from biomechanical loading when compared to the body of the mandible. When assessing variations in the bone mineral density across areas of the body of the mandible, bone mineral density in the anterior regions was lower than

that of the middle and posterior regions of the body. In addition, the bone mineral density of the buccal surface of the newborn pig mandible was significantly less mineralized than that of the lingual and apical regions (Mulder *et al*., 2006). While variations in the bone mineral density were considered across the condyle and body of the mandible, the potential impact of the developing dentition and occlusal forces on the bone mineral density with growth were not considered (Mulder *et al*., 2006).

In contrast to the juvenile mandible, the biomechanical impact and spatial demands of the dentition on the morphology of the adult mandible have been extensively studied within the context of changes in the state of the dentition *i.e.* from dentate to edentulous states of the dentition (Cawood & Howell, 1988, 1991; Chrcanovic *et al*., 2011; Ozturk *et al*., 2013). Studies assessing changes in the morphology of the mandible relative to alterations in the state of the dentition have been focused towards investigating the effects of tooth loss on the alveolar region and body of the mandible (Cawood & Howell, 1988, 1991; Tsakiris *et al*., 1996). In addition, studies investigating the effects of tooth loss on the morphology of the mandible have restricted their assessments to comparisons of the dentate and edentulous states (Chrcanovic *et al*., 2011) or the dentate and partially edentulous states of the dentition (Ozturk *et al*., 2013).

While there have been studies assessing what kind of an effect tooth loss would have on other areas of the mandible (Oettle *et al*., 2009; Chrcanovic *et al*., 2011; Ozturk *et al*., 2013; Oettle *et al*., 2016), these assessments have been focused on changes in the general dimensions of the mandible (Chrcanovic *et al*., 2011; Ozturk *et al*., 2013) and in particular the angle of the mandible (Oettle *et al*., 2009; 2016). Little is known about the effects of tooth loss on the changes in the morphology of the mandible across areas related to the attachment of the muscles of mastication. This is thought to be a significant clinical factor considering how the biomechanical environment of the oral cavity is thought to change with an alteration in the state of the dentition.

1.2 Aim and objectives

The structure of the mandible is thought to change significantly during periods of craniofacial growth and development as well as during periods of transition in the development and functioning of the masticatory apparatus and the dentition with age. These changes are largely thought to emanate from the spatiotemporal demands of the growing craniofacial complex as well as the changing biomechanical environment of the oral cavity with growth. Moreover these changes in the morphology and general structure of the mandible may serve as reliable indicators of the age of an individual.

Thus, the aim of this study was to assess the osteological changes in the mandible, throughout development and growth. To fully determine the applicability of osteological changes in the mandible as indicators of age within a forensic and clinical context, it was necessary to first understand the role each of the associated tissues of the oral cavity *i.e.* the dentition and tongue, in the overall growth of the mandible.

The morphometric growth relationships of the immature mandible and tongue were investigated using a combination of geometric morphometrics and linear measurements. It was hypothesized that the tongue would be elemental not only in maintaining normal mandibular ontogeny, but also in its postnatal development and growth (Chapter 3).

Considering the proposed role of the tongue in maintaining normal mandibular ontogeny and the effect of a changing biomechanical environment on the modelling and remodelling of bone, this study evaluated changes in bone mineral density across the body of the immature mandible during the early stages of dental development (Chapter 4). It was hypothesized that the bone mineral density of the mandible would change in response to variations in the biomechanical loading associated with an increase in the functioning of the tongue as well as the development, emergence and eruption of the dentition.

Furthermore as the dentition was thought to have significant spatial and biomechanical influences on the morphology of the mandible, this study investigated differences in the morphology of the human mandible across the various developmental and functional states of the dentition. This was done as a means of ascertaining what the importance of the teeth are in maintaining the morphology of the adult mandible in humans (Chapter 5). It was hypothesized that the progression of edentulism would be significantly associated with the size and shape of the mandible, with a morphometric gradient from the fully dentate to the fully edentulous state.

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CHAPTER 2:

MATERIALS AND METHODS

As this thesis is being undertaken by publication, the specific methodologies applied to each study are outlined within the relevant section of the article (Chapters 3 to 5). However, an expanded explanation of the methodology, where appropriate, appears in this materials and methods section in order to explain the rationale for the methodology used in this study.

2.1 Materials

The study material consisted of 717 fetal, neonatal and adult mandibles sourced from cadaveric and skeletonized remains of all population groups, forming part of the Paediatric Collection and Raymond A. Dart Collection of Human Skeletons, School of Anatomical Sciences and the Johannesburg Forensic Paediatric Collection, Division of Forensic Medicine and Pathology, University of the Witwatersrand. Of these 174 paediatric mandibles were used in the evaluation of the growth relationship between the tongue and the mandible, 45 paediatric mandibles were analyzed in the assessment of variations in bone density and 498 adult mandibles were evaluated when investigating how the dentition influences the morphology of the mandible. The mandibles utilized were those of a modern South African population. Identical protocols were followed in processing the fresh individual remains from each collection. However, individuals showing evidence of damage to the mandible or tongue, congenital abnormalities, soft or bony tissue pathologies or surgical intervention, were excluded from the study. In addition, evidence of previous orthodontic treatment, such as lingual retainers, also led to exclusion from the study.

The use of mandibles sourced from the Raymond A. Dart Collection of Human Skeletons and the Paediatric Collection in the School of Anatomical Sciences was granted an ethical clearance waiver (W-CJ-140604-1) from the Human Research Ethics Committee (HREC-medical) (Appendix 1). In addition, full ethical clearance (M121145) was also obtained from the Human Research Ethics Committee (HREC-medical), owing to the forensic nature of part of the sample (Appendix 2).

A total of 174 fetal and neonatal cadavers were used in the assessment of the morphometric relationships of the immature mandible and tongue (Chapter 3). This study sample consisted of complete human cadaveric remains of unknown provenance donated under the Human Tissue Act 65 of 1983. In the assessment of variations in the bone density of the immature mandible a total of 45 fetal and neonatal mandibles were utilized, of these 28 mandibles were sourced from fresh cadaveric tissue of unknown provenance and 17 mandibles were from skeletonized individuals (Chapter 4).

The effects of the state of the dentition on the morphology of the mandible were assessed across 498 adult mandibles, which included 262 male and 236 female skeletons (Chapter 5). This study sample made use of skeletonized individuals accessioned into the Raymond A. Dart Collection of Human Skeletons between 1924 and 2003. As such, individuals accessioned in the early 1920s were born as early as 1851. Thus, due to the extensive time period covered and the possibility of specimens being included in this study from various stages of this time period, there is a potential for the influence of a secular trend. The concept of a secular trend is generally applied within the context of an overall increase or decrease in the dimensions of the body over a substantial period of time and has been widely discussed in growth related studies (Tobias, 1985; Henneberg & van den Berg, 1990; Martin & Danforth, 2009; Antoun *et al*., 2014). As the magnitude of trends across South Africans of varying origins is similar despite their substantial socioeconomic differences over time (Henneberg & van den Berg, 1990), the potential for the influence of a secular trend in the present study's sample is diminished.

A combination of donated and forensic cadavers as well as skeletonized samples comprised the sample used in this thesis. While the chronological ages of the skeletonized samples and donated cadavers were known, the forensic cadaveric samples had to be assigned an age estimate as there were no records available for these specimens. To standardize the age estimations of the individuals being studied across both sample types, the approximate age of each individual was estimated based on the stage of their dental development. Dental age estimates were determined from dental scans taken with a Nomad dental X-ray scanner and based

on dental age estimation standards proposed by Alqahtani *et al*. (2010). While dental age estimation standards for an African sample are currently being developed, it should be noted that the dental age estimation standards proposed by Alqahtani *et al*. (2010) are based on samples from individuals of Bangladeshi and White origin respectively.

2.1.1 Rationale for the use of Micro-focus Computed Tomography X-ray scanning

The study also investigated changes in the bone density across the body of the immature mandible (Chapter 4). While dual energy X-ray absorptiometry (DXA) is a favoured application across investigations of bone density (Henderson, 1997; Pierce *et al*., 2000; Henderson *et al*., 2002; Schreiber *et al*., 2014), the present study made use of micro-CT. Computed tomography scans have been applied more frequently to density assessments as they are more volumetric in nature, may be limited to specific regions of interest and conventionally make use of the Hounsfield unit scale (Schreiber *et al*., 2014). In contrast, dual energy X-ray absorptiometry is limited to larger areas of assessment such as the posterior elements of the spine, and makes use of planar measurements of density (Schreiber *et al*., 2014). The Hounsfield unit scale, which assesses density values between the air (-1000) and the bone (+1000) is considered to be a normalized index, representative of the radiodensity of distilled water (0) at standard pressure and temperature (Schreiber *et al*., 2014). This scale has gained popularity in modern radiology imaging as a result of the software permitting its calculation from a selected region of interest on CT scans (Schreiber *et al*., 2014). Furthermore, this type of assessment may be conducted in conjunction with other related CT assessments. The advantage of using this method is that there is no additional cost or added radiation exposure.

The use of Micro-CT scanning was selected, as this technique provides the facility to study internal architectural features of bone as well as higher resolution images than those produced by conventional CT as well as histomorphometry and microradiograhy (Mulder *et al*., 2004). Furthermore, Micro-CT scanning was preferred over conventional cone beam CT and general CT, due to its capacity to produce both

three-dimensional and two-dimensional reconstructions as well as volumetric measurements (Gielkens *et al*., 2008). While synchrotron micro-tomography is thought to be more accurate in the quantification of various bone properties, this technique is both time consuming and has limited availability, making micro-CT more accessible and appropriate for studies of both mineralized and immature bone types (Mulder *et al*., 2004). Furthermore, while the acquisition of volume data through the application of micro-CT is considered to be expensive and time consuming and often requires specialised software, the volume data sets generated by this technology offer a wealth of options in terms of data analysis (Weber *et al*., 2001). In addition, the volume data generated was suitable specifically for the assessment of variations in bone density across the immature human mandible (Chapter 4).

2.2 Methods

As this study assessed a combination of immature and adult mandibles, it was necessary to make use of a morphometrics approach (Weber *et al*., 2001; Franklin & Cardini, 2007; Slice, 2007; Hale *et al*., 2014; Algee-Hewitt & Wheat, 2016). Morphometrics assesses patterns of variation associated with both differences in size and shape (Slice, 2007). Therefore, it is a method suitable for understanding changes in the growth and development of the mandible as produced by the tongue (Chapter 3), as well as the effects of tooth loss on the morphology of the adult mandible (Chapter 5). Three-dimensional data was captured using the Immersion Microscribe G2 portable digitizer and a Nikon XTH 225L microfocus X-ray CT scanner. The selection of the digitizer and the scanner was attributed to the recent evolution of virtual anthropology, which has been directed towards the capturing of three-dimensional landmark and volumetric data, using sophisticated 3D scanners and portable landmark digitizers (Weber *et al*., 2001; Hale *et al*., 2014; Algee-Hewitt & Wheat, 2016).

The Immersion Microscribe G2 portable digitizer, used to capture 3D data across 21 pre-selected osteological landmarks (Chapters 3 and 5), was selected on the basis of its affordability, robusticity and portability. In addition, this digitizer is also considered to be suitable for the acquisition of surface landmark data (Franklin *et al*., 2006;

Franklin & Cardini, 2007; Slice, 2007). While three-dimensional laser scanning has over the years emerged as an alternative to portable digitizers, the methodological procedures associated with this type of scanning have yet to be standardized through the publication of disciplinary standards (Algee-Hewitt & Wheat, 2016).

2.2.1 Landmarks

A total of 21 osteological landmarks were primarily selected for the purposes of investigating the changes in the morphology of the mandible associated with the loss of the dentition (Chapter 5) or growth of the tongue (Chapter 3). These landmarks were selected as they represent areas of the mandible which are known to be significantly impacted by changes in growth as well as the level of masticatory function (Lee *et al*., 2001; Koolstra, 2002; Radlanski *et al*., 2002, 2003). In general, the present study made use of type I (points defined by a juxtaposition of different tissues *e.g.* symphysis menti), type II (landmarks defined as points on the maximum curvature or tips of processes *e.g.* antegonial notch) and type III (reflect extreme points that can only be defined relative to the geometry of an entire structure) landmarks. Types I and II landmarks are considered to be less prone to scanning and measurement error when compared to type III (*e.g.* glabella) landmarks (Algee-Hewitt & Wheat, 2016). In addition, to investigate the variations in the bone density across the body of the mandible, eight density points were selected along the lingual and buccal surfaces of the immature mandible (Chapter 4). These points were selected to evaluate changes in bone density relative to the alveolar and basal areas of the mandible (Jäger *et al*. 1990; Park *et al*. 2008; Hsu *et al*. 2011; Chang *et al*. 2012).

2.2.2 Data analysis

In the analysis of the generated three-dimensional landmark data, the study made use of a Generalized Procrustes Analysis (GPA) as well as a Principal Component Analysis (PCA). These analyses were conducted primarily for the purposes of assessing size and shape variations in the morphology of the mandible across the various states of the dentition (Chapter 5). While the application of co-ordinate data was suitable for the assessment of morphological data, the analysis of co-ordinate data is complicated. The co-ordinates must be captured with respect to a defined set

of co-ordinate axes and in a specific sequence. This may be attributed to the numerical values of each co-ordinate being a reflection of their location, and the size of the specimen being encoded in each recorded co-ordinate (Zelditch *et al*., 2004; Slice, 2007). To standardize the co-ordinate sets for location, orientation and size, the data was converted to reflect a set of distances between landmarks and registered to a common reference point (Zelditch *et al*., 2004; Slice, 2007). The standardization of the co-ordinate data was achieved by using a GPA.

A GPA is suitable for shape assessments, as it addresses the associated issues of translation, rotation and scaling of each specimen by squaring the summed distances between corresponding landmarks on each configuration (Slice, 2007; Mitterocker & Gunz, 2009). As such, specimens are individually scaled to a standard unit *i.e.* centroid size. This process standardizes the data by bringing the landmark configurations of all the specimens within the study sample into a common co-ordinate system, effectively superimposing the landmark configurations. Thus, differences in the values of landmark co-ordination reflect differences in configuration shapes (Zelditch *et al*., 2004; Slice, 2007). Furthermore, while the newly transformed configurations are all a common size, the scale factor used to standardize these configurations may be applied to subsequent size comparisons between samples (Slice, 2007). This allows for further size analyses using the generated landmark data.

In the overall analysis of shape, differences may be visualized either by means of a graphical representation such as a thin-plate spline (Chapter 5) or by means of quantifying the shape differences using Mosimann Shape variables (Chapter 3) (Mosimann, 1970; Zelditch *et al*., 2004). This study made use of both approaches. While the thin-plate splines gave an excellent visual representation of the changes in the shape of the mandible across the various dentition states (Zelditch *et al*., 2004), the Mosimann shape variables assisted in quantifying the difference in shape across the morphometric growth relationships of the mandible and tongue (Mosimann, 1970; Jungers *et al*., 1995). Mosimann shape variables were used as they adjust the differences in scale between individuals by dividing the measurement value by the calculated geometric mean. This effectively facilitates the assessment of shape

relative to size (Mosimann, 1970; Jungers *et al*., 1995). In general the Mosimann shape variables gave a sense of how significant the changes in shape were across the various assessments. In addition, to further control for any potential size influences on the shape comparisons, a multivariate analysis of covariance (MANCOVA) was conducted, with the geometric mean being identified as the covariate (Riffenberg, 2006). In adopting this approach, the potential influences of size were as much as possible alleviated from the shape analysis.

The sample was also analyzed for changes in the size of the mandible relative to variations in the state of the dentition, subsequent to the assessment of the changes in shape (Chapter 5). The geometric mean was used to indicate the overall size of a particular specimen relative to the other individuals within the sample. Furthermore, changes in the size dimensions of the mandible relative to the state of the dentition were also evaluated using a series of osteological measurements. These measurements and the geometric mean were then examined using a multivariate analysis of variance (MANOVA). This type of analysis is recommended for data derived from landmark co-ordinates (Slice, 2007). A MANOVA is also recommended for testing the interaction between variables (Zar, 1999), and is useful in assessing which areas of the mandible are significantly affected by the state of the dentition (Chapter 5).

In addition, the sample was subjected to a PCA. This analysis was conducted to investigate the level of covariance in the sample between variables such as age relative to a set parameter *e.g.* size. Alternatively the PCA also served to estimate the correlation between age groups with reference to size and shape. In general, a PCA is a dimension reduction technique, which aims to identify orthogonal linear combinations associated with the original variable that are most likely to reflect the sample variability (Slice, 2007). Owing to its value as an indicator of both covariance as well as correlations, the PCA was conducted across all three sub-investigations of the current study.

Detailed geometric morphometric analyses were necessary for extrapolating information about the influences of the growth of the tongue (Chapter 3) and for the effect of a change in the state of the dentition on the morphology of the mandible (Chapter 5). However, it was important to ascertain if either age or the state of the dentition influenced the changes observed in the morphology of the mandible. Thus, a two-way analysis of variance (ANOVA) was conducted (Chapter 5). This analysis would assess if the changes observed in the morphology of the mandible were the result of general aging or if the state of the dentition was the primary factor in determining mandibular form (Zar, 1999). The two-way ANOVA was also performed in the assessment of variations in the bone density of the immature mandible (Chapter 4). An alternative method of assessing this type of relationship would be to run a pairwise comparison. However, a two-way ANOVA is more sensitive to potential interactions between the various parameters. A one-way analysis of variance and a linear correlation analysis were also conducted when the morphometric relationships of the tongue and mandible were assessed (Chapter 3).

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CHAPTER 3:

MORPHOMETRIC GROWTH RELATIONSHIPS OF THE IMMATURE HUMAN MANDIBLE AND TONGUE

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European Journal of Oral Sciences 2014; 122:181-189 (DOI: 10.1111/eos.12126)
(Appendix 3)

3.1 Abstract:

The masticatory apparatus is a highly adaptive musculoskeletal complex comprising several relatively independent structural components, which assist in functions including feeding and breathing. We hypothesized that the tongue was elemental in the maintenance of normal ontogeny of the mandible and in its post-natal growth and development and tested it using a morphometric approach. We assessed tongue and mandibular measurements in 174 (97 male) human cadavers. Landmark lingual and mandibular data were gathered individuals aged between 20 gestational weeks and 3 years postnatal. In this analysis, geometric morphometrics assisted in visualizing the morphometrical growth changes in the mandible and tongue. A linear correlation in conjunction with principal component analysis further visualized the growth relationship between these structures. We found the growth of the tongue and mandible were intrinsically linked in size and shape between 20 gestational weeks and 24 months postnatal. However, the mandible continued to change in shape and size into the 3rd year of life, whereas the tongue only increased in size over this same period of time. These findings provide valuable insights into the allometric growth relationship between these structures, potentially assisting the clinician in predicting the behaviour of these structures in the assessment of malocclusions.

Key Words: Tongue, mandible, development, principal component analysis

3.2 Introduction

The masticatory apparatus is a highly adaptive musculoskeletal complex comprising several relatively independent structural and functional components (Atchley & Hall, 1991; Koski, 2007). This complex of structures collectively assists in the functions of feeding, phonation, swallowing and breathing (Humphrey, 1971; Coquerelle *et al*., 2013). Understanding how the structure and function of the craniofacial elements, such as bone, muscle and cartilage change during late fetal development is a topic of considerable interest to biologists and clinicians alike (Chin & Werb, 1997; Nie *et al*., 2006; Fukase, 2012). In recent times, molecular biology has provided much needed knowledge regarding mechanisms of development through the identification of genes and growth factors (Chin & Werb, 1997; Martinez-Maza et al., 2012; Boell, 2013; Boell et al., 2013). However, morphological studies have proven to be a valuable approach for understanding the relative roles of growth over time (Radlanski *et al*., 2002; 2003). Morphological studies have placed great emphasis on the interactions of the various masticatory structures in older age cohorts (Lee *et al*., 2001; Alqahtani *et al*., 2010; Hutchinson, 2010; Melink *et al*., 2010; Hutchinson *et al*., 2012), leaving a significant knowledge gap in relation to the earlier stages of development. We have attempted to address this gap by investigating the static allometry of the tongue and mandible during the morphologically significant growth period of 20 gestational weeks to 3 years of life. This period is particularly significant as it involves the transition from a prenatal to postnatal environment, which inherently introduces different biomechanical interactions associated with suckling, further tooth development and tooth eruption and increasing degrees of mastication. Furthermore the current study is innovative in its direct assessment of the relationship between the tongue and mandible and considers the effects of the muscles of mastication and the growth of the cranial base on the allometric growth relationship of the mandible and tongue.

The early morphological growth and development of the mandible as a central component of the masticatory apparatus has been extensively studied. Lee *et al.* (2001) considered the sequential growth pattern of the human mandible and observed the radiating expansion of the mandible from the primary growth centre as early as the fifth week of development (Lee *et al*., 2001). The ossification of the

mandible commences at the sixth week of development (Radlanski *et al*., 2002, 2003) with insular cartilages between weeks 10 and 14 near the posterior region of Meckel's cartilage. The mandible alters its morphology to accommodate the development of the deciduous dentition and the increase in mastication between 30 weeks of gestation and 3 years of age (Hutchinson, 2010; Hutchinson *et al*., 2012). However, these studies, although alluding to the potential influence of the tongue on the normal growth of the mandible, did not elaborate further on this fundamental relationship.

The relative roles and relationships of the masticatory apparatus within the developing oral cavity are of clinical importance in understanding the aetiology of various cases of malocclusion. The emergence and eruption of the posterior permanent dentition, which in some cases may occur as early as 4.5 years of age (Alqahtani *et al*., 2010), presents the clinician with the possibility of a posterior cross-bite malocclusion (Melink *et al*., 2010). This type of malocclusion not only is considered as the most prevalent in relation to the deciduous dentition, but may result in facial asymmetry and temporomandibular disorders later in adulthood if left undiagnosed (Melink *et al*., 2010). One of the suggested diagnostic criteria for a posterior cross-bite malocclusion is a short lingual frenulum, which results in the tongue assuming a lower position within the oral cavity (Melink *et al*., 2010). This change in tongue posture leads to a disturbance in the dynamic equilibrium of the oral cavity and possibly manifests later as a narrow and shortened maxilla (Swinehart, 1950; Lowe *et al*., 1985; Melink *et al*., 2010). Although there is a suspected link between the hard and soft tissues of the oral cavity in the aetiology of cases of malocclusion, most of these investigations have been pursued in older age-cohorts.

In an early study Swinehart (1950) summarized the relationship between the growth of the tongue relative to the mandible in patients of malocclusion and concluded that the tongue exerted considerable expansive forces on the mandibular arch during growth. However, Swinehart (1950) postulated that these forces only had a significant effect on the normal occlusion when the tongue had completed its descent in the oral cavity (Swinehart, 1950). Lowe *et al*. (1985) further reinforced these

hypotheses by investigating the role of the tongue in malocclusions in adults. He concluded that not only was the position of the tongue a significant variable in establishing a normal occlusion pattern, but that the degree of tongue protrusion relative to mandibular rotation was also significant (Lowe *et al*., 1985). Whilst these studies addressed the potential role of the tongue in cases of malocclusion, they did not consider the normal prenatal and postnatal interactions between the involved components.

Prenatal and postnatal interactions between the tongue and the mandible are particularly significant in the understanding and subsequent prevention and treatment of cases of maldevelopment. Developmental abnormalities associated with the masticatory complex vary considerably, from extremely rare cases of aglossia to the more common cases of bifid tongue (Kuroda & Ohyama, 1981; Sinn *et al*., 1981; Frohberg & Lange, 1993; Chidzonga *et al*., 1996; Britto *et al*., 2000; Salles *et al*., 2008). Whilst extensive literature exists describing the abnormalities of the masticatory apparatus, few studies have explained the relationship between the tongue and mandible in any of the assessed patients. This would be important in understanding clinical conditions such as malocclusions, and micrognathia. The possible association of microglossia with glossoptosis and the possibility of micrognathia being outgrown later in life (Sinn *et al*., 1981), raise the question of the tongue's influence in the normal growth of the mandible. Furthermore cases of aglossia and hypoglossia are accompanied by retarded development of the mandible and teeth (Kuroda & Ohyama, 1981; Salles *et al*., 2008). This emphasizes the potentially significant role of the tongue in the general growth of the oral cavity.

Based on the results of earlier studies (Swinehart, 1950; Kuroda & Ohyama, 1981; Lowe *et al*., 1985; Salles *et al*., 2008; Hutchinson, 2010; Hutchinson *et al*., 2012), we hypothesized that the tongue is elemental not only in maintaining normal mandibular ontogeny, but also in its post-natal growth and development. To test this hypothesis, we utilized a morphometric approach. First, we used fetal cadavers to determine the changes in the maximum length and breadth dimensions of the tongue associated with growth. Second, we measured landmark data of the external surface of the mandible in individuals aged between 20 weeks of gestation and 3 years postnatally.

In this analysis we used principal component analysis (PCA) to visualize the morphometrical changes in the mandible and tongue that are associated with growth. Additionally a linear correlation analysis was used in conjunction with PCA to further visualize the growth relationship *i.e.* changes in size and shape between the tongue and mandible within the sample.

3.3 Materials and Methods

3.3.1 Materials

Fetal and neonatal human cadavers were sourced from the Paediatric Collection, School of Anatomical Sciences, University of the Witwatersrand. The study sample consisted of complete human cadaveric remains of unknown provenance donated under the Human Tissue Act 65 of 1983. In addition, ethical clearance (M121145) was obtained from the Human Research Ethics Committee (HREC) (Appendices 1 and 2). The mandibles of 97 male and 77 female cadavers were assessed (Table 1). Specimens ranged in age from 20 weeks of gestation to 3 years postnatally. Ages were determined using dental age standards proposed by Alqahtani *et al*. (2010). In addition, standard body measurements (*e.g*. crown-rump length, toe-heel length, crown-heel length and head circumference), were also utilized in the determination of age (Lubchenco *et al*., 1966; Fenton, 2003; Hansen *et al*., 2003). The sample was sub-divided into four groups: group 1 (20-30 weeks of gestation), group 2 (31-40 weeks of gestation), group 3 (0-12 months postnatal) and group 4 (12.5-36 months postnatal). Specimens were further classified, according to the state of the dentition, as dentulous, partially edentulous and edentulous (Table 1) (Hutchinson *et al*., 2012).

Table 1. The age, sex and dental state of the specimens sourced from the School of Anatomical Sciences, University of the Witwatersrand.

	Males	Females	Dentulous	Partially Edentulous	Edentulous
Group 1 (n=41) 20 - 30 wks	20	21	0	0	41
Group 2 (n= 60) 30.5 - 40 wks	36	24	0	0	60
Group 3 (n=56) 0 - 12 mnths	32	24	0	10	46
Group 4 (n=17) 12.5 - 36 mnths	9	8	7	10	0
Total	97	77	7	20	147

Individuals showing evidence of damage to the mandible or tongue, congenital abnormalities, soft- or bony-tissue pathologies or surgical intervention, were excluded from the study.

Cadavers were fixed in 5% formalin. This solution has been shown to have no significant level of tissue shrinkage and therefore correction for shrinkage was not undertaken (Blanton & Biggs, 1980; Boonstra *et al*., 1983).

3.3.2 Methods

Soft-tissue dissection was performed to expose the antero-lateral surface as well as the inferior border of the mandible (Fig 1). The tissues were reflected in order to preserve the overlying soft tissues for future studies.

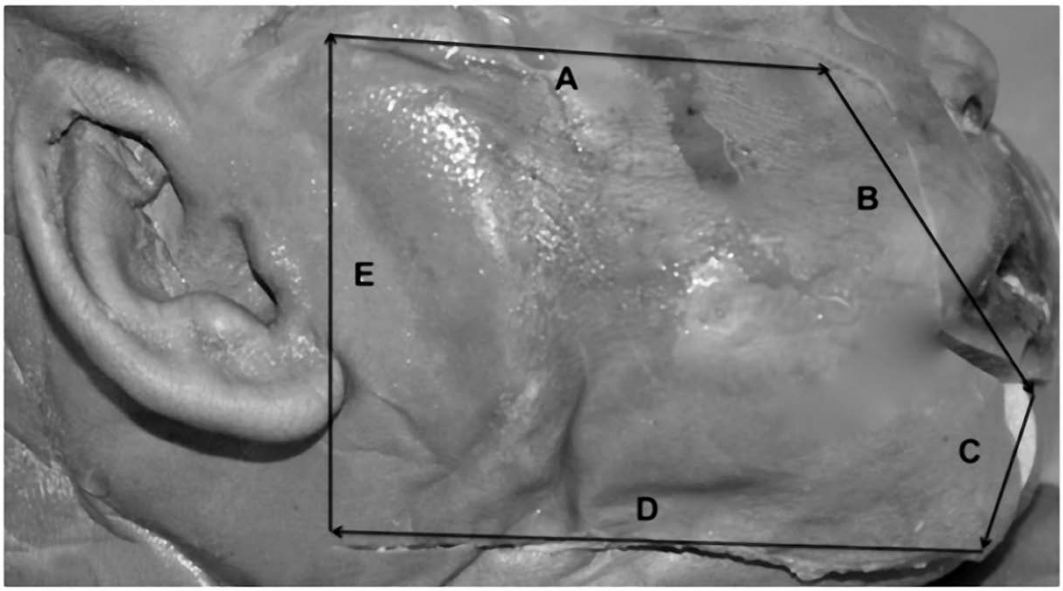


Figure 1: Planned incision lines A-E for dissection of a neonate, lateral view (Hutchinson *et al*., 2012)

In cadavers where the mouth could not be opened, a series of incisions were performed in order to maintain the position of the tongue relative to the mandible. The first incision was made along the capsule of the temporomandibular joint. The mandible was not removed from the specimen as the attachments of the lateral and medial pterygoid muscles were kept intact. There was no further dissection of the oral cavity.

An Immersion Microscribe G2 (Immersion, San Jose, CA, USA), with an accuracy of 0.38mm, was used to record 21 homologous landmarks on the mandible (Fig 2). These landmarks included the points necessary to calculate the linear measurements and to define the shape of the mandible. Landmark points were digitized on the mandible *in situ* and were recorded in a pre-set sequence (clockwise direction).

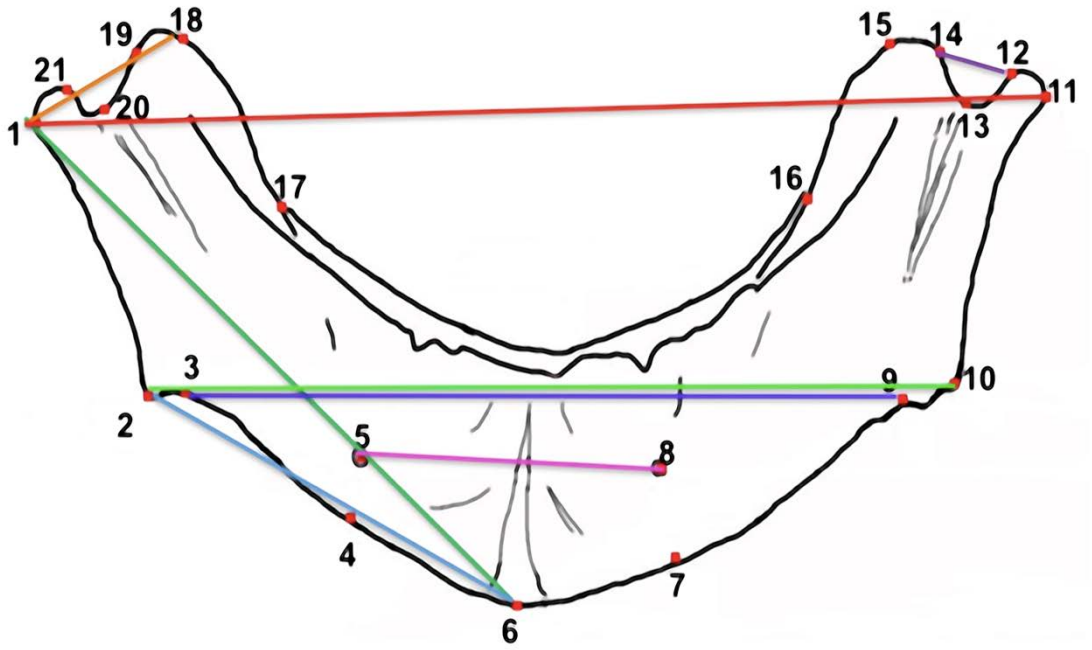


Figure 2: Diagrammatic view of the whole mandible from an anterior position indicating the 21 homologous landmarks. Lines indicate measurements: Mandibular body length (light blue), maximum length of the mandible (dark green), bicondylar width (red), mandibular basilar width bigonial (light green), mandibular basilar width biantegonial (dark blue), mental interforaminal distance (pink), notch width (purple) and maximum width of the mandible (orange) (Hutchinson *et al*., 2012).

Using each landmark, three coordinates (X, Y, Z) were recorded and represent the horizontal, vertical and depth planes, respectively. The three-dimensional coordinates were then imported into a Microsoft Excel worksheet and the Pythagorean formula was used to convert the data into linear distances:

$$= \sqrt{(x_1-x_2)^2 + (y_1-y_2)^2 + (z_1-z_2)^2}$$

where x_1, y_1, z_1 and x_2, y_2, z_2 refer to the coordinates of the first and second landmarks respectively.

Eight standard anthropometric measurements derived from De Villiers (1968), Loth (1995), Scheuer & Black (2000), Hesby *et al*. (2006), White et al. (2011) and Hutchinson *et al.* (2012) were recorded using the previously digitized points. In

addition, the maximum bicondylar width was recorded according to a procedure specifically designed for the purposes of this study. From these measurements, morphological changes of the mandible and tongue in the sagittal and coronal planes were derived.

Measurements assessing changes in the sagittal plane included the mandibular body length (measured from the mental tubercle to the angle of the mandible) and the maximum length of the mandible (measured from the mental tubercle to the posterior tip of the mandibular condyle) (Loth, 1995). In the assessment of the maximum length and mandibular body length measurements, the most anterior point on the mental symphysis cartilage ridge was used as the reference point in unfused mandibles. Additionally, the mandibular notch width (measured from the most posterior tip of the coronoid process to the most anterior tip of the condylar process) (De Villiers, 1968), and maximum width of the mandible (measured between the most anterior point on the coronoid process and the most posterior point on the condyle of the mandible) (Hutchinson, 2010; Scheuer & Black, 2000) were also assessed.

Measurements assessing changes in the coronal plane included the mandibular basilar bigonial width, the width of the mandibular arch between the mandibular angles (the point where posterior and inferior borders meet), as well as the mandibular basilar biantegonial width (the width of the mandibular arch from the bilateral points of the most superior point of the antegonial notch) (Hesby *et al*., 2006). Additionally, the mental interforaminal distance was measured between the two mental foramina on the anterior surface of the mandible (De Villiers, 1968), the maximum bicondylar width was the distance measured between the most lateral points on the lateral surfaces of the mandibular condyles and the mental angle, which was formed at the mental symphysis between the mandibular bodies of both hemi-mandibles (Hutchinson, 2010), was also assessed.

The measurements used to calculate the mental angle included the mandibular body length of each side (a+b) as well as the mandibular basilar width bigonial (c). The cosine rule was then used for mental angle calculation, as the triangle formed by the

measurements was not considered to be a right-angled triangle; thus, the Pythagorean equation could not be used.

The mental angle was calculated as follows:

$$CosC = (a^2 + b^2 - c^2)/(2ab)$$

where a and *b* represent the left and right mandibular body lengths, respectively; *c* represents the mandibular basilar width bigonial, and C represents the calculated mental angle.

A sliding caliper (0.02-mm accuracy) was used for measuring the tongue. The tongue was extended to flatten out its natural curvature, conventionally induced by the confined space of the oral cavity. Three measurements were used to assess the dimensions of the tongue relative to the mandibular arch in both the sagittal and coronal planes respectively.

Measurements in the sagittal plane included the whole tongue length (measured from the tip of the tongue anteriorly to the posteriorly located median glossoepiglottic fold at the junction of the root of the tongue and epiglottis) and the anterior tongue length (measured from the tip of the tongue anteriorly to the opening of the foramen cecum). Measurements in the coronal plane included the maximum breadth of the tongue (measured just anterior to the laterally located palatoglossal arches). All tongue measurements were made on the dorsal surface of the tongue.

3.3.3 Data analysis

Descriptive statistics, including mean and SD, were calculated for all mandibular and soft tissue (tongue) measurements. The distribution of the sample was tested for normality using the Shapiro-Wilk's test and further analyses were based on this result

(Zar, 1999). All statistical analyses were conducted using the PAST (Paleontological Statistics) statistical program (Hammer *et al*., 2001).

ANOVA was performed using the calculated geometric mean, to assess variations in the dimensions of the tongue and mandible relative to age. The geometric mean assesses the total mean of all values associated with a specific individual and establishes the overall standing of an individual's size relative to the rest of the sample (Mosimann, 1970; Jungers *et al*., 1995). Between- and within-group variances were represented by the *F*-ratio, which indicated the degree of similarity in the means. A MANOVA was performed using the calculated Mosimann shape variables. The Mosimann shape variables were used as they alter the differences in scale between individuals by dividing each measurement value by the geometric mean, so that shape may be assessed relatively independently of size (Mosimann, 1970; Jungers *et al*., 1995). The use of parametric statistics in conjunction with the geometric mean and Mosimann shape variables was performed to correct for possible inferior distribution properties associated with these analyses (Jungers *et al*., 1995).

The relationship between the tongue and the mandible was further assessed using a linear correlation coefficient. A significance level of alpha<0.05 was considered statistically significant and all correlation values of >0.7 were considered significant.

To visualize the differences in the size and shape of the mandible and tongue with growth, the geometric mean and Mosimann shape variables were independently subjected to a PCA using the PAST (Paleontological Statistics) statistical program (Hammer *et al*., 2001).

Inter- and intra-observer errors were evaluated using 10 specimens and then tested using the Lin's concordance correlation co-efficient of reproducibility, which provides information on the level of repeatability of the method and the degree of association between measurements (Allan, 1982).

3.4 Results

Analysis of the mean and SD of the measurements of the sample showed a general increase in the size of the mandible and tongue in all dimensions, except for the calculated mental angle, across groups 1-4 (Table 2). As no significant differences were observed between measurements on the left and right sides, the mean values of the right and left sides are given. The distribution of the sample data was observed to be normal.

The relationship between the tongue and the mandible was first tested, in terms of size, by both ANOVA and PCA. Both the tongue and mandible showed statistically significant increases in size in all group comparisons (Table 3). This growth relationship was further emphasized by the high degree of overlap across the first three groups of the size-associated PC1 in both the mandible (Fig 3a) and the tongue (Fig 3b). The high degree of overlap indicated a consistent degree of growth in the lingual and mandibular dimensions across the prenatal, perinatal and postnatal age ranges. Additionally the growth relationship between the tongue and the mandible was further emphasized by the significant degree of overlap between groups 3 and 4 (Fig 3a,b). Furthermore, the principal component plots emphasized the effect of the significant size increases observed in the tongue and the mandible in the particular dimensions across the entire sample (Fig 3a,b).

Table 2: Growth increases in the sagittal and coronal plane dimensions of the tongue and mandible between 20 gestational weeks and 36 months postnatal. Values are given in mm for each age group, mean±SD (range) indicating an overall increase in all tongue and mandible dimensions except the mental angle.

Measurement	Group 1 (n=41): (20-30 wks)	Group 2 (n=60): (30.5-40 wks)	Group 3 (n=56): (0-12 mnths)	Group 4 (n=17): (12.5-36 mnths)
MXTL	25.07 ±2.82	30.87 ±4.84	33.44 ±5.39	37.3 ±3.74
MNTL	21.99 ±2.56	27.63 ±4.20	29.31 ±5.46	32.83 ±3.72
TB	21.42 ±2.12	25.75 ±3.08	29.60 ±4.31	35.2 ±4.48
MBS_BG	34.12 ±3.39	39.25 ±4.16	48.24 ±5.83	58.42 ±7.96
MBS_BAG	30.21 ±3.26	34.80 ±3.73	43.34 ±3.14	53.58 ±7.51
BWMF	21.02 ±2.43	24.84 ±2.83	29.74 ±3.14	34.11 ±3.59
MBL	29.30 ±2.82	34.57 ±3.05	42.35 ±4.46	51.91 ±7.35
LLM	38.45 ±3.09	45.18 ±3.88	56.15 ±6.98	72.31 ±10.77
MW	13.70 ±1.93	15.64 ±2.07	20.10 ±3.72	25.88 ±4.58
NW	6.67 ±0.95	7.36 ±1.24	9.70 ±2.24	13.29 ±2.69
MEA	71.42 ±6.29	69.24 ±6.15	69.45 ±5.50	68.46 ±2.98
MBW	45.20 ±3.37	52.91 ±4.62	62.43 ±6.24	74.42 ±9.87

MXTL, maximum tongue length; MNTL, minimum tongue length; TB, tongue breadth; MBS_BG, mandibular basilar width bigonial; MBS_BAG, mandibular basilar width biantegonial; BWMF, mental interforaminal distance; MBL, mandibular body length; LLM, maximum length of the mandible; MW, maximum width of the mandible; NW, width of the mandibular notch; MEA, mental angle; MBW, maximum bicondylar width.

Table 3. Multivariate analysis of variance (MANOVA) comparison between tongue and mandible measurements using the calculated Mosimann shape variable for groups 1 to 4. Differences observed within the mandible between groups 1 to 4 were substantially more significant when compared to those within the tongue. Comparisons between tongue groups 1 and 3 and 3 and 4 all indicated differences, which were not considered to be statistically significant in terms of shape.

	ANOVA (Mandible)	**ANOVA (Tongue)**
Tukey's pairwise comparison	***Q/p(same)**	***Q/p(same)**
Group 1 vs. Group 2	6.13 (9.2 E-05)	7.79 (8 E-06)
Group 1 vs. Group 3	16.96 (8 E-06)	11.84 (8 E-06)
Group 1 vs. Group 4	31.12 (8 E-06)	18.32 (8 E-06)
Group 2 vs. Group 3	10.84 (8 E-06)	4.04 (0.02206)
Group 2 vs. Group 4	24.99 (8 E-06)	10.53 (8 E-06)
Group 3 vs. Group 4	14.16 (8 E-06)	6.48 (3.4 E-05)
Within group variance (F ratio)	150.5	52.9

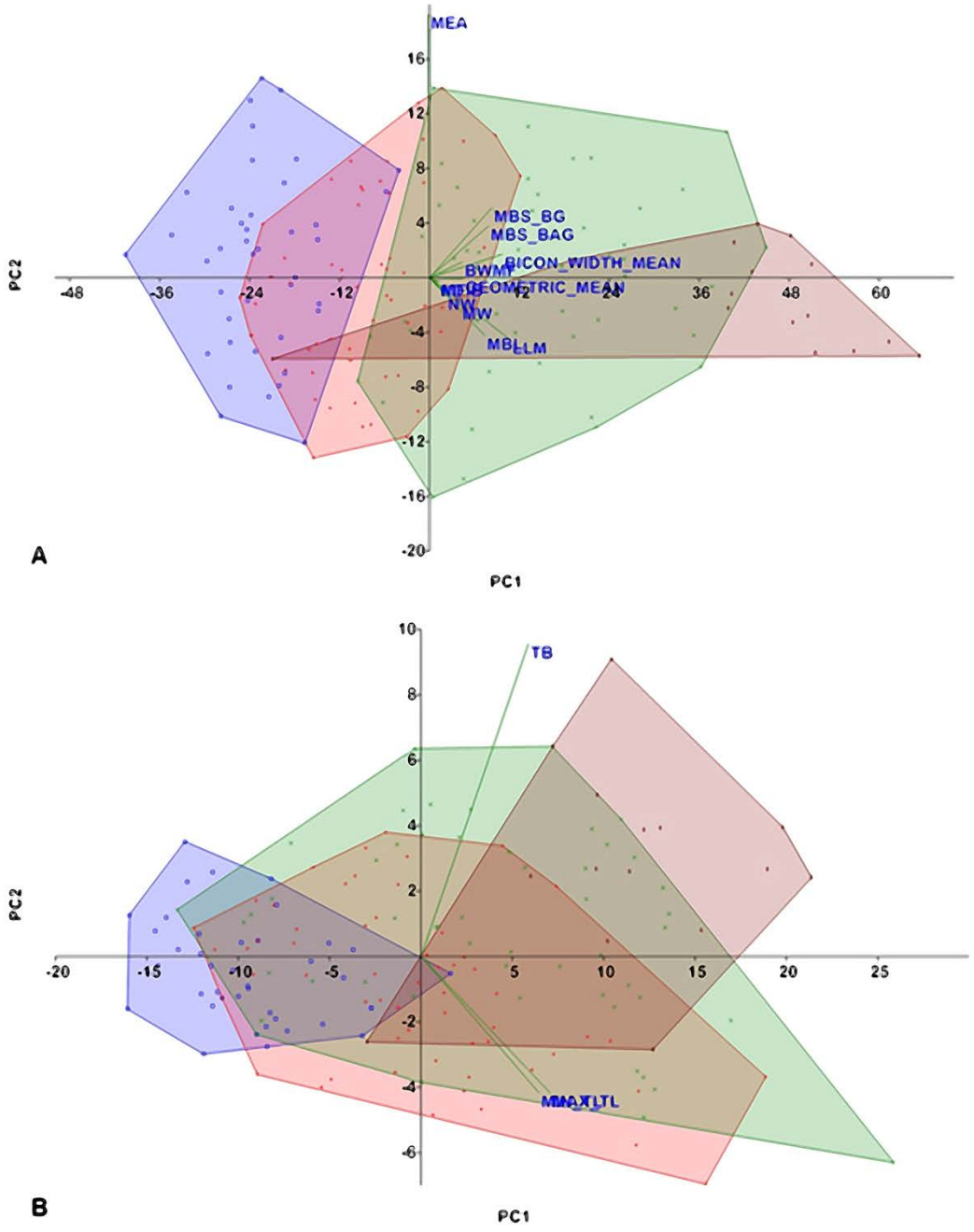


Figure 3: Principal component analysis assessing the level of size variance for the mandible (a) and tongue (b), between each of the four groups, group 1 (blue: 20-30 gestational weeks), group 2 (red: 31-40 gestational weeks), group 3 (green: 0-12 months) and group 4 (brown: 12.5-36 months). Plots illustrated the effect of each measurement on PC1 and PC2.

The mandible and the tongue were found to lengthen significantly along the sagittal plane, in all age groups (Fig 3a,b). The mandibular width also increased slightly, indicating a widening in the mandibular notch area. The mandible was also observed to widen in the coronal plane where a significant increase in the maximum bicondylar width was observed over time (Fig 3a). The posterior region of the mandible increased in width in the gonial region, where increases were observed in the mandibular basilar width bigonial and the biantegonial width. Tongue breadth increased at the same time (Fig 3a,b). In the anterior region of the mandible, the mental angle and mental interforaminal distance showed a very small degree of widening (Fig 3a).

The relationship between the tongue and mandible was then tested in terms of shape by using the Mosimann shape variables in both the MANOVA and PCA. Both the tongue and the mandible showed significant alterations in shape, across all age groups (Table 4). The lingual dimensions showed a statistically significant increase between groups 1 and 2, as well as between groups 2 and 3; however, no such increase in the dimensions of the shape of the tongue was observed between groups 3 and 4. This is in contrast to the mandible, which underwent significant increases in the dimensions of shape across all age groups (Table 4).

Significant increases in the dimensions of the shape of the mandible and tongue were observed by MANOVA as a result of the significant degree of overlap in the tongue and mandible assessments across groups 1 to 3 of the PCA. A lesser degree of overlap was observed between groups 3 and 4 (Fig 4a,b). Additionally, the mental angle exerted a significant degree of influence on the developing mandible (Fig 4a,b).

Table 4. Multivariate analysis of variance (MANOVA) comparison between tongue and mandible measurements using the calculated Mosimann shape variable for groups 1 to 4. Differences observed within the mandible between groups 1 to 4 were substantially more significant when compared to those within the tongue. Comparisons between tongue groups 1 and 3 and 3 and 4 all indicated differences which were not considered to be statistically significant in terms of shape.

	MANOVA (Mandible)	**MANOVA (Tongue)**
Group 1 vs. Group 2	1.34 E-12	0.0275
Group 1 vs. Group 3	1.55 E-24	0.0567
Group 1 vs. Group 4	2.85 E-16	0.0025
Group 2 vs. Group 3	3.45 E-12	0.0017
Group 2 vs. Group 4	1.98 E-15	0.0009
Group 3 vs. Group 4	2.12 E-06	0.1676

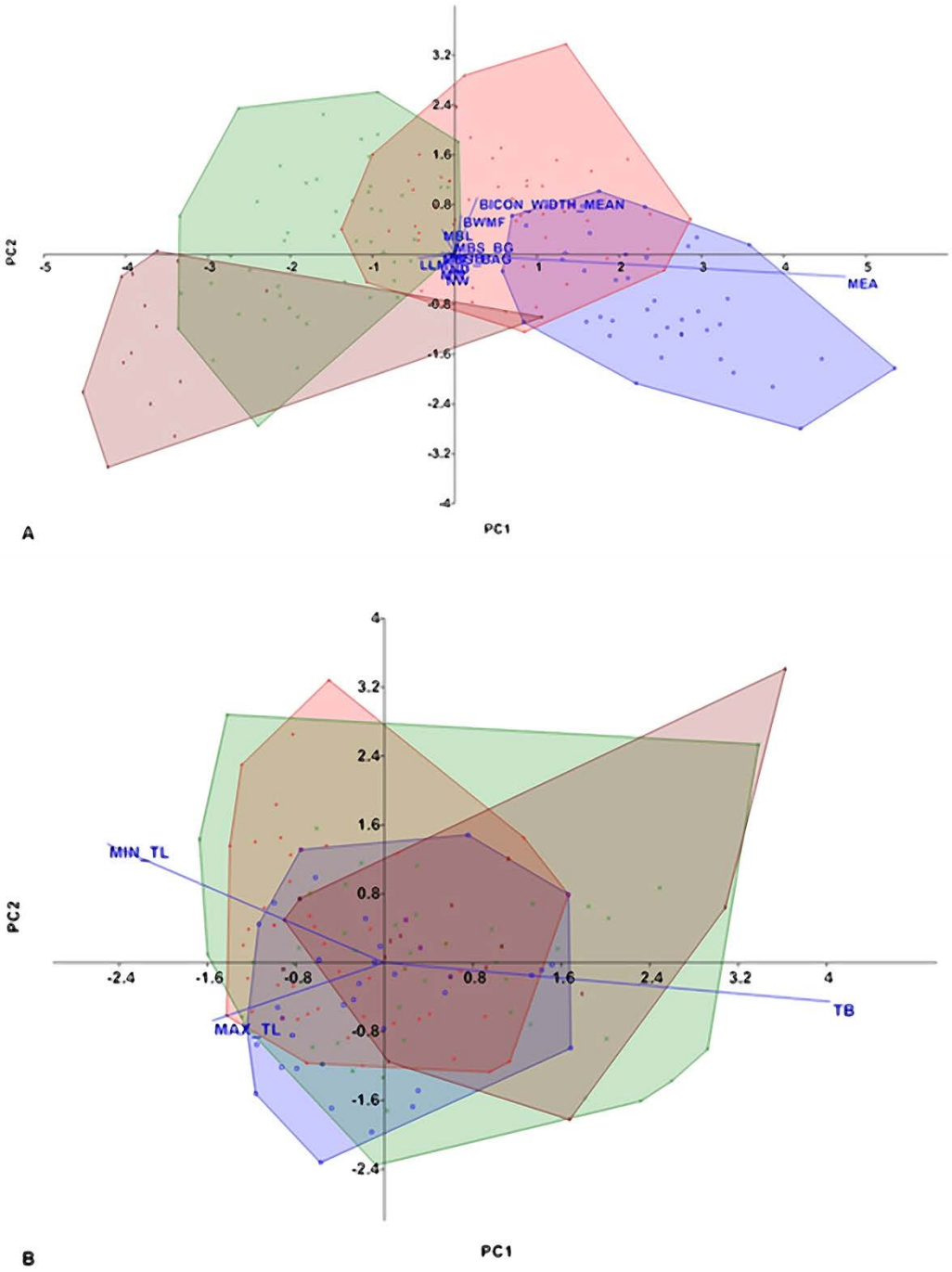


Figure 4: Principal component analysis assessing the level of shape variance for the mandible (a) and tongue (b), between each of the four groups, group 1 (blue: 20-30 gestational weeks), group 2 (red: 31-40 gestational weeks), group 3 (green: 0-12 months) and group 4 (brown: 12.5-36 months). Plots illustrated the effect of each measurement on PC1 and PC2.

The plot of the mental angle indicated a major change in the shape of the mandible anteriorly. The overall lengthening of the mandible in the sagittal plane resulted in the ramus of the mandible assuming a more upright position as observed in the decrease in the dimensions of the mandibular body length and the maximum length of the mandible. No major change was observed in the tongue length dimension over the same period of time. The decrease in the tongue breadth, and in the mandibular basilar width bigonial and biantegonial dimensions, indicated a flattening of the tongue in the coronal plane coupled with a small degree of eversion in the gonial region of the mandible (Fig 4a,b). The significance of the overall relationship observed between the mandible and the tongue was further clarified in the linear correlation assessment (Table 5), with strong correlations being observed in tongue breadth and in the posteriorly assessed dimensions of the mandible.

The significant degree of correlation observed between the minimum and maximum tongue length and breadth indicated that this organ increased its length at a proportional rate to its breadth. The tongue breadth was also observed to widen at a very similar rate to the observed widening of the maximum bicondylar width. Furthermore, as the tongue lengthened, the posterior bicondylar region of the mandible widened, and a small widening was also observed in the mental interforaminal distance (Table 5).

Table 5. Linear correlation analysis between tongue and mandible measurements across groups 1 to 4. Values above 0.7 indicated a high degree of correlation. This was observed predominantly in the coronal plane measures, alternatively good to moderate correlations were observed in the sagittal plane measures.

	MXTL	MNTL	TB
MBL	0.656	0.616	0.809
LLM	0.649	0.611	0.809
MW	0.575	0.539	0.724
NW	0.518	0.485	0.669
MBS_BG	0.674	0.644	0.834
MBS_BAG	0.662	0.637	0.834
BWMF	0.694	0.678	0.803
MBW	0.699	0.674	0.864
MEA	0.015	0.040	0.038
MXTL		0.962	0.806

MXTL, maximum tongue length; MNTL, minimum tongue length; TB, tongue breadth; MBS_BG, mandibular basilar width bigonial; MBS_BAG, mandibular basilar width biantegonial; BWMF, mental interforaminal distance; MBL, mandibular body length; LLM, maximum length of the mandible; MW, maximum width of the mandible; NW, width of the mandibular notch; MEA, mental angle; MBW, maximum bicondylar width.

3.5 Discussion

Recently, attention has focused on the molecular aspects of mandibular development (Martinez-Maza *et al*., 2012; Boell, 2013), and especially on the fundamental role of metallo- proteinases in craniofacial matrix development (Chin & Werb, 1997). By contrast, few current studies have investigated morphological patterns in development and growth of the lingual-mandibular complex during the late trimester and early postpartum period. However, Barron *et al*. (2011) have recently produced interesting molecular data illustrating that the transciption factor, Hand2, plays a key role in mandibular arch development, which also initiates morphogenesis of the tongue mesenchyme (Barron *et al*., 2011). Previously, it was suggested that the tongue was elemental not only in maintaining normal mandibular ontogeny but also in its post-natal growth and development (Swinehart, 1950; Lowe *et al*., 1985; Coquerelle *et al*., 2013). To test our hypothesis that the tongue plays a crucial role not only in determining shape and attachment sites of individual jaw muscles but also in the evolution of complex bone architecture, we performed a series of comparative analyses using cadaveric human fetuses. We found that the tongue and mandible were intrinsically linked *i.e.* showed a positive correlation in the allometric growth relationship up to the second year of life. During the third year of life, no further significant changes took place in the morphology of the tongue, in contrast to the changes in the overall size and shape of the mandible. This contrast in growth patterns between the tongue and the mandible may be the result of increasing biomechanical and space demands on the mandible from the emerging deciduous dentition as well as the successive developing permanent dentition during this time (Alfaqeeh *et al*., 2013).

We used the geometric mean and Mosimann shape variables as tools to visualize the effects of variations in size and shape in the sample. By subjecting each of these variables to a PCA, with the main focus of each PCA being either size or shape, we effectively studied the influence of size and shape as independently as possible. This process of analysis further enabled us to visualize the influence of each of the assessed measurements on the size and shape changes of the sample with the aid of loading values obtained from the PCA. Significant age-related size increases were observed in both the mandible and the tongue in individuals aged between 20 weeks

of gestation and 3 years postnatally. The size increases were emphasized in our study by increases in the posterior region of the mandible, which may be associated with an increase in the width of the cranial base (Swinehart, 1950; Nie, 2005) or the lateral expansion of the tongue, as shown in the present study. Mandibular growth and position relative to the posterior cranial base may be influenced by variations in the growth rate, length and inclination of the posterior cranial base components (Moss & Rankow, 1968; Nie, 2005). The mandibular condyle, as a principle centre of growth of the mandible, through its association with the mandibular fossa of the temporal bone from 14 weeks of development, will grow synchronously with the rapid lateral expansion of this region (Moss & Rankow, 1968; Nie, 2005). This is observed in the current study in the significant increase in the bicondylar width of the mandible. There is, at this stage a strong correlation between the growth of the posterior cranial base components and the lateral expansion of the mandible. However, it should also be considered that there may be additional influences from the anterior cranial base, in terms of varying growth rates and their effect on muscle attachment sites (Lee *et al*., 1996; Nie, 2005).

It has been suggested by Moss & Rankow (1968) and Koski (2007) that the significant increase in the bicondylar width of the mandible may result from muscular tension on the condyle of the mandible resulting from varying cranial base growth rates. The increased growth of the anterior cranial base when compared to the posterior cranial base may further influence the growth of the mandible, through vectorial forces from the various attachments of the muscles of mastication (Moss & Rankow, 1968; Nie, 2005; Koski, 2007).

Additionally it appears that the lateral expansion of the growing tongue may influence the growth of the posterior regions of the mandible. This is evident in the highly correlated increased lateral expansion of the maximum breadth of the tongue with the mandibular gonial region seen here. Swinehart (1950) postulated that it was the growing tongue, through its natural expansive forces, that increased the dimensions of the mandibular arch sufficiently to accommodate the dentition. Evidence of the tongue’s natural expansive forces in normal development is emphasized by the

significantly retarded transverse development of the mandibular arch in cases of aglossia (Salles *et al*., 2008).

As the current study delves into a particularly complex period of oral-cavity development, an appreciation of the preceding developmental events is necessary to fully understand the relationships observed within the current context. In the current study, the consistent widening of the posterior region of the mandible suggests a consistent lateral expansive force of the tongue descending into the oral cavity. Age, in this instance, should also be considered in evaluations of the tongue, as children are noted to have higher dorsal tongue heights when compared to adults (Lowe *et al*., 1985). This is consistent with previous descriptions of growth where the tongue commences its descent only after the enlargement of the oral cavity, thus allowing the closure of the palatal shelves (Humphrey, 1971).

However, in addition to the expansive forces exerted by the growing tongue on the mandible, there are movement related forces that also progressively exert their effects. During the eighth week of development, the mouth has been found to open and close in response to reflex activity in the depressor and elevator muscle groups associated with the fetal mandible (Humphrey, 1971; Herring, 1985; Koolstra, 2002). These reflex activities produce low-magnitude and high-frequency strains, which have been shown to have a significant effect on the determination of bone structure (Daegling, 2012). They further serve as precursors to the development of the skeletal attachment sites of the associated muscle groups (*i.e.* the condylar and coronoid processes) (Spyropoulos, 1977; Herring, 1985; Koolstra, 2002). These actions are subsequently followed by more powerful and complex suckling interactions between the tongue and the oral cavity (Gasser, 1965; Humphrey, 1971; Herring, 1985; Reissland *et al*., 2012). Therefore, suckling during infancy is described as a combination of suction (negative pressure within the oral cavity) and expression (positive pressure exerted by the tongue), actions complicated by varying cycles of nutritive and non-nutritive suckling, which requires the independent functioning of both the anterior and posterior areas of the tongue (Herring, 1985). This suggests that there are functionally different demands on the anterior and posterior regions of the developing mandible with the onset of suckling, and subsequently with the onset

of mastication during the juvenile period of growth (Humphrey, 1971; Herring, 1985; Koolstra, 2002; Reissland *et al*., 2012). The influence of various types of suckling and the associated change in suckling dynamics is highly prevalent in the occurrence of malocclusions. In a recent study conducted by Melink *et al*. (2010), it was concluded that infants who were bottle-fed adopted an incorrect tongue posture and function, which led to a change in the normal dynamics of the oral cavity (Melink *et al*., 2010). The change in oral cavity dynamics through incorrect tongue posture and subsequent lengthening of the lingual frenulum, led to the development of a posterior cross-bite (Melink *et al*., 2010). In turn, this change resulted in a broadening of the mandible arch on the cross-bite side (Melink *et al*., 2010).

In the current study, significant lengthening in the sagittal plane of the mandible was noted. However, there was no significant widening in the interforaminal distance between the mental foramina. This suggests that the mandible maintains a constant growth rate anteriorly, despite considerable morphological changes resulting from early suckling. The relationship between the anterior region of the mandible and tongue has been reported to commence as early as the seventh week of development (Lee *et al*., 2001). The attachment of the genioglossus muscle, first observed on the inferior side of the anterior portion of Meckel's cartilage, reattaches to the trabeculae of the mandibular symphysis by the end of the eighth week (Lee *et al*., 2001). During the sixth week, the primary ossification centre of the mandible is first observed near the mental foramen (Lee *et al*., 2001; Radlanski *et al*., 2003). The trabeculae of the mandible then extend radially towards the future mandibular symphysis, alveolar bone, mandibular body and coronoid process (Lee *et al*., 2001). In a study of development in a porcine model, the radial extension of the trabeculae resulted in an increased level of mineralization in the body of the mandible when compared to the condylar region (Mulder *et al*., 2006). Thus, a higher turnover of bone, resulting from an increased growth rate in the condylar region of the mandible is indicative of the direction of mandibular growth (Mulder *et al*., 2006).

The high degree of mineralization in the body of the mandible in humans compared with pigs may also be related to the arrangement of muscle fibres associated with the muscles in the floor of the mouth (Radlanski *et al*., 2001). These closely reflect the

size and orientation changes in the bony architecture of the mandible during growth (Radlanski *et al*., 2001). As the mandible grows, the mesenchymal tissue is stretched and indicates the muscle fibre arrangement in the direction of the imposed force (Radlanski *et al*., 2001). Furthermore, the dimensions of the mandibular arch increase to accommodate the growth of the tongue and development and emergence of the deciduous dentition (Hutchinson, 2010; Hutchinson *et al*., 2012).

In the current study the normal growth of the mandible and tongue is reflected as a synchronous relationship in which significant size increases in the length of the mandible and tongue are observed. Liu *et al*. (2008) analyzed the effects of tongue reduction on mandibular growth and observed significant reductions in the anterior mandibular length, mandibular symphysis length, dorsal width of the anterior mandible and mandibular dental arch at the canine teeth (Liu *et al*., 2008). These observations suggest that a reduction in the volume of the tongue during periods of growth would result in alterations in the craniofacial skeletal growth and dental arch formation (Liu *et al*., 2008). Salles *et al.* (2008) similarly observed retardation in the growth of the mandible in a 14-year old patient diagnosed with aglossia. The alveolar arches had not developed transversally and there was significant retardation in the anterior growth of the mandible (Salles *et al.,* 2008). There was also a significant reduction in the width of the mandibular arch, leading to a reduction in the space between the mandibular bodies (Salles *et al.,* 2008). Retardation in the growth of the mandible was attributed to the absence of muscular stimulus between the alveolar arches (Salles *et al.,* 2008), usually provided by the tongue in the initial stages of suckling and later during mastication (Humphrey, 1971; Herring, 1985). Additionally, similar observations were made in a case of hypoglossia, in which the patient presented with a smaller tongue as well as with a retruded lower lip and micrognathia (Kuroda & Ohyama, 1981; Salles *et al*., 2008). Although the tongue was present, the significant reduction in tongue size was considered to result in severe retardation of the downward and forward growth of the mandible (Kuroda & Ohyama, 1981).

Although the mental angle was the only parameter that indicated a significant change in the shape of the mandible in our study during the time-period of assessment, it showed no significant change in the size. The change in the shape of the mental

region may be caused by the tongue and suprahyoid muscles (Coquerelle *et al*., 2013). These muscular structures act as significant contributors to the positioning and orientation of the anterior teeth through bone remodelling, which ultimately shapes the prominence of the mental region (Coquerelle *et al*., 2013). Furthermore changes in the magnitude and frequency of strain produced by various muscle groups associated with speech production also play a significant role in shaping the mental region (Daegling, 2012). High-frequency and low-magnitude forces associated with speech production, significant increases in the trabecular density and bone volume may account for the significant postnatal development of this region (Daegling, 2012). However, the significant change in the shape of the mental region may also be the result of the fusion of the hemi-mandibles and subsequent eruption of the deciduous dentition (Hutchinson, 2010).

The increases in the posterior mandibular dimensions in the older specimens (*i.e.* those from cadavers at a later stage of development) indicated the possibility of the mandible increasing in width to accommodate the lateral expansion of the posterior cranial base (Nie, 2005). However, the consistent increase in the bigonial and biantegonial measurements does not preclude the mandible increasing in width to accommodate the descent of the growing tongue into the oral cavity (Swinehart, 1950; Lowe *et al*., 1985; Coquerelle *et al*., 2013). The consistent lengthening of the mandible and tongue along the sagittal plane further reinforces the necessity of an increase in the mandibular arch to accommodate the growing and descending tongue (Swinehart, 1950; Kuroda & Ohyama, 1981; Lowe *et al*., 1985; Salles et al., 2008; Hutchinson, 2010). Thus, it may be concluded that the growth of the mandible is intrinsically linked to that of the mandible particularly during the functionally complex late prenatal and early postnatal stages of growth. Furthermore these findings provide insights into the allometric growth relationship between the tongue and mandible, potentially assisting the clinician in predicting the behaviour of these structures in the assessment of malocclusions.

3.6 References

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CHAPTER 4:

VARIATIONS IN BONE DENSITY ACROSS THE BODY OF THE IMMATURE HUMAN MANDIBLE

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Accepted Journal of Anatomy (December 2016)

4.1 Abstract

During growth the mandible accommodates increases in biomechanical loading resulting from changes in the function of structures of the oral cavity. Biomechanical loads are thought to play an intricate and vital role in the modelling and remodelling of bone, with site-specific effects on bone mineral density. It is anticipated that the effects of this loading on bone mineral density is intensified during the functional transition from prenatal to postnatal stages. The aim of this study was thus to evaluate changes in bone mineral density across the body of the immature human mandible during the early stages of dental development. The study sample included 45 human mandibles, subdivided into three age groups: prenatal (30 gestational weeks to birth; n=15), early postnatal (birth to 12 months; n=18) and late postnatal (1 to 5 years; n=12). Mandibles were scanned using X-ray micro-CT. Eight landmarks were selected along the buccal/labial and lingual surfaces of each dental crypt for evaluation of the bone mineral density. Bone mineral density values were calculated using a reference standard and analysed using multivariate statistics. The bone mineral density of the lingual surface was found to be significantly higher ($p \leq 0.000$) than that of the buccal/labial surface. Furthermore, bone mineral density in the alveolar region of the buccal/labial surface of the deciduous central incisor ($p \leq 0.001$), the deciduous first molar ($p \leq 0.013$), and lingual alveolar area of the deciduous second molar ($p \leq 0.032$) were significantly greater in the early postnatal period than in the prenatal period. While changes in bone mineral density across the lingual surface were consistent with the progression of development and the biomechanical demand of the tongue as previously demonstrated, changes observed across the buccal/labial surface of the mandible appeared to accompany advancing dental development. Thus, changes in bone mineral density across the mandible appear to be reflective of the stage of dental development and the level of biomechanical loading.

Key words: Embryology, functional morphology, growth/development, imaging, jaw biomechanics, paediatrics

4.2 Introduction

During the transition from the prenatal to postnatal stages, the mandible and the masticatory apparatus adapt to significant variations in the biomechanical forces which are associated with the development, emergence and eruption of the dentition (Herring, 1985; Mavropoulos *et al*. 2005; Sato *et al*. 2005). Loading conditions acting on the mandible are thought to play a marked and influential role in the modelling and remodelling of bone (Herring, 1985; van Eijden, 2000; Koolstra, 2002), with site-specific effects on the bone mineral density (van Eijden, 2000; Ruffoni *et al*. 2007; Hernandez, 2008; Roschger *et al*. 2008; Cioffi *et al*. 2010; Hichijo *et al*. 2015). As such, it may be expected that the effects of increased biomechanical loading on bone mineral density are particularly evident during the functional transition from the prenatal to the postnatal stages of growth, within the immature mandible.

During fetal growth, as the upper digestive tract adapts to increased functional demands and as various mouth opening reflexes and perinatal oral sensorimotor functions commence, the functionality of the tongue and associated oral cavity musculature is intensified (Miller *et al*. 2003; Delaney & Arvedson, 2008; Coquerelle *et al*. 2013). This surge in the functionality of the oral cavity musculature results in increased strain on the muscle attachment sites as well as escalation in early mandibular movements, which are thought to be initiated by tongue movements (Lee *et al*. 2001; Coquerelle *et al*. 2013). During the early postnatal period, the functioning of the masticatory apparatus becomes more sophisticated due to the development, emergence and eruption of the dentition as well as changes in tongue posture. These changes are thought to result in variations in the loading conditions imposed on the mandible. This may lead to a possible change in the bone mineral density of the associated areas of the mandible (Miller *et al*. 2003; Delaney & Arvedson, 2008; Coquerelle *et al*. 2013).

Studies assessing variations in mandibular bone mineral density have contrasted hard and soft diet types to assess the impact of forces arising from the muscles of mastication on their respective mandibular attachment sites (Mavropoulos *et al*. 2004, 2005). However, the influences of increased biomechanical forces resulting

from the dentition have not been investigated (Mavropoulos *et al*. 2004, 2005). Furthermore, while these studies focused on the volumetric density changes *i.e.* the amount of mineralized bone associated with bone density, they did not specifically assess changes in the degree of mineralization of the mineral component of bone density (Mavropoulos *et al*. 2004, 2005; Roschger *et al*. 2008). Volumetric density changes serve as valuable indicators of the biomechanical potential of bone, while bone mineral density distributions provide an indication of the strength, stiffness and toughness of the bone (Ruffoni *et al*. 2007; Hernandez, 2008; Roschger *et al*. 2008; Cioffi *et al*. 2010; Hichijo *et al*. 2015). Furthermore, bone mineral density distributions assist in predicting the pattern of biomechanical force and strain across a developing bone, through the identification of areas of bone presenting with low levels of mineralization and a higher degree of bone modelling and remodelling (Roschger *et al*. 2008; Cioffi *et al*. 2010; Hichijo *et al*. 2015).

Furthermore, studies assessing the prenatal growth of the mandible have mostly limited their focus to the embryonic or early fetal periods of development (Merida Velasco *et al*. 1990; Lee *et al*. 2001; Radlanski *et al*. 2002, 2003, 2004). While the mandible undergoes a significant degree of development during these periods, it is the transition across functional states from the fetal to the early postnatal periods, which significantly influences the mandibular morphology (Miller *et al*. 2003; Delaney & Arvedson, 2008; Coquerelle *et al*. 2013; Hutchinson *et al*. 2014). In addition, while studies have assessed the changes in the mandibular morphology during this functionally complex period; there is a paucity of information on how the bone mineral density varies, particularly over periods of dental development and eruption. As such, it was hypothesized that the bone mineral density of the mandible will change in response to variations in biomechanical loading associated with an increase the development, emergence and eruption of the dentition. Thus, the aim of this study was to evaluate changes in bone mineral density across the body of the immature mandible during the early stages of dental development. While imaging modalities such as DXA (Dual-Energy X-ray) provide a basis for studying volumetric density changes, modalities such as micro-CT provide detailed information about the bone mineral density distributions and as such the degree of mineralization of bone (Ruffoni *et al*. 2007; Roschger *et al*. 2008). Thus, the study made use of micro-CT scanning to investigate changes in the bone mineral density distribution across areas

of the body of the immature mandible associated with the tongue and developing dentition.

4.3 Materials and Method

4.3.1 Materials

Forty-five fetal and neonatal mandibles were utilised, of which 28 were sourced from fresh cadaveric tissue of unknown provenance from the Johannesburg Forensic Paediatric Collection, Division of Forensic Medicine and Pathology. Soft tissue dissection was performed to extract the mandibles from the fresh cadaveric remains. The remaining 17 mandibles were from skeletonized individuals of the Raymond A. Dart Collection of Human Skeletons, School of Anatomical Sciences. Identical protocols were followed in processing the individual remains from each collection. Use of the skeletonised mandibles is permissible under the Human Tissue Act 65 of 1983, while ethical clearance for the study was obtained from the University of the Witwatersrand's Human Ethics Research Committee (M121145) (Appendices 1 and 2).

Specimens ranged in age from 30 gestational weeks to 5 years postnatal. The ages of all specimens were estimated by means of small dental radiographs (two sets: anterior view showing the anterior dental crypts and associated dentition and a lateral view showing the posterior dental crypts and the associated developing dentition) obtained with a NOMAD portable X-ray unit (Aribex Inc., Charlotte, NC, USA) and dental development standards previously proposed by Alqahtani *et al.* (2010). The sample was subdivided into three groups: prenatal (30 gestational weeks to birth, n=15), early postnatal (birth to 12 months, n=18) and late postnatal (1 to 5 years, n=12). The sample was subdivided according to these chronological intervals as the mandible is thought to undergo a significant amount of change during these designated periods with the progression of oral and masticatory function. A visual examination was conducted across the study sample and individuals showing obvious evidence of congenital abnormalities, surgical intervention or general damage to the mandible were excluded from the study. As such, based on this

exclusion criteria 15 specimens were excluded from the total study sample (n=60) and only 45 mandibles were found to be suitable for further analysis.

All mandibles were scanned using a Nikon XTH 225L micro-focus CT X-ray unit (Nikon Metrology, Leuven, Belgium), located at the MIXRAD laboratory at the South African Nuclear Energy Corporation, Pelindaba. Scanning parameters were set to 100KV / 100µA and 100µm as a means of standardising the scanning conditions across the sample. A 0.1mm thick aluminium filter was used to approximate a homogeneous X-ray beam spectrum by removing the lower energy photons. Specimens were securely mounted in a polystyrene mould, with the body of the mandible vertically oriented. In addition a small plastic peg was used as a density reference sample owing to its uniform material composition. The reference sample was placed within the polystyrene mould for each scan to further assist in standardising scanning conditions across scans. The mounted specimens were then placed on to a rotating sample manipulator, which facilitated scanning at 360^0. A thousand projection images were obtained in the 360^0 which resulted in a good quality scan with optimized time. The scanning setup was optimized for highest spatial resolution by setting the sample with maximum magnification (magnification is a property of a cone beam). Consequently a resolution of between 0.023 and 0.050 µm was obtained. The scans were then reconstructed using Nikon CTPro software (Nikon Metrology, Leuven, Belgium).

Following 3-dimensional reconstruction, all the scans were imported into VGStudio Max V2.2 (Volume Graphics GmbH, Heidelberg, Germany) as volume files for further analysis. All imported volume files were then subjected to a surface determination analysis to allow for clear determination of surface landmarks. The orientation of the micro-CT slices of the mandible was then standardized by aligning each slice with an established transverse plane. This alignment allowed for further analysis of the dental crypt in a horizontal cross-section. The transverse reference plane was established by selecting a minimum of three reference points (fiducial markers) along the inferior region of the mandible (Fig. 1). These points which were located between the antegonial notch and the dental crypt of the canine (Fig. 1), assisted in standardizing the alignment of the micro-CT slices with the transverse plane. All slices of the

mandible were then aligned to these reference points and the transverse reference plane was then established parallel to the inferior region of the mandible.

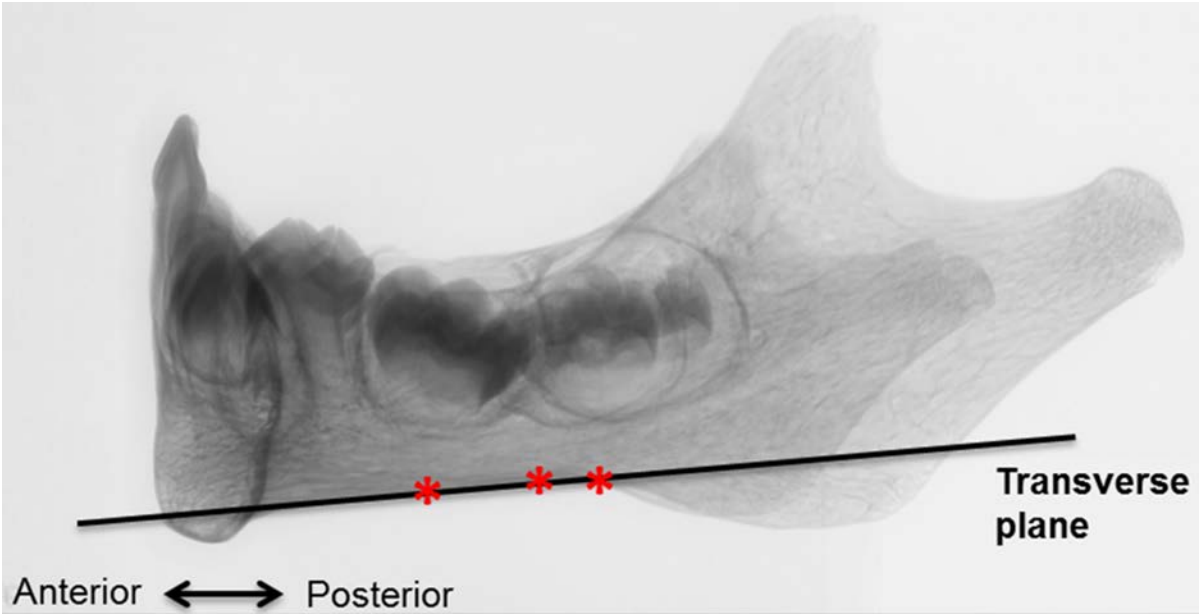


Figure 1: Lateral view of the immature mandible indicating the reference (fiducial) points and transverse plane used in the alignment of the micro-CT slices.

After the micro-CT slices of the mandible were aligned with the established transverse plane, the mesiodistal mid-point of each individual dental crypt was determined (Fig. 2A). All dental crypts were included in the study assessment, with the exception of the M2 molar crypt which was only present in one individual. The maximum mesiodistal diameter used to determine the mid-point of each dental crypt was established tangential to the buccal/labial surface (Fig. 2A). The mid-point of the maximum mesiodistal diameter of the dental crypt was then located and a section was taken at this point (Fig. 2A). A single mid-point section was used for the anterior dental crypts as they were not as long as the posterior dental crypts (Fig. 2A). In the case of the posterior dentition, the maximum mesiodistal diameter was subdivided into three sections (distal, midpoint and mesial) (Fig. 2A).

To further study the mineral density of the lingual and buccal/labial surfaces of the mandible relative to each dental crypt, the mandible was again subjected to a surface determination analysis (Fig. 2A). The surface determination analysis defines the

surface of the mandible in three-dimensional space, which then allows for the selection of points or areas of interest. Following the surface determination analysis, all micro-CT slices were then re-aligned to the sagittal plane at the midpoint of the mesiodistal diameter of each dental crypt and subjected to further cross-sectional analysis (Fig. 2B). The re-alignment of the micro-CT slices from the transverse plane to the sagittal plane provided the opportunity to study changes in the bone mineral density along the superior, middle and inferior points of the lingual and buccal/labial surfaces respectively (Fig. 2B). This process was repeated for all subsequent dental crypts.

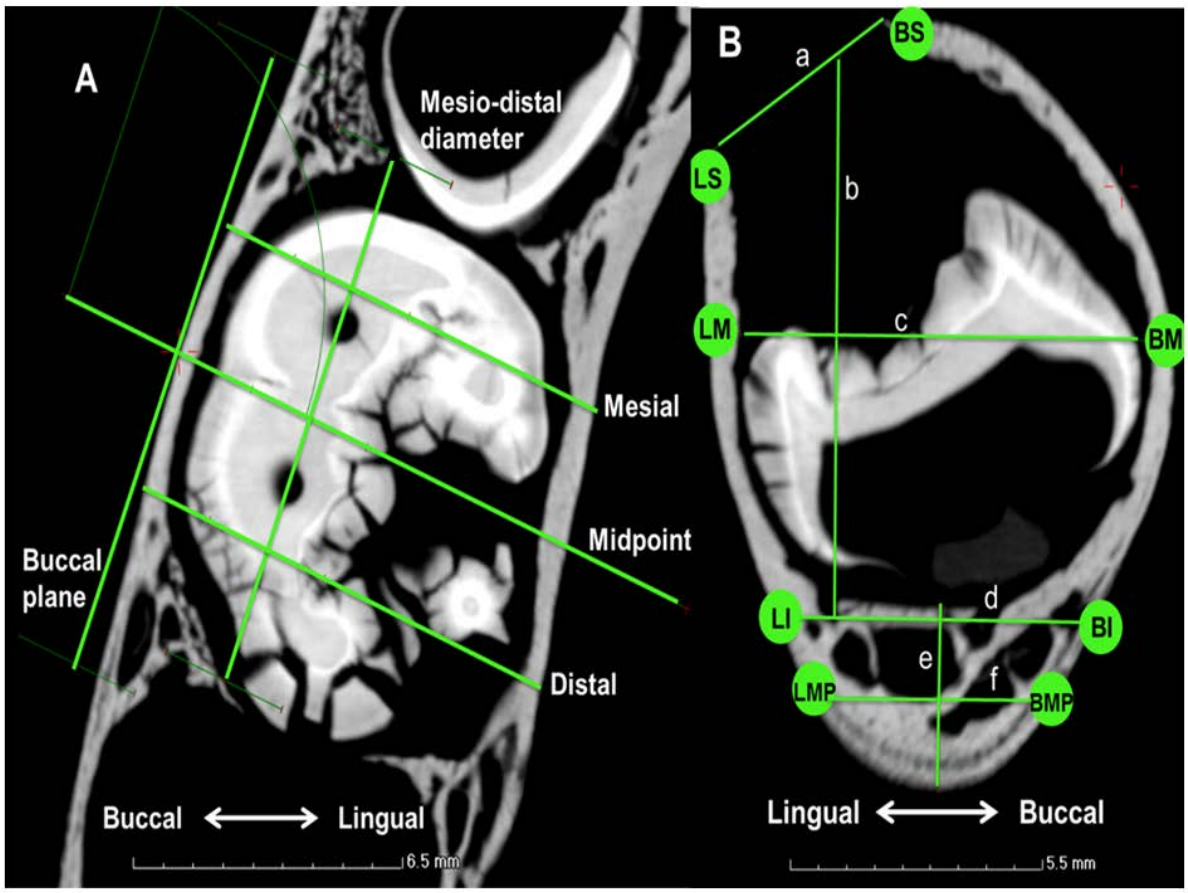


Figure 2: Micro-CT scan of the second deciduous molar dental crypt of a paediatric mandible **(2A and B).** Superior view of a transverse section of the left deciduous molar dental crypt indicating the position at which the coronal sections were taken at the mesial, midpoint and distal positions of the dental crypt **(2A)**. Lateral view of a midpoint coronal section of the left deciduous molar crypt indicating the four buccal and four lingual density points. (BS: Buccal superior; BM: Buccal middle; BI: Buccal

inferior and BMP: Buccal basal midpoint; LS: Lingual superior; LM: Lingual middle; LI: Lingual inferior and LMP: Lingual basal midpoint) **(2B)**.

4.3.2 Measurements

To assess the bone mineral density on both the buccal/labial and lingual surfaces of each dental crypt, a total of eight points were selected along these surfaces (Fig. 2B) on the basis of the functionality and stage of dental development (Jäger et al. 1990; Park et al. 2008; Hsu et al. 2011; Chang et al. 2012). The size of each assessment point was adapted to accommodate the change in the size of the body of the immature mandible. Thus, the area of each assessment point included a radius of 0.4mm and a depth of 1mm. The depth of each assessment point included multiple slices through an area. Superior, middle and inferior points were located in the alveolar region and a midpoint within the basal region of the each surface.

The superior points of the lingual and buccal/labial surfaces were located at the level of the opening of the dental crypt (a), which corresponded to the bony crests of each surface (Fig. 2B). The middle points of the lingual and buccal/labial surfaces were located at the level of the maximum width of the dental crypt (c), which was associated with the midpoint of the dental crypt height (b). The dental crypt height was measured between the most inferior point of the dental crypt and a point directly perpendicular to it, which was located at the level of the opening of the dental crypt (Fig. 2B). The inferior point on both the lingual and buccal/labial surfaces, were aligned with the inferior border of the dental crypt (d) (Fig. 2B). The lingual and buccal/labial surface midpoints were located at the midpoint of the basal region (f) indicated at the midpoint of the basal bone height (e). The basal bone height was measured from the most inferior point located along the inferior border of the mandible to a perpendicular point located along the inferior border of the dental crypt (Fig. 2B).

4.3.3 Data analysis

The grey value assessed at each point was used as an estimate of bone mineral density. This value was standardized using the grey value of the reference material included in each scan *i.e*. the grey value of each assessment point was subdivided by the grey value of reference. This resulted in the data for each assessment point being presented as ratios or absolute values, which allowed for further statistical analysis and comparisons of the bone mineral density distribution values across the sample.

Descriptive statistics, including means and standard deviations, were calculated for all the density ratios (since absolute density values cannot be used with polychromatic X-ray beams) associated with each dental crypt. A Shapiro-Wilk's test assessed the distribution of the sample, which further determined statistical analyses based on the distribution type. All statistical analyses were conducted using SPSS v.23 (IBM, Armonk, NY, USA) and PAST (Paleontological Statistics) statistical programs (Paleontological Association, London, UK) (Hammer *et al*. 2001).

Considering the parametric distribution of the data, an analysis of covariance (ANCOVA) was first conducted to evaluate the influence of age on the bone mineral density distribution patterns of the buccal/labial and lingual surfaces respectively (Zar, 1999). Furthermore a principal component analysis (PCA) was conducted to visualize the level of interaction between the buccal/labial and lingual surfaces of the mandible and their associated alveolar and basal areas across the prenatal, early postnatal and late postnatal groups.

In the cases where a significant degree of interaction was observed between the buccal/labial and lingual surfaces a multivariate analysis of variance (MANOVA) was conducted to evaluate the level of interaction between the independent factors i.e. age group and tooth type and the dependent factors i.e. mineral density ratios associated with each surface (Zar, 1999). A Tukey's honest significant difference (HSD) posthoc test and pairwise comparisons were also conducted. A significance

level of alpha<0.05 was considered statistically significant. Inter- and intra-observer errors were evaluated using ten specimens and then tested using Lin's concordance correlation co-efficient of reproducibility, which provides information on the level of repeatability of the method and the degree of association between measurements (Allan, 1982).

4.4 Results

A high level of repeatability (77.3% to 99.9%) was observed across all measurements within the study. As no significant differences were observed between the superior, middle and inferior mineral density landmarks of the alveolar areas of each surface, these values were averaged to provide a value for the general alveolar area. Furthermore, as no significant differences were observed between the left and right measurements these values were also averaged. While the age group had no effect on variations in bone mineral density across the alveolar and basal areas of the lingual surface ($p<0.153$), both areas of the buccal/labial surface were well correlated with changes in age ($p<0.001$) (Fig. 3).

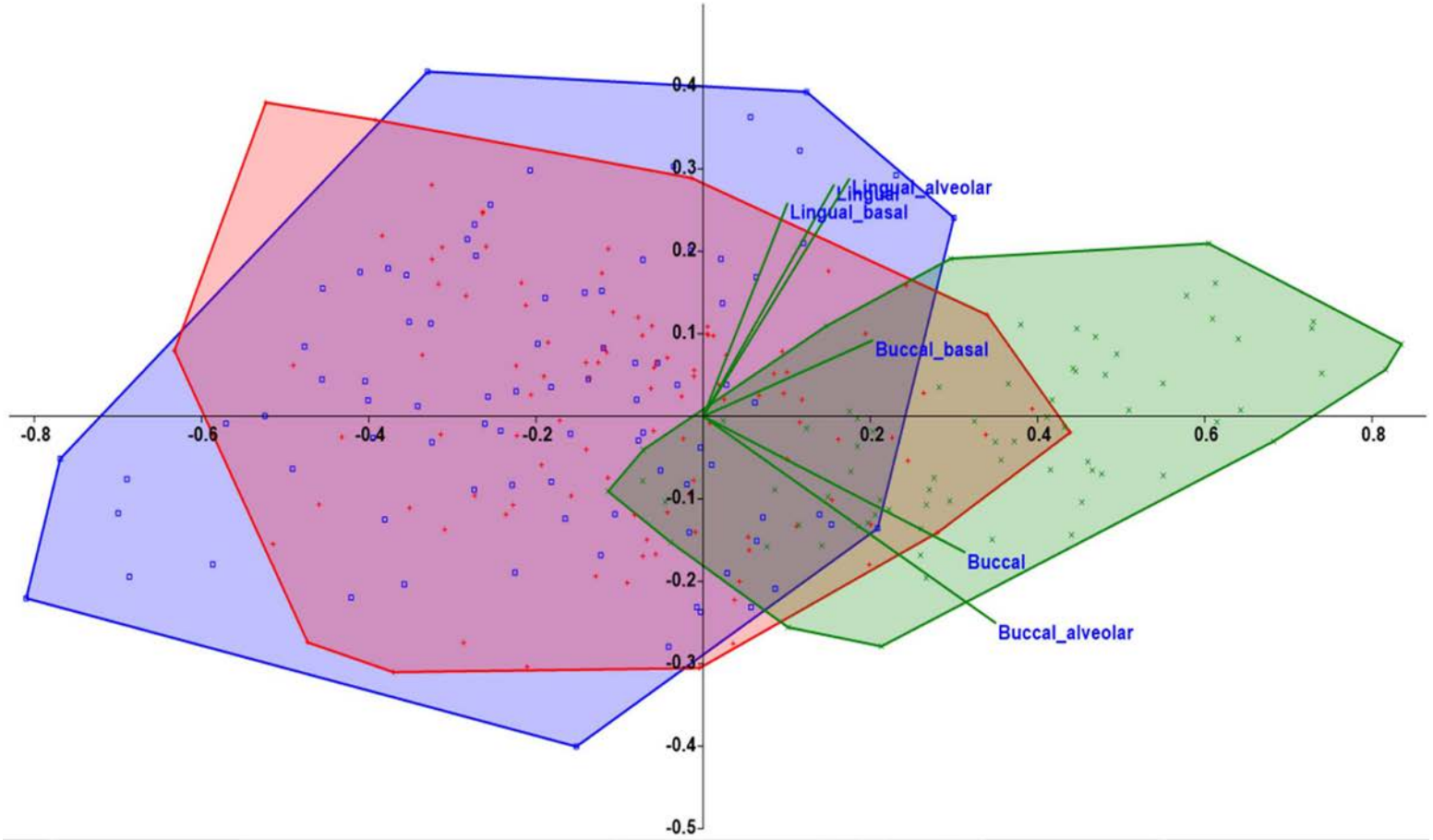


Figure 3: Principal component analysis (PCA) indicating the level of correlation between lingual and buccal surface measurements across the prenatal, early postnatal and late postnatal periods of growth. (Prenatal: blue; Early postnatal: red; Late postnatal: green).

4.4.1 The buccal/labial surface versus the lingual surface of the mandible

Bone mineral density values across the lingual surface were significantly higher ($p<0.001$) than those of the buccal/labial surface across all dental crypts, with the exception of the first permanent molar crypt in the prenatal and early postnatal periods (Fig. 4A, D; Table 1). Furthermore in the late postnatal period, bone mineral density values across the lingual surface were significantly higher ($p<0.001$) across the lateral incisor crypt, canine crypt and first deciduous molar dental crypt when compared to the buccal surface (Fig. 4A, D; Table 1). In the early postnatal period, bone mineral density along the lingual surface in the area of the lateral incisor crypt (4.4%, $p< 0.015$) was significantly greater than that of the central incisor crypt and bone mineral density of the second deciduous molar crypt (8.2%, $p<0.001$) was significantly greater than that of the first permanent molar crypt (Fig. 4A, Table 1). Bone mineral density across the lingual surface of the second deciduous molar crypt in the early postnatal period was significantly greater (3.7%, $p<0.046$) than that of the prenatal period (Fig. 4A, Table 2). In addition, bone mineral density along the lingual surface was significantly greater in the late postnatal period (CI: 7.5%, $p<0.001$; LI: 5.4%, $p<0.013$; C: 4.8%, $p<0.029$; DM1: 5.9%, $p<0.005$; DM2: 4.7%, $p<0.023$; M1: 12.2%, $p<0.001$), when compared to the early postnatal period (Fig 4A, Table 2).

In the prenatal and early postnatal periods, bone mineral density along the buccal/labial surface of the mandible, in the area of the lateral incisor crypt, was significantly greater than that of the central incisor crypt (8.1%, $p<0.004$) and canine crypt (9.5%, $p<0.001$) areas respectively (Fig. 4D, Table 1). In addition, bone mineral density in the area of the first deciduous molar crypt was significantly greater (7.0%, $p<0.001$) than that of the canine crypt area (Fig. 4D, Table 1). In the late postnatal period, only bone mineral density in the area of the second deciduous molar crypt was significantly greater (5.1%, $p<0.026$) than that of the first deciduous molar crypt (Fig. 4D, Table 1). Along the general buccal/labial surface of the mandible, bone mineral density was significantly greater in the early postnatal period (CI: 8.8%, $p<0.002$; LI: 4.8%, $p<0.045$; DM1: 6.3%, $p<0.011$) when compared to the prenatal period (Fig. 4D, Table 2). In addition, bone mineral density along the buccal/labial surface was significantly greater in the late postnatal period (CI: 16.9%, $p<0.001$; LI:

11.0%, p<0.001; C: 23.9%, p<0.001; DM1: 15.1%, p<0.001; DM2: 16.5%, p<0.001; M1: 13.4%, p<0.001), when compared to the early postnatal period (Fig 4D, Table 2).

4.4.2 Alveolar region

In the early postnatal period, bone mineral density across the lingual surface was significantly greater in the area of the lateral incisor crypt (5.2%, p< 0.029) and the second deciduous molar crypt (10.2%, p<0.001) when compared to the central incisor crypt and the first permanent molar crypt respectively (Fig. 4B, Table 1). Bone mineral density in the area of the second deciduous molar crypt was significantly greater (5.0%, p<0.032) in the early postnatal period than the prenatal period (Fig. 4B). In addition, bone mineral density along the lingual surface was significantly greater in the late postnatal period (CI: 10.3%, p<0.001; LI: 6.7%, p<0.002; C: 6.0%, p<0.011; DM1: 6.6%, p<0.003; DM2: 6.6%, p<0.002; M1: 16.7%, p<0.001), when compared to the early postnatal period (Fig 4B, Table 2).

Along the buccal/labial surface of the mandible, bone mineral density variations observed across the alveolar region followed a similar pattern of change to that previously observed along the general buccal/labial surface of the mandible (Fig. 4D and 4E, Table 1). Along the buccal/labial surface of the mandible, bone mineral density was significantly greater in the early postnatal period (CI: 12.6%, p<0.001; DM1: 7.4%, p<0.013) when compared to the prenatal period (Fig. 4E, Table 2). In addition, bone mineral density along the buccal/labial surface was significantly greater in the late postnatal period (CI: 20.3%, p<0.001; LI: 11.8%, p<0.001; C: 31.0%, p<0.001; DM1: 17.8%, p<0.001; DM2: 20.5%, p<0.001; M1: 12.8%, p<0.001), when compared to the early postnatal period (Fig 4E, Table 2).

4.4.3 Basal region

In the prenatal period, bone mineral density across the lingual surface was significantly greater in the area of the lateral incisor crypt (6.6%, p<0.004) when compared to the central incisor crypt area (Fig. 4C, Table 1). In the early postnatal period, bone mineral density in the areas of the lateral incisor crypt (5.3%, p<0.009)

and second deciduous molar crypt (5.5%, $p<0.005$) were significantly greater when compared to the central incisor crypt and first permanent molar crypt areas respectively (Fig. 4C, Table 1). Bone mineral density across the buccal/labial surface, in the areas of the central incisor crypt (6.7%, $p<0.005$) and canine crypt (5.9%, $p<0.016$) was significantly greater than that of the lateral incisor crypt area within the prenatal period (Fig. 4F, Table 1). In the early postnatal period, bone mineral density in the areas of the first (4.9%, $p<0.013$) and second (9.6%, $p<0.001$) deciduous molar crypts were significantly greater when compared to the canine crypt and first permanent molar crypt areas respectively (Fig. 4F, Table 1).

Along the buccal/labial surface of the mandible, bone mineral density was significantly greater in the early postnatal period (LI: 5.2%, $p<0.032$; DM2: 4.1%, $p<0.044$) when compared to the prenatal period (Fig. 4F, Table 2). In addition, bone mineral density along the buccal/labial surface was significantly greater in the late postnatal period (CI: 8.0%, $p<0.001$; LI: 9.9%, $p<0.001$; C: 10.4%, $p<0.001$; DM1: 7.0%, $p<0.002$; DM2: 7.3%, $p<0.001$; M1: 15.6%, $p<0.001$), when compared to the early postnatal period (Fig 4F, Table 2).

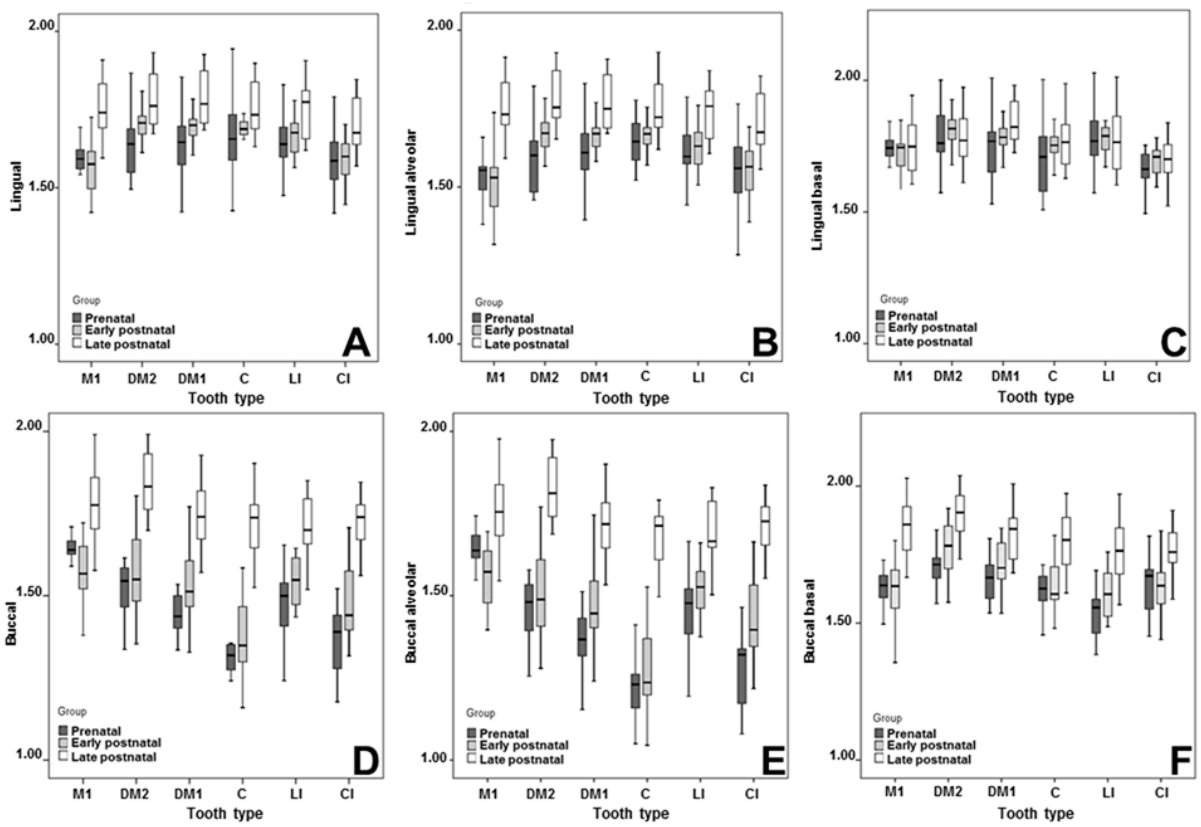


Figure 4: Box-whisker plots indicating differences in the bone mineral density values across the general lingual (A), lingual alveolar (B), lingual basal (C), general buccal/labial (D), buccal/labial alveolar (E) and buccal/labial basal (F) surfaces of the mandible during the prenatal (30 gestational weeks to birth), early postnatal (birth to 1 year) and late postnatal (1 to 5 years) periods. Density differences between each of the dental crypts are also indicated across all assessed age groups for each surface. (CI: central incisor; LI: lateral incisor; C: canine; DM1: first deciduous molar; DM2: second deciduous molar and M1: first permanent molar).

Table 1: Multivariate analysis of variance (MANOVA) indicating the pairwise comparisons of density ratios between dental crypts for each of the assessed mandibular surfaces within each age group

		Mean ± SD						Pairwise comparisons (p<0.05)					
		CI	LI	C	DM1	DM2	M1	Univariate F (p<0.05)	CI vs. LI	LI vs. C	C vs. DM1	DM1 vs. DM2	DM2 vs. M1
	Buccal	1.36 ± 0.11	1.47 ± 0.11	1.33 ± 0.10	1.43 ± 0.09	1.51 ± 0.09	1.64 ± 0.05	15.580 (0.001)	0.004	0.001	0.009	0.028	0.002
Group 1	**Lingual**	1.58 ± 0.12	1.65 ± 0.14	1.67 ± 0.14	1.63 ± 0.12	1.64 ± 0.11	1.59 ± 0.07	2.072 (0.069)	0.047	0.470	0.236	0.802	0.166
(n=15):	**Buccal alveolar**	1.27 ± 0.13	1.45 ± 0.12	1.24 ± 0.13	1.36 ± 0.10	1.45 ± 0.11	1.64 ± 0.06	21.703 (0.001)	0.001	0.001	0.004	0.029	0.001
30 gw to	**Lingual alveolar**	1.55 ± 0.13	1.60 ± 0.15	1.66 ± 0.14	1.60 ± 0.12	1.59 ± 0.11	1.54 ± 0.08	2.653 (0.023)	0.125	0.124	0.084	0.940	0.173
birth	**Buccal basal**	1.64 ± 0.11	1.53 ± 0.09	1.62 ± 0.11	1.65 ± 0.11	1.70 ± 0.07	1.63 ± 0.07	4.468 (0.001)	0.005	0.016	0.459	0.153	0.077
	Lingual basal	1.67 ± 0.11	1.78 ± 0.13	1.71 ± 0.15	1.73 ± 0.14	1.78 ± 0.12	1.73 ± 0.09	2.557 (0.028)	0.004	0.060	0.477	0.240	0.248
	Buccal	1.48 ± 0.11	1.54 ± 0.07	1.38 ± 0.12	1.52 ± 0.14	1.58 ± 0.12	1.57 ± 0.09	9.654 (0.001)	0.054	0.001	0.001	0.124	0.936
Group 2	**Lingual**	1.59 ± 0.08	1.66 ± 0.06	1.68 ± 0.06	1.69 ± 0.06	1.70 ± 0.05	1.56 ± 0.08	7.550 (0.00)	0.015	0.506	0.800	0.689	0.001
(n=18):	**Buccal alveolar**	1.43 ± 0.13	1.52 ± 0.08	1.29 ± 0.15	1.46 ± 0.16	1.51 ± 0.13	1.56 ± 0.09	12.335 (0.001)	0.015	0.001	0.001	0.192	0.171
Birth to 1	**Lingual alveolar**	1.55 ± 0.09	1.63 ± 0.07	1.66 ± 0.06	1.66 ± 0.06	1.67 ± 0.06	1.50 ± 0.10	8.622 (0.001)	0.029	0.270	0.992	0.874	0.001
year	**Buccal basal**	1.63 ± 0.10	1.61 ± 0.09	1.63 ± 0.10	1.71 ± 0.09	1.77 ± 0.10	1.60 ± 0.14	8.258 (0.001)	0.582	0.535	0.013	0.076	0.001
	Lingual basal	1.69 ± 0.08	1.78 ± 0.05	1.75 ± 0.06	1.78 ± 0.06	1.82 ± 0.06	1.72 ± 0.07	3.795 (0.002)	0.009	0.420	0.321	0.300	0.005
	Buccal	1.73 ± 0.08	1.71 ± 0.10	1.71 ± 0.11	1.75 ± 0.10	1.84 ± 0.10	1.78 ± 0.12	2.804 (0.001)	0.768	0.978	0.418	0.026	0.150
Group 3	**Lingual**	1.71 ± 0.09	1.75 ± 0.09	1.76 ± 0.09	1.79 ± 0.09	1.78 ± 0.09	1.75 ± 0.10	1.199 (0.310)	0.263	0.779	0.446	0.878	0.422
(n=12):	**Buccal alveolar**	1.72 ± 0.08	1.70 ± 0.10	1.69 ± 0.12	1.72 ± 0.10	1.82 ± 0.10	1.76 ± 0.12	2.291 (0.046)	0.671	0.830	0.487	0.029	0.177
1 to 5	**Lingual alveolar**	1.71 ± 0.10	1.74 ± 0.08	1.76 ± 0.09	1.77 ± 0.09	1.78 ± 0.09	1.75 ± 0.10	0.827 (0.531)	0.415	0.730	0.690	0.772	0.445
years	**Buccal Basal**	1.76 ± 0.09	1.77 ± 0.12	1.80 ± 0.12	1.83 ± 0.10	1.90 ± 0.10	1.85 ± 0.11	3.176 (0.008)	0.797	0.535	0.368	0.121	0.221
	Lingual basal	1.70 ± 0.09	1.77 ± 0.12	1.77 ± 0.11	1.84 ± 0.09	1.78 ± 0.11	1.75 ± 0.10	2.580 (0.027)	0.074	0.979	0.092	0.147	0.457

CI: Central Incisor; LI: Lateral Incisor; C: Canine; DM1: Deciduous molar 1; DM2: Deciduous molar 2; M1: Permanent molar 1

Table 2: Multivariate analysis of variance (MANOVA) indicating the pairwise comparisons of density ratios between age groups for each of the assessed mandibular surfaces across all associated dental crypts

	Buccal			Lingual			Buccal Alveolar			Lingual Alveolar			Buccal Basal			Lingual Basal		
	Univariate F (p<0.05)	Group 1 vs. Group 2	Group 2 vs. Group 3	Univariate F (p<0.05)	Group 1 vs. Group 2	Group 2 vs. Group 3	Univariate F (p<0.05)	Group 1 vs. Group 2	Group 2 vs. Group 3	Univariate F (p<0.05)	Group 1 vs. Group 2	Group 2 vs. Group 3	Univariate F (p<0.05)	Group 1 vs. Group 2	Group 2 vs. Group 3	Univariate F (p<0.05)	Group 1 vs. Group 2	Group 2 vs. Group 3
CI	42.745 (0.001)	0.002	0.001	7.818 (0.001)	0.777	0.001	49.797 (0.001)	0.001	0.001	11.433 (0.001)	0.850	0.001	6.913 (0.001)	0.784	0.001	0.290 (0.749)	0.615	0.763
LI	19.081 (0.001)	0.045	0.001	4.635 (0.011)	0.599	0.013	15.304 (0.001)	0.081	0.001	7.536 (0.001)	0.498	0.002	18.648 (0.001)	0.032	0.001	0.010 (0.990)	0.961	0.919
C	53.430 (0.001)	0.215	0.001	3.525 (0.031)	0.687	0.029	58.439 (0.001)	0.159	0.001	4.170 (0.017)	0.902	0.011	12.201 (0.001)	0.828	0.001	1.468 (0.232)	0.248	0.517
DM1	32.518 (0.001)	0.011	0.001	9.774 (0.001)	0.060	0.005	33.544 (0.001)	0.013	0.001	10.794 (0.001)	0.055	0.003	10.980 (0.001)	0.069	0.002	3.868 (0.022)	0.175	0.108
DM2	37.138 (0.001)	0.087	0.001	8.039 (0.001)	0.046	0.023	38.724 (0.001)	0.150	0.001	12.586 (0.001)	0.032	0.002	12.339 (0.001)	0.044	0.001	0.743 (0.477)	0.263	0.364
M1	14.666 (0.001)	0.088	0.001	16.731 (0.001)	0.393	0.001	10.244 (0.001)	0.070	0.001	24.709 (0.001)	0.321	0.001	22.230 (0.001)	0.467	0.001	0.307 (0.736)	0.802	0.463

Group 1: 30 gestational weeks to birth, Group 2: Birth to 1 year, Group 3: 1 to 5 years.

CI: Central Incisor; LI: Lateral Incisor; C: Canine; DM1: Deciduous molar 1; DM2: Deciduous molar 2; M1: Permanent molar 1

4.5 Discussion

The transition between the late prenatal and early postnatal periods of growth represents a functionally complex period. It is during this period, that the growth of the mandible and the associated masticatory apparatus is synchronized with the development of functions such as sucking, suckling and mastication, which are required for successful nutritional intake (Miller *et al*. 2003; Delaney & Arvedson, 2008). As the oral cavity structures adapt to accommodate the change in function, it is expected that bone mineral density will be significantly affected by both changes in the state of the dentition as well as the functionality of the masticatory apparatus during the late prenatal and early postnatal periods of growth. This change in bone mineral density distribution illustrates the impact of functional changes on the developing mandible during its early period of growth. The change may also be significant when considering that many of the bone disorders e.g. osteogenesis imperfecta, affect children (Roschger *et al*. 2008). Furthermore the bone mineral density distribution across the cortical bone of the mandible observed in this study, provides valuable clinical insights into the impact of biomechanical changes arising from dental development on the mandibular bone during growth (Roschger *et al*. 2008).

While this study offers valuable insights into the growth and development of the mandible during a particularly complex period of growth, there were some limitations in the sample which should be noted. The cross sectional nature of the study, ultimately limits the level of causal inferences regarding changes in age or function. In addition, part of the sample was sourced from a forensic setting, thus detailed demographic data for each specimen was not available. Furthermore the nutritional status and any underlying metabolic diseases of the study sample are not known and as such inferences concerning the effects of any metabolic disturbances on the structure and morphology of the mandible fall outside the scope of this study. Thus, the study made use of ratios or absolute values rather than Hounsfield's units, as a means to describe a pattern of change in bone mineral density versus quantifying the bone density of an area. While the present study assessed changes in bone mineral density across the body of the immature mandible, it did not specifically assess

changes in the amount of mineralised bone tissue or change in the bone volume. Thus, the depth of inferences regarding changes in bone density across the immature mandible is limited to bone mineral density distribution.

Considering that the development and growth of the oral cavity soft tissues, precedes that of the developing dentition, the findings of the current study will be discussed in accordance with this timeline. In general, bone mineral density across the lingual surface was observed to be significantly higher than the buccal/labial surface, across the prenatal and early postnatal periods. This disparity in bone mineral density between the surfaces of the mandible may be attributed to the early development of the mandible. During the 6th week of gestation, the intramembranous ossification of the mandible commences relative to the facial surface of Meckel's cartilage (Lee *et al*. 2001). Trabecular bone originating from the primary growth center of the mandible advances in a buccal direction (Lee *et al*. 2001; Radlanski *et al*. 2003). Thus, the bone of the lingual surface is further advanced in its growth as well as degree of mineralization, when compared to the buccal/labial surface of the mandible.

As the morphology of the mandible is thought to change with the development of the associated masticatory structures and an increase in masticatory activity, variations in bone mineral density within the alveolar and basal areas of the lingual and buccal/labial surfaces of the mandible were investigated further. The higher bone mineral density of the lateral incisor crypt region, particularly during the prenatal and early postnatal periods of growth, may be attributed to changes in both the morphology of the symphyseal region of the mandible (Scheuer & Black 2000; Lee *et al*. 2001; Radlanski *et al*. 2003; Hutchinson *et al*. 2014) as well as in the functioning of the tongue (Miller *et al*. 2003; Delaney & Arvedson, 2008).

During the 12th week of gestation, the ventral symphyseal cartilages commence the process of endochondral ossification (Scheuer & Black, 2000; Radlanski *et al*. 2003). This process, which includes increased bone modelling and remodelling, continues until fusion of the hemi-mandibles is completed (Scheuer & Black, 2000; Hutchinson *et al*. 2012, 2014). Thus, bone in the symphyseal region and associated central

incisor crypt region is younger and as such lower in density (Hernandez, 2008), when compared to the more established bone of the lateral incisor region. The lower density bone of the central incisor crypt area and the associated symphyseal region of the mandible may also be attributed to a high level of biomechanical activity originating from the tongue. The level of biomechanical demand is linked to the level of functional activity, which is associated with a variation in the degree of strain imposed on the lingual surface of the mandible. In a study investigating the effects of a reduction in the volume of the tongue on craniofacial growth, Liu *et al*. (2008a, 2008b) observed a reduction in the symphysis width and anterior dental arch width as well as a significant reduction in the bone mineral density (Liu *et al*. 2008a, 2008b). The observations made in these investigations were based on a porcine sample, making direct inferences to the human difficult. However, Hutchinson *et al*. (2014) observed similar patterns in the relationship between the growth of the tongue and the mandible in prenatal and early postnatal human samples.

The significant difference observed in bone mineral density between the second deciduous molar and the first permanent molar crypt regions along the lingual surface may be a result of a change in the orientation of the ramus. The ramus of the mandible undergoes a significant transition both in its orientation and morphology across the late prenatal and early postnatal periods of growth as a result of the increase in functional and spatial demands from the developing dentition (Hutchinson *et al*. 2012, 2014). These demands result in increased bone modelling and remodelling, which leads to the ramus transitioning from a horizontal disposition prenatally to a more vertical orientation postnatally (Scheuer & Black, 2000; Hutchinson *et al*. 2012, 2014). Furthermore, there is also a change internally within the body of the mandible, as the development and growth of the mandibular canal is thought to be synchronized with the dentition (Hutchinson *et al*. 2016).

The differences in bone mineral density across the buccal/labial surface of the mandible may be reflective of the stage of dental development. The bone mineral density in the area of the lateral incisor crypt was significantly higher than that of the canine and central incisor crypt regions, which may be attributed to the level of dental development across the three dental crypt regions. While the canine tooth only starts

development after the lateral incisor, the central incisor tooth is thought to be more advanced in its growth and development when compared to the lateral incisor (Alqahtani *et al.* 2010). The lower bone mineral density in the area of the canine may be attributed to the early stage of dental development, while the lower bone mineral density of the central incisor crypt area may be the result of an increase in bone modelling owing to increased physiological tooth movement in the area (Melsen, 1999; Dolce *et al*. 2002; Wise & King, 2008). The effect of the level of tooth development may also account for the progressively higher bone mineral density found across successional crypts in a posterior direction in the current study.

In conclusion, variations in the bone mineral density associated with the lingual surface of the mandible were observed to be more reflective of the level of development of the tongue and muscles of mastication as well as a change in the level of biomechanical demand of these structures on the lingual surface. In contrast, the variations in the bone mineral density associated with the buccal/labial surface of the mandible are more reflective of the state of dental development and associated stage of physiological tooth movement. As such, changes in bone mineral density across the mandible appear to be reflective of the stage of dental development and the level of biomechanical loading consistent with the transition from the fetal to early postnatal stages of growth.

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CHAPTER 5:

IMPORTANCE OF TEETH IN MAINTAINING THE MORPHOLOGY OF THE ADULT MANDIBLE IN HUMANS

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European Journal of Oral Sciences 2015; 123:341-349 (DOI: 10.1111/eos.12203)
(Appendix 4)

5.1 Abstract:

Edentulism can negatively affect both masticatory performance and dietary intake by altering the patterns of biomechanical stress and strain, which in turn modifies the morphology of the different regions of the mandible. The morphological changes in the mandible caused by these stressors are unknown when comparing mandibles across varying dentition states. This study investigated differences in the morphology of human mandibles across various states of the dentition. In total, 498 mandibles of individuals between 20 and 80 yr of age at death were sourced from the Raymond A. Dart Collection of Human Skeletons. These included fully dentate (n=95), partially edentulous (n=333) and fully edentulous (n=70) mandibles. Twelve linear anthropometric measurements of the mandible were derived from digitized landmarks located on its external surface. Data analysis included geometric morphometrics and multivariate analyses. Compared with dentate and partially edentulous mandibles, fully edentulous mandibles showed a shorter alveolar height (-0.4 mm), a shorter mandibular body length (-4 mm), a larger gonial angle ($+4^0$, $p<0.001$), a more obtuse mental angle ($+4.7^0$), a broader bicondylar width (+3.8 mm) and a taller ramus height (+1.6 mm). Thus, edentulous states are associated with an altered mandibular morphology, which may result from a reduction in jaw function.

Key Words: Dentition, mandible, edentulism, morphology

5.2 Introduction

The influence of diet on the general performance of the masticatory system and mandibular morphology has been a focus in a variety of studies (Carlson & van Gerven, 1977; Fukase & Suwa, 2008; Holmes & Ruff, 2011). The overall shift in food consistency from a hard to a soft diet is believed to cause a reduction in the size of the masticatory musculature as well as in the growth of the maxillomandibular complex in modern Nubians when compared to the earlier Mesolithic population (Carlson & van Gerven, 1977). This reduction led to a compensatory decrease in the size of the teeth and the associated alveolar bone (Carlson & van Gerven, 1977). Fukase & Suwa (2008) observed a similar trend in the symphyseal region of prehistoric Jomon and modern Japanese populations with a shift from a hard diet to a soft diet type. The posterior region of the Jomon mandible (hard-diet consumers) was observed to have greater body height and thicker cortical bone in comparison to their modern counterparts (Fukase & Suwa, 2008), although this was not statistically significant. A progressive decline in the general dimensions of the mandible and size of the dentition with the adoption of a more refined diet was also observed in an assessment of the Tigara and Arikara population groups (Holmes & Ruff, 2011). Thus, it may be hypothesized that refinement in the diet could lead to a more gracile mandibular morphology, with a smaller dentition, as a result of a reduction in masticatory performance (Carlson & van Gerven, 1977; Fukase & Suwa, 2008; Holmes & Ruff, 2011; Holton *et al*., 2014). However, although these studies have documented a change in the morphology of the mandible in relation to diet, they did not investigate the potential effect of different dentition states (*e.g*. dentate versus partial edentulism) on the overall morphology of the mandible.

The majority of studies investigating the effect of varying dentition states on the morphology of the mandible have focused on the relationship between alveolar bone resorption and the position of the mandibular canal (Cawood & Howell, 1988, 1991; Wyatt, 1998; Chrcanovic *et al*., 2011; Ozturk *et al*., 2013). Cawood and Howell (1988) found no significant changes in the morphology of the basal bone of edentulous adult mandibles, except in cases where the bone was subjected to harmful load effects (*e.g.* the overloading of ill-fitting dentures). However, significant decreases in both the vertical and horizontal axes of the alveolar bone were

observed following edentulism (Cawood & Howell, 1988, 1991). These significant changes in the dimensions of the alveolar bone may largely be attributed to the onset of disuse atrophy (Ortman, 1962) and residual ridge resorption (Atwood, 1979; Jahangiri *et al*., 1998) following tooth loss. Chrcanovic *et al*. (2011) observed that the overall dimensions of the mandible were larger in dentulous individuals than in edentulous individuals. They concluded that with tooth loss, the resorption of the crest of the alveolar bone influenced the position of the mental foramen (Chrcanovic *et al*., 2011). In addition, Ozturk *et al*. (2013), in their assessment of the ramus height and general corpus dimensions of dentulous and partially edentulous adult mandibles, found that the number of teeth was the primary determinant of changes in the size and shape of the mandible. Although the effect of each dentition state on the mandibular morphology has been previously documented (Cawood & Howell, 1988, 1991; Chrcanovic *et al*., 2011; Ozturk *et al*., 2013), the influence of the varying tooth combinations on the general morphology of the mandible has not been investigated across all states in a particular sample of mandibles. Studies investigating the changes in the morphology of the mandible relative to a particular dentition state have also made use of small samples and undefined age ranges, which may act as confounders in the overall analysis of these samples. In addition, the majority of these studies have made limited use of geometric morphometrics and multivariate means of analysis, which effectively limits the degree of inferences that may be made with regards to changes in the morphology of the mandible.

Considering the results of earlier studies in which morphological changes in the mandible were observed in isolation in either the dentulous, partially edentulous or edentulous states specifically (Carlson & van Gerven, 1977; Cawood & Howell, 1988, 1991; Wyatt, 1998; Fukase & Suwa, 2008; Chrcanovic *et al*., 2011; Holmes & Ruff, 2011; Ozturk *et al*., 2013; Holton et al., 2014;), we hypothesized that the severity of edentulism would be significantly associated with the size and shape of the mandible, with a morphometric gradient from the fully dentate to the fully edentulous state. Thus, the aim of this study was to investigate how the morphology of the mandible changed with the onset of edentulism.

5.3 Materials and methods

5.3.1 Materials

Mandibles from early adulthood extending to the advanced stages of senescence were sourced from the Raymond A. Dart Collection of Human Skeletons, School of Anatomical Sciences, University of the Witwatersrand, South Africa. This collection of skeletons has been accumulated since 1924 to present. Four-hundred and ninety-eight mandibles were assessed, from 262 male and 236 female skeletons (Table 1). Skeletal samples were obtained under the Human Tissue Act 65 of 1983 (Appendix 1), and ethical clearance (M121145) to utilize the sample was obtained from the Human Research Ethics Committee – medical (Appendix 2).

The age of specimens ranged from 20 to 80 yr. The ages of the skeletal specimens were assessed based on the state of the ante-mortem dentition in addition to the recorded age at death of each individual. The sample was subdivided into three age groups: group 1, 20-40 yr; group 2, 41-60 yr; and group 3, 61-80 yr (Table 1). The state of the dentition of the sample was determined based on the condition of the dental crypts and limited to the mandible. Individuals presenting with a full permanent dentition (third molars included) were classified as dentulous. Edentulous individuals were classified based on the complete/partial closure of the dental crypts and absence of the entire dentition (Cawood & Howell, 1988, 1991). In the case of individuals presenting with an incomplete dentition and/or intact dental crypts (postmortem tooth loss), the mandible was classified as partially edentulous (Table 1). Individuals classified as partially edentulous presented with dentition sets that ranged from the presence of only one tooth to 15 mandibular teeth.

Table 1*:* Age, sex and dental state of the specimens sourced from the School of Anatomical Sciences, University of the Witwatersrand

Age group	Sex	Dentulous	Partially Edentulous	Edentulous	Total
Group 1 (n=174)	M	36	47	4	87
20-40 yr	F	24	61	2	87
Group 2 (n=166)	M	14	64	8	86
41-60 yr	F	11	58	11	80
Group 3 (n=158)	M	8	56	25	89
61-80 yr	F	2	47	20	69

F: Female; M: male

Individuals showing evidence of damage to the mandible, congenital abnormalities, bony tissue pathologies or surgical intervention, were excluded from the study. Evidence of previous orthodontic treatment, such as lingual retainers, also led to exclusion from the study.

5.3.2 Methods

An Immersion Microscribe G2 (Solution technologies, Oella, MD, USA), with an accuracy of 0.38 mm, was used to record 21 landmarks on the mandible (Fig. 1A). These landmarks included the points necessary to calculate the linear measurements and to define the shape of the mandible. To standardize the collection of landmark data, the dry mandibles were placed in a fixed position (Fig. 1). Landmarks were recorded in a pre-set sequence (clockwise direction) (Fig. 1).

Using each landmark, three coordinates (X, Y, Z) were recorded and represent the horizontal, vertical and depth planes, respectively. The three-dimensional coordinates were then imported into a Microsoft Excel worksheet and the Pythagorean formula used to convert the data into linear distances:

$$= \sqrt{(x_1-x_2)^2 + (y_1-y_2)^2 + (z_1-z_2)^2}$$

where x_1, y_1, z_1 and x_2, y_2, z_2 refer to the coordinates of the first and second landmarks, respectively.

Twelve standard anthropometric measurements, derived from previous research (De Villiers, 1968; Loth, 1995; Scheuer & Black, 2000; Hesby *et al*., 2006; White *et al.*, 2011; Hutchinson *et al*., 2012), were recorded using digitized points. In addition, the measurements for the maximum bicondylar width (MBW) and the distance between the mental foramina and the superior border of the mandible (MF_SB) were specifically designed for the purposes of this study (Fig. 1). All measurements assessed morphological and size changes in the sagittal, coronal and transverse planes of the mandible and are described in detail in Hutchinson *et al*. (2014).

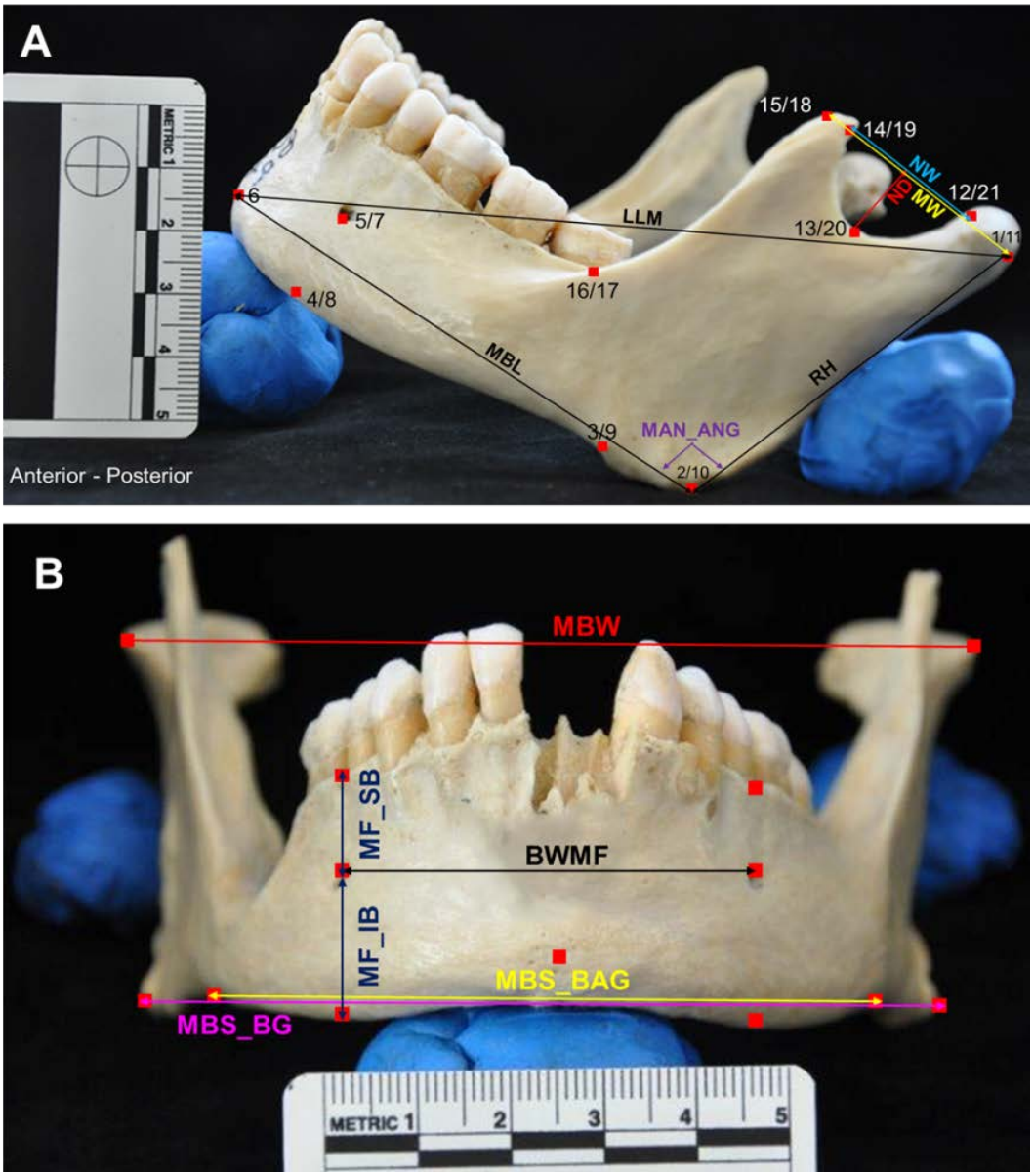


Figure 1: (A) Lateral view of the left side of the mandible indicating the digitizing position used for osteological specimens (Raymond A. Dart Collection), as well as the measurements corresponding to the sagittal plane of assessment. The mental region is elevated and secured in place anteriorly by morphometric putty. The ramus of the mandible is secured posteriorly mid-way along the ramus to expose all necessary points for digitization. Twenty-one landmarks were recorded across each mandible (landmarks 1-5 and 17-21 recorded on the right side of the mandible and landmarks 7-16 on the left side of the mandible). (B) Anterior view of the mandible indicating the transverse and vertical measurements. BWMF, mental interforaminal distance; LLM, maximum length of the mandible; MBL, mandibular body length; MBS_BAG, mandibular basilar width bigonial; MBW, maximum bicondylar width; MEA, mental angle; MF_IB, distance between the inferior border of the mandible and the mental foramen; MF_SB, distance between the mental foramina and the superior border of the mandible; MW, maximum width of the mandible; ND, depth of the mandibular notch; NW, width of the mandibular notch; RH, height of the ramus.

Furthermore the measurements used to calculate the mental angle (MEA) included the length of the mandibular body of each side, as well as the mandibular basilar width bigonial. The gonial angle (MAN_ANG) was calculated using the height of the ramus, the length of the mandibular body, and maximum length of the mandible measurements for the left and right sides, respectively (Fig. 1). The cosine rule $CosC= (a^2 + b^2 - c^2)/(2ab)$ was then used to calculate the MEA and the MAN_ANG because the triangles formed by the measurements were not considered to be right-angled triangles; thus the Pythagorean equation could not be used.

5.3.3 Statistical and morphometric analysis

Descriptive statistics, including means and SD, were calculated for all mandibular measurements. The Shapiro-Wilk's test was used to assess the distribution of the sample, which further determined statistical analyses based on the distribution type (Zar, 1999). All statistical analyses were conducted using PAST (Paleontological Statistics, Paleontological Association, London, UK) (Hammer *et al*., 2001) and SPSS v. 20 (IBM, Armonk, NY, USA) statistical programs.

A two-way ANOVA was conducted to evaluate the relationship between the dependent variables *i.e*. the anthropometric measurements and the independent variables, which included age and dentition state. The degree of interdependency between factors was also evaluated (Zar, 1999). In cases where a significant degree of interaction occurred between factors, a Tukey's honest significant difference (HSD) post-hoc test and pairwise comparisons were conducted to compare the independence of factors and the associated level of significance (Zar, 1999). Additionally, the relationship between size and shape was tested using a multivariate analysis of variance (MANOVA) (Zar, 1999). Furthermore a multivariate analysis of covariance (MANCOVA) was conducted in which the geometric mean was selected as the covariate (Riffenberg, 2006). In general, the effects of age and dentition, and the interaction between age and dentition, were investigated further if variables between subjects were significant ($P < 0.05$).

To visualize the differences in the size and shape of the mandible with growth and varying state of the dentition, the sample data set was subjected to a generalized full Procrustes analysis and principal component analysis using PAST (Hammer *et al*., 2001) and MORPHOLOGIKA 2 v. 2.5 (University College London, UK) (Rohlf, 1999; Mitterocker & Gunz, 2009) respectively. A generalized full Procrustes analysis (Mitterocker & Gunz, 2009) was performed, which eliminates the influence of size, rotation, and translation of the mandible. Data was then organized according to dentition state (dentulous, partially edentulous and edentulous) and the mean shape of each dentition state was assessed using wireframes.

Inter- and intra-observer errors were evaluated using repeated measurements on 10 random specimens that were then tested using the Lin's concordance correlation co-efficient of reproducibility, which provides information on the level of repeatability of the method and the degree of association between measurements (Allan, 1982).

5.4 Results

A very high degree of repeatability (79-99%) was observed across the 14 measurements assessed in the current study. However, a moderate level of repeatability was also observed in the distance between the inferior border of the mandible and the mental foramen (60%).

Table 2: Two-way analysis of variance (ANOVA) investigating the between-group effects of age and dentition on all assessed measurements

		Tukey's post-hoc test (P < 0.05)				Tukey's post-hoc test (P < 0.05)		
Measurement	Age (P < 0.05)	Group 1 vs. Group 2	Group 2 vs. Group 3	Group 1 vs. Group 3	Dentition (P < 0.05)	Group 1 vs. Group 2	Group 2 vs. Group 3	Group 1 vs. Group 3
GM	0.69	-	-	-	0.01	0.00	0.48	0.00
MBS_BG	0.28	-	-	-	0.35	-	-	-
MBS_BAG	0.20	-	-	-	0.09	-	-	-
BWMF	0.11	-	-	-	0.05	0.00	0.09	0.00
MF_IB	0.13	-	-	-	0.56	-	-	-
MF_SB	0.20	-	-	-	0.00	0.00	0.00	0.00
MBL	0.24	-	-	-	0.00	0.00	0.16	0.01
LLM	0.14	-	-	-	0.37	-	-	-
MW	0.55	-	-	-	0.01	0.00	0.35	0.00
NW	0.41	-	-	-	0.06	-	-	-
ND	0.42	-	-	-	0.46	-	-	-
RH	0.24	-	-	-	0.00	0.13	0.92	0.02
MEA	0.86	-	-	-	0.00	0.00	0.02	0.00
MBW	0.04	0.00	0.22	0.00	0.01	0.00	0.52	0.00
MAN_ANG	0.05	0.16	0.87	0.05	0.00	0.00	0.00	0.06

BWMF, mental interforaminal distance; GM, geometric mean; LLM, maximum length of the mandible; MAN_ANG, gonial angle; MBL, mandibular body length; MBS_BAG, mandibular basilar width biantegonial; MBS_BG, mandibular basilar width bigonial; MBW, maximum bicondylar width; MEA, mental angle; MF_IB, distance between the inferior border of the mandible and the mental foramen; MF_SB, distance between the mental foramina and the superior border of the mandible; MW, maximum width of the mandible; ND, depth of the mandibular notch; NW, width of the mandibular notch; RH, height of the ramus; -, indicates absence of data.

The overall effect of age and the state of the dentition on all measurements assessed was first tested independently using a two-way ANOVA. Both age and the state of the dentition were observed to have an effect on the MBW ($P<0.04$ and $P<0.01$, respectively) and on the MAN_ANG ($P<0.05$ and $P = 0.00$, respectively). Whilst the age of the individual had no further effect on the mandibular measurements, the state of the dentition influenced the geometric mean (GM) ($p<0.01$), the mental interforaminal distance (BWMF) ($p<0.05$), the MF_SB ($P = 0.00$), and the mandibular body length (MBL) ($P = 0.00$). In addition, the state of the dentition further influenced the mandibular width (MW) ($P < 0.01$), the height of the ramus (RH) ($P = 0.00$) and the MEA ($P = 0.00$) (Table 2).

The only differences in the overall size and shape (MANOVA) of the mandible associated with age, were found for MBW ($P < 0.036$) and MAN_ANG ($P < 0.046$), which were larger in the older age group (61-80 yr) than in the younger age groups (Table 3, Fig. 2). The differences in the position of the ramus became more obvious with the comparison between dentate and edentulous mandibles, in which the MBW ($P < 0.012$), the MAN_ANG ($P = 0.00$), and the RH ($P < 0.00$) were significantly larger in edentulous than in dentate mandibles (Table 3, Fig. 2).

The width of the mandible was significantly smaller ($P < 0.006$) in the edentulous mandibles than in the dentate and partially edentulous mandibles across the 41-80 yr age range ($P < 0.014$) (Tables 2 and 3). In addition to the overall size and shape variations observed in the rameal region of the mandible, the arch of the mandible was significantly shorter across the length of the mandibular body ($P < 0.000$) in the edentulous mandible in comparison to the dentate and partially edentulous mandibles (Table 3). The height of the mandibular body was also significantly reduced in the edentulous mandible. The MF_SB was significantly shorter ($P = 0.00$) in the alveolar region of edentulous mandibles, particularly when compared to the partially edentulous group (Tables 2 and 3). Furthermore, along the anterior mental region of the mandible, the influence of age and the absence of the dentition ($P < 0.001$) culminated in a significantly smaller BWMF ($P < 0.047$) (Tables 2 and 3, Fig. 2). No further significant changes in the BWMF were observed when comparing the partially edentulous and edentulous groups. In contrast, the MEA was significantly

larger (P =0.000) in the edentulous individuals, whereas that in the dentulous individuals, and particularly the partially edentulous individuals, was small (Table 3, Fig. 2). The edentulous mandible was also observed to be significantly smaller than partially edentulous and dentate mandibles (GM, P = 0.009).

Table 3: Multivariate analysis of variance (MANOVA) indicating the pairwise comparisons for each of the age groups and dentition states associated with the individual measurements

	Age	Mean ± SD (mm)			Dentition (p<0.05)				Mean ± SD (mm)		
Measurement	Univariate F (p<0.05)	Group 1: (20-40 yr) (n=174)	Group 2: (41-60 yr) (n=166)	Group 3: (61-80 yr) (n=158)	Univariate F (p<0.05)	Dentulous vs. Edentulous	Dentulous vs. Partially Edentulous	Partially Edentulous vs. Edentulous	Dentulous	Partially Edentulous	Edentulous
GM	0.4 (0.69)	47.6 ± 2.4	47.7 ± 2.7	47.0 ± 2.7	4.7 (0.01)	0.00	0.09	0.02	47.9 ± 2.5	47.6 ± 2.6	46.1 ± 2.6
MBS_BG	1.3 (0.28)	89.1 ± 6.2	91.6 ± 6.8	91.7 ± 6.3	1.1 (0.35)	-	-	-	90.0 ± 6.2	90.6 ± 6.7	92.3 ± 6.3
MBS_BAG	1.6 (0.20)	81.7 ± 5.0	84.1 ± 4.9	83.9 ± 4.73	2.4 (0.09)	-	-	-	82.6 ± 5.1	83.1 ± 5.0	84.5 ± 4.5
BWMF	2.2 (0.11)	44.8 ± 3.0	44.8 ± 2.8	44.6 ± 3.1	3.1 (0.05)	0.06	0.02	0.79	45.5 ± 3.0	44.8 ± 2.9	43.5 ± 2.8
MF_SB	1.6 (0.20)	15.1 ± 2.5	13.9 ± 3.3	12.3 ± 3.5	69.2 (0.00)	0.00	0.00	0.00	13.4 ± 1.6	13.2 ± 1.6	13.0 ± 1.7
MF_IB	2.0 (0.13)	13.5 ± 1.6	13.0 ± 1.5	13.2 ± 1.6	0.6 (0.56)	-	-	-	15.4 ± 2.2	14.3 ± 2.8	9.1 ± 3.0
MBL	1.4 (0.24)	82.7 ± 5.1	84.3 ± 5.2	83.2 ± 5.3	8.5 (0.00)	0.00	0.00	0.12	84.5 ± 5.8	83.4 ± 5.1	81.5 ± 4.6
LLM	2.0 (0.14)	114.8 ± 5.8	117.6 ± 6.2	116.4 ± 5.8	1.0 (0.37)	-	-	-	115.8 ± 6.5	116.2 ± 5.9	117.0 ± 5.7
MW	0.6 (0.55)	38.9 ± 3.8	38.5 ± 3.9	38.0 ± 3.8	5.1 (0.01)	0.00	0.03	0.04	39.2 ± 4.0	38.6 ± 3.8	36.7 ± 3.4
NW	0.9 (0.41)	26.5 ± 3.3	27.0 ± 3.2	26.9 ± 3.3	2.9 (0.06)	-	-	-	27.1 ± 3.6	26.8 ± 3.2	26.3 ± 2.9
ND	0.9 (0.42)	12.0 ± 3.4	12.0 ± 3.4	11.5 ± 3.5	0.8 (0.49)	-	-	-	12.0 ± 3.6	11.7 ± 3.4	11.8 ± 3.1
RH	1.4 (0.24)	56.0 ± 5.1	57.1 ± 5.8	56.5 ± 5.8	5.9 (0.00)	0.01	0.67	0.00	56.5 ± 4.9	56.2 ± 5.6	58.1 ± 5.8
MBW*	3.3 (0.04)	104.7 ± 5.4	107.6 ± 6.0	108.7 ± 5.6	4.5 (0.01)	0.01	0.81	0.00	105.9 ± 6.3	106.7 ± 5.6	109.7 ± 6.0
MEA	0.1 (0.86)	65.2 ± 4.5	66.0 ± 5.0	67.1 ± 5.6	12.1 (0.00)	0.00	0.00	0.00	64.4 ± 4.0	65.9 ± 5.0	69.1 ± 5.5
MAN_ANG**	3.1 (0.05)	110.4 ± 4.9	111.4 ± 4.9	111.7 ± 5.2	8.5 (0.00)	0.01	0.00	0.96	108.9 ± 4.7	111.4 ± 4.9	112.9 ± 5.0

*Statistically significant differences in MBW were found for the group 1 vs. group 2 ($P < 0.01$) post-hoc comparison, within the age assessment.

**Statistically significant differences in MAN_ANG were found for the group 1 vs. group 2 ($P < 0.01$) post-hoc comparison, within the age assessment.

BWMF, mental interforaminal distance; GM, geometric mean; LLM, maximum length of the mandible; MAN_ANG, gonial angle; MBL, mandibular body length; MBS_BAG, mandibular basilar width biantegonial; MBS_BG, mandibular basilar width bigonial; MBW, maximum bicondylar width; MEA, mental angle; MF_IB, distance between the inferior border of the mandible and the mental foramen; MF_SB, distance between the mental foramina and the superior border of the mandible; MW, maximum width of the mandible; ND, depth of the mandibular notch; NW, width of the mandibular notch; RH, height of the ramus; -, indicates absence of data.

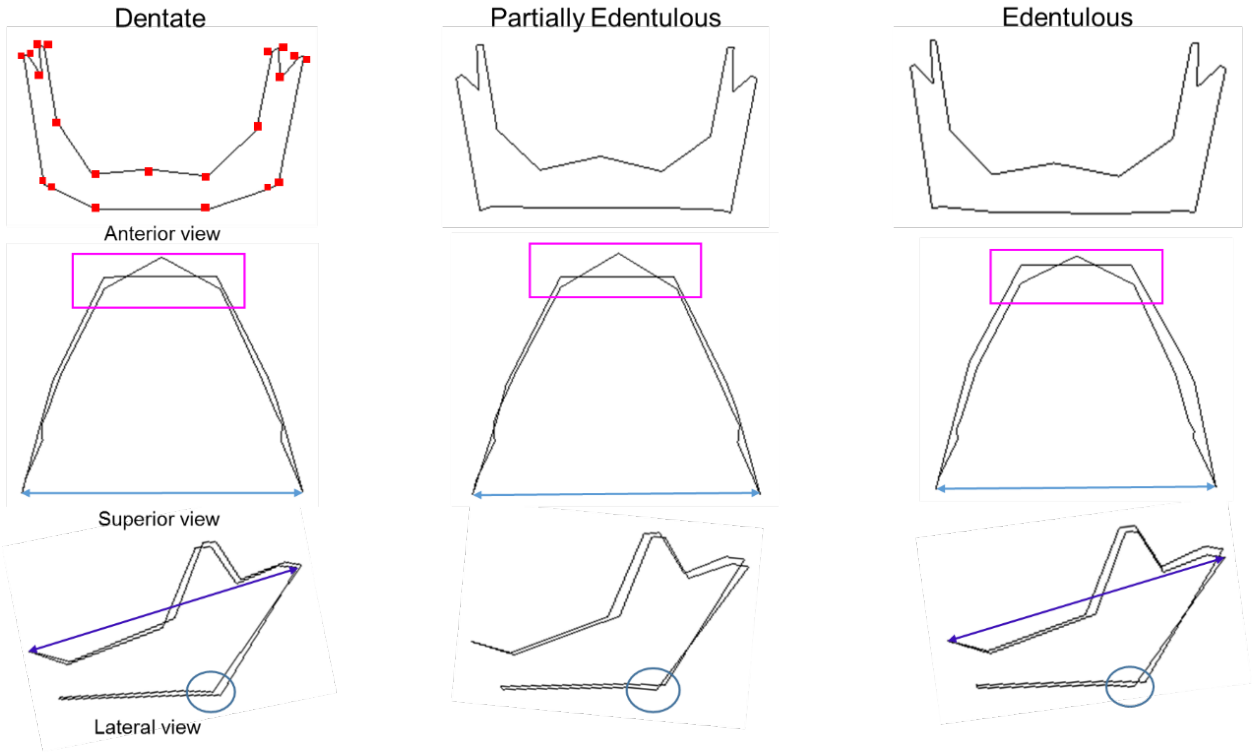


Figure 2. Principal component analysis wireframes illustrating the superimposed superior and inferior surfaces (superior view) and left and right lateral surfaces (lateral view) and anterior view. A widening of the mandibular arch in the mental region (pink rectangle) and bigonial width (light blue line) in the superior view, as well as a reclining of the ramus indicated by an increase in the length of the mandible (dark blue line) and deepening of the antegonial notch (blue circle) in the lateral view, were all indicated across the assessed dentition states

When comparing mandibles across age groups, the MAN_ANG of elderly individuals was significantly larger ($P < 0.046$) than that of individuals in the younger groups, and the body of the mandible was significantly longer in elderly individuals than in individuals of younger groups, both in the maximum length of the mandible (LLM) ($P < 0.004$) and in the MBL ($P < 0.031$). The mandibular arch was broader postero-superiorly, with a significantly wider MBW ($P < 0.006$) in the elderly group (Table 4, Fig. 2).

When comparing mandibles across the dentate, partially edentulous and edentulous dentition states, the significantly larger MAN-ANG ($P = 0.001$) in the edentulous group gave the ramus of the mandible a reclined appearance (Table 4). This gave the edentulous mandibular arch a longer appearance in the sagittal plane. This longer appearance was further emphasized by a significantly taller ramus ($P < 0.000$) and increased maximum length of the mandible (LLM) ($P = 0.000$) in the edentulous group (Fig. 2). A significantly shorter MBL ($P < 0.005$) and MF_SB ($P = 0.000$) resulted in the appearance of a vertically compressed mandibular body in the edentulous mandible (Table 4, Fig. 2). The compression of the body was further accentuated by a significantly wider MEA ($P = 0.000$), mandibular basilar bigonial (MBS_BG) ($P < 0.001$) and biantegonial widths (MBS_BAG) ($P = 0.000$) across the transverse plane in the edentulous mandibles. In addition, the generally everted appearance of the ramus was further emphasized by a significantly larger MBW of the mandible ($P < 0.000$) and mandibular notch depth (ND) ($P < 0.012$), particularly when comparing the edentulous and partially edentulous groups (Fig. 2).

Table 4: Multivariate analysis of co-variance (MANCOVA) indicating the pairwise comparisons for age and the dentition states associated with the individual measurements

Measurement	Age Univariate F (P < 0.05)	Dentition (P < 0.05) Univariate F (P < 0.05)	Dentate vs. Edentulous	Dentate vs. Partially edentulous	Partially edentulous vs. Edentulous
MBS_BG	2.6 (0.08)	7.0 (0.00)	0.00	0.17	0.00
MBS_BAG	2.7 (0.07)	10.6 (0.00)	0.00	0.24	0.00
BWMF	2.2 (0.11)	1.9 (0.15)	-	-	-
MF_SB	1.3 (0.28)	72.7 (0.00)	0.00	0.01	0.00
MF_IB	1.9 (0.14)	1.9 (0.15)	-	-	-
MBL*	3.5 (0.03)	5.3 (0.01)	0.02	0.00	0.85
LLM**	5.5 (0.00)	9.8 (0.00)	0.00	0.60	0.00
MW	1.0 (0.38)	1.3 (0.26)	-	-	-
NW	2.5 (0.09)	1.3 (0.28)	-	-	-
ND	0.9 (0.41)	4.5 (0.01)	0.01	0.93	0.00
RH	1.1 (0.35)	19.4 (0.00)	0.00	0.46	0.00
MBW*	5.1 (0.01)	12.2 (0.00)	0.00	0.23	0.00
MEA	0.1 (0.87)	12.8 (0.00)	0.00	0.00	0.00
MAN_ANG***	3.1 (0.05)	7.1 (0.00)	0.04	0.00	0.56

*Statistically significant difference in MBL was found for group 1 vs. group 3 (P < 0.01). **Statistically significant differences in LLM and MBW were found for group 1 vs. group 2 (P < 0.00) and for group 1 vs. group 3 (P < 0.01). *** Statistically significant difference in MAN_ANG was found for group 1 vs. group 2 (P < 0.01) post-hoc age comparisons.BWMF, mental interforaminal distance; LLM, maximum length of the mandible; MAN_ANG, gonial angle; MBL, mandibular body length; MBS_BAG, mandibular basilar width biantegonial; MBS_BG, mandibular basilar width bigonial; MBW, maximum bicondylar width; MEA, mental angle; MF_IB, distance between the inferior border of the mandible and the mental foramen; MF_SB, distance between the mental foramina and the superior border of the mandible; MW, maximum width of the mandible; ND, depth of the mandibular notch; NW, width of the mandibular notch; RH, height of the ramus; -, indicates absence of data.

5.5 Discussion

The state of the dentition may influence the functional ability of the jaw (Julien *et al*., 1996; Lepley *et al*., 2011), and this, in turn, may affect the remodelling of mandibular bone (Holton *et al*., 2014). As a result of this interaction, we hypothesized that variation in the state of the dentition would have an effect on the mandibular morphology.

The overall dimensions of the edentulous mandibles were significantly smaller than those of mandibles where teeth were present, with the exception of the MEA and MAN_ANG. Therefore, the presence of the dentition appears to maintain an upright ramus and a morphologically distinct mental region.

The significantly lower height of the mandibular corpus observed in the edentulous mandible when compared to the dentate mandible, may be explained by alveolar bone resorption resulting from tooth loss (Cawood & Howell, 1988), disuse atrophy (Ortman, 1962) and residual ridge resorption (Atwood, 1979; Jahangiri *et al*., 1998). Bone resorption and formation may persist throughout life at a reduced rate (Tallgren, 1972; Atwood, 1979; Jahangiri *et al*., 1998). The subsequent alteration in the morphology of the residual alveolar ridge affects the retention and functioning of dentures, which both rely on the quantity of bone, the architecture of the mandible (Jahangiri *et al*., 1998), and the overall efficiency of the masticatory apparatus.

Tooth loss accompanied by the resorption of the residual alveolar ridge can influence the overall relationship between the maxilla and mandible, as well as muscle function and facial morphology (Cawood & Howell, 1991). These changes may have occurred in our study sample because a significant decrease in the overall size as well as the length of mandibular body was observed in edentulous individuals when compared to their dentate counterparts. In addition, a significantly increased MBW and MEA were observed in the edentulous mandibles when compared to their dentulous counterparts. The shorter mandibular body and increased MBW and MEA may be attributed to bone resorption expedited by changes in bone density (Kribbs &

Chesnut, 1984; Kribbs, 1990; Krall *et al*., 1994). The shortening and widening of the dental arch found in individuals aged 40 years and older can be the result of resorption of the buccal cortex of incisor alveolar bone and of the lingual cortex of molar alveolar bone, which, in turn, may be facilitated by a decrease in bone density (Atkinson & Woodhead, 1968). In addition, the larger MBW of the mandible and the wider MEA observed in our study, can be explained by an increase in the buccal cortical thickness of the mandible, accompanied by bone resorption lingually. The shorter mandibular body would also have an effect on the overall geometry of the mandibular arch.

In the current study, the LLM and the MAN_ANG were significantly larger in edentulous individuals when compared to their dentate counterparts. The ramus of the mandible in edentulous individuals also appears to be in a reclined position. The overall increase in the MAN_ANG with molar tooth loss (Oettle *et al*., 2009) has been previously attributed to the so-called ‘moulding effect’ of the masticatory muscles on the mandibular morphology in an attempt to restore the occlusal plane and maintain optimal mastication (Oettle *et al*., 2009). This effect on mandibular morphology is thought to be dependent on the functionality of the masticatory muscles (Raustia *et al*., 1996). However, in the present study, as specimens were derived from a skeletal population, no inference can be made regarding the anatomy or level of activity of the muscles of mastication. In addition, as specimens presenting with pathologies were excluded from our study sample, the potential influence of systemic influences, such as osteoporosis and osteopenia, on the individual mandible have not been considered.

While a larger MAN_ANG is in agreement with previous studies assessing the transition between dentulism and edentulism (Ohm & Silness, 1999; Dutra *et al*., 2004; Huumonen *et al*., 2010; Joo *et al*., 2013; Uma *et al*., 2013), the larger RH is not consistent with the literature. The progressive loss of the natural dentition results in an alteration of occlusal relationships, rotations of the mandible, realignment of the mandibular corpus and changes in the regional morphometry of the mandible (Uma *et al*., 2013). The overall change in the occlusal relationship further results in an altered pattern of the intensity and frequency of stress and strain imposed on the

bone by the surrounding structures, which affect the balance between bone resorption and remodelling (Frost, 1987, 2003; Groning *et al*., 2013). This may assist in explaining why the RH in edentulous mandibles was larger than their dentate counterparts. Indeed, this may be the result of condylar remodelling due to the different physiological demand associated with edentulism. Interestingly, as the loss of the dentition proceeds, there is a reduction in the overall facial height and vertical dimensions of occlusion, resulting in mandibular prognathism (Raustia *et al*., 1998; Kumar, 2014; Small *et al*., 2015).

The overall prognathism of the mandible with the onset of edentulism is further accentuated by the glenoid fossa adopting a more anterior position in edentulous individuals compared to its more posterior position in dentate individuals (Raustia *et al*., 1998). While there is a change in the antero-posterior position of the glenoid fossa with a reduction in the vertical facial dimensions, there is no further change in the lateral position of the glenoid fossa in edentulous individuals (Small *et al*., 2015). However, the maximum bite force of the muscles of mastication is also influenced by a change in the vertical dimensions, with the elevator muscles having a greater mechanical advantage when the ramus is more vertical and the MAN_ANG more acute (Bakke, 2006). In the current study, the ramus was observed to be significantly narrower in the edentulous mandible than in the mandibles of dentate and partially edentulous individuals. No additional significant differences were observed in the dimensions of the mandibular notch across the various dentition states. The narrowness of the ramus with edentulism may be attributed to bone resorption resulting from a decrease in bite force associated with tooth loss and denture use (Frost, 1987, 2003; Huumonen et al., 2010; Groning *et al*., 2013).

The present study has a number of methodological limitations that should be considered. The study design is cross sectional and not longitudinal. This effectively limits any causal inferences regarding changes in morphology with age. Furthermore, there is a potential confounding effect of secular trends. The concept of a secular trend is described within the context of an overall increase in the dimensions of the body over a substantial period of time and is widely discussed within the scope of growth related studies (Henneberg & van der Berg, 1990; Martin & Danforth, 2009;

Antoun *et al*., 2014). In addition, the occurrence of secular trends has previously been ascribed to either an individual's genetic potential or their socioeconomic status (Tobias, 1985). However, the genetic admixture and magnitude of trends across various socioeconomic settings within the South African population have essentially reduced the potential for secular trends within this population group (Henneberg & van der Berg, 1990).

The strengths of this study include a relatively large number of observations, the use of geometric morphometrics to describe shape templates, and the use of multivariate data analysis. As a result, the multivariate analysis used in the current study allowed for the investigation of the individual influences of age and the state of the dentition as potential confounders on the sample.

In conclusion, mandibular edentulism is associated with site-specific effects on mandibular size and shape. The morphological differences that were found between the various states of the dentition may be ascribed to the altered functional activities of the jaw that accompany the onset of edentulism.

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CHAPTER 6:

DISCUSSION

The human mandible has been intimately studied to assess how it develops and grows. However, investigations have either focused on the early prenatal period of its growth (Spyropoulos, 1977; Lee *et al*., 2001; Radlanski *et al*., 2002; 2003; 2015) or have considered aspects in older age cohorts (Lowe *et al*., 1985; Koolstra, 2002; Karup *et al*., 2005; Coquerelle *et al*., 2012). Those studies which have assessed how the mandible adapts to its changing functional environment, have made use of a variety of animal based models (Mulder *et al*., 2006; Liu *et al*., 2008a; Radlanski *et al*., 2016). However, as the animals used in these models have varying periods of gestation, direct inferences between these models and that of the growth of the human mandible is difficult. Thus, the present study endeavoured to gain an understanding of the development and growth of the human mandible across the functionally complex late prenatal and early postnatal period of growth.

While this study offers insights into the development and growth of the mandible during a particularly complex period of growth, there were some limitations in the nature of the sample that should be noted. The current study is cross sectional and not longitudinal in design. The cross sectional nature of the study, as well as the exclusive use of the mandible alone, ultimately limits the level of causal inferences regarding changes in age or function. This is particularly relevant within a functional dental context, where the inclusion of the maxilla may have provided some clarity around the biomechanical relationships associated with varying states of malocclusion. However, in a forensic context, the juvenile mandible is recovered more often than the maxilla and is thus often used in the estimation of age of unidentified individuals. Thus, the mandible alone was the focus of the present study.

In the assessment of the effects of a change in the state of the dentition on the morphology of the mandible, the study made use of dentate, partially edentulous and edentulous mandibles. However, when investigating the effects of partial edentulism on the morphology of the mandible, no distinction was made between the various tooth combinations present. This again effectively limits the level of causal inferences that may be made, as different tooth combinations will have an impact on the level of mastication, which in turn will influence the morphology of the mandible over time.

Furthermore, as part of the sample was sourced from a forensic setting and detailed demographic data for each specimen was not available, there is an osteological paradox that should be considered. The osteological paradox considers three main sample influences namely: demographic non-stationarity, selective mortality and hidden heterogeneity in risks (Wood *et al*., 1992; De Witte & Stojanowski, 2015). Demographic non-stationarity is broadly defined as a population's departure from a stationary state, which may be characterized by a closure to migration, zero growth rates or an equilibrium being reached in the age distribution of the population (Wood *et al*., 1992; De Witte & Stojanowski, 2015). Selective mortality in particular is related to the selection of a sample. This concept highlights the limitations in sample selection, where a study sample may never fully include all individuals within a population that may have been at risk of the disease under investigation (Wood *et al*., 1992; De Witte & Stojanowski, 2015). Hidden heterogeneity in risks draws attention to the limitation that the population from which a skeletal sample is drawn, may have comprised an unknown mixture of individuals with varying levels of underlying frailty or potential susceptibility to disease or death (Wood *et al*., 1992). While the osteological paradox has mostly been applied to the interpretation of skeletal lesions within a paleobiology and paleopathological setting (Wood *et al*., 1992; De Witte & Stojanowski, 2015), the principle should be considered within the context of the current study. Any specimens presenting with obvious pathologies or developmental abnormalities were excluded. As the nutritional status and any underlying metabolic diseases of the present sample are not known, inferences concerning the effects of any metabolic disturbances on the structure and morphology of the mandible fall outside the scope of this study. Thus, the study made use of ratios or absolute values rather than Hounsfield's units, as a means to describe a pattern of change in bone mineral density versus quantifying the bone density of an area.

Bone density is a reflection of both the total amount of mineralized bone tissue as well as the volume of bone (Burr & Allen, 2014). While the present study assessed changes in bone mineral density across the body of the immature mandible, it did not specifically assess changes in the amount of mineralized bone tissue or change in the volume of bone. Thus, the depth of inferences regarding changes in bone density

across the immature mandible are limited to bone mineral density changes as a whole, and cannot include direct inferences to the degree of mineralization of the assessed area of tissue or changes to the volume of bone studied.

The present study first investigated the nature of the growth relationship between the immature mandible and the tongue, because of the significant role the tongue plays in performing actions such as sucking and suckling. As early as the 12th week of gestation, the elevation, protrusion and retraction of the tongue assists in the process of swallowing (Humphrey, 1971). During the perinatal stage of growth, the protrusive and retractive actions of the tongue are intensified in response to increased suckling demands (Delayney & Arvedson, 2008). In the first and subsequent years of postnatal growth, the changes in posture and functioning of the tongue are further increased with the onset of mastication (Delayney & Arvedson, 2008). These changes in the posture and function of the tongue coincide with a dynamic period in the development and growth of the mandible. As such, understanding the nature of the growth relationship between the tongue and mandible is fundamental when interpreting the changes in the morphology and function of these structures. A strong correlation between the changes in the dimensions of the mandible and the tongue was observed across age group comparisons between 20 gestational weeks and 2 years of age. It was thus hypothesized that the growth of the immature mandible may be directed by the growth of the tongue. This could result in areas of increased bone modelling and remodelling, directly associated with the attachment of the tongue to the body of the mandible, which could be representative of an increase in loading conditions. The increase in loading conditions could manifest as areas of bone with a lower density, as a result of the high level of bone modelling and remodelling (Hernandez, 2008).This, in fact, was evident from the subsequent study of bone mineral density.

Variations in bone mineral density of the body of the immature mandible were assessed, to ascertain if a change in the loading conditions of the tongue influenced the growth and morphology of the mandible. The lingual surface of the mandible had significantly higher bone mineral density values when compared to the external surface of the mandible across the assessed period of 30 gestational weeks to 5

years of growth. In addition, the variations in the bone mineral density across the external surface of the mandible followed the patterns of dental development during the postnatal periods of growth. Thus, bone mineral density was higher across those dental crypts where the dentition was in the advanced stages of development relative to those dental crypts with teeth in the early stages of dental development. This led to the conclusion that the development of the dentition may play an important role in the remodelling of the mandible, which could thus not be solely attributed to the tongue.

The present study also considered what kind of effect a change in the state of the dentition would have on the morphology of the adult mandible, as a change in bone density due to dental development was found. Edentulism resulted in significant changes across the morphology of the whole mandibular complex. The edentulous mandible had a shorter alveolar height and mandibular body length as well as more obtuse gonial and mental angles, when compared to the dentate mandible. In addition, the bicondylar width was broader and the ramus was taller in the edentulous mandible when compared to the dentate mandible. These changes in the morphology of the mandible appear to be indicative of an altered morphology resulting from a reduction in jaw function. These have previously also been well described (Atkinson & Woodhead, 1968; Tallgren, 1972; Huumonen *et al*., 2010; Kumar, 2014).

With respect to the general dimensions of the mandible between 20 gestational weeks and 3 years of age and considering that both the tongue and the dentition influenced the growth of the mandible, the general dimensions of the mandible increased significantly across the consecutive age group comparisons over the late prenatal and early postnatal period of growth. These dimensions included the length and breadth of the arch of the mandible as well as those related to the areas directly associated with the muscles of mastication. However, the mental angle of the mandible, which was calculated using the length of the body of each hemi-mandible and the basilar bigonial width of the mandible, appeared to decrease significantly across the same period of assessment. These changes in the dimensions and morphology of the arch of the mandible may be the result of passive growth

pressures exerted on the mandible by the growing cranial base, during the prenatal and early postnatal stages of growth.

Furthermore, increases in the width of the mandible, which were observed in the present study across the bicondylar, bigonial and biantegonial measurements, may also be the result of passive basicranial growth pressures imposed on the ramus of the mandible through its association with the temporomandibular joint and glenoid fossa. However, the contribution of the posterior cranial base to the growth of the craniofacial region, in particular the position of the glenoid fossa, is limited as a result of the early ossification of this area (Nie, 2005).

When considering the growth trajectory and positioning of the mandible relative to the cranial base, the position of the glenoid fossa and the growth rates of the anterior and posterior basicranium are important factors to consider. These factors are particularly significant when studying the functional and occlusal relationships between the mandible and the maxilla with growth (Anderson & Popovich, 1983; Kerr & Adams, 1988; Kasai *et al*., 1995; Jeffery, 2002; Hayashi, 2003; Polat & Kaya, 2007), as the growth rate of the anterior cranial base is twice that of the posterior cranial base (Nie, 2005). In addition, the degree of flexion of the cranial base is also considered to have a significant effect on the occlusal relationships between the mandible and the maxilla.

The effect of cranial base flexion on the skeletal relationships of the maxilla and mandible were investigated using 3D-CT across a sample of skeletal class I and class II juvenile and sub-adult individuals (Hayashi, 2003). While the horizontal (anterior-posterior) position of the glenoid fossa was positively correlated with increases in the angle of flexion, the vertical orientation of the craniofacial complex appeared to be negatively correlated with the glenoid fossa position (Hayashi, 2003). Thus, it was suggested that the inclination of the posterior cranial base directly influences the position of the glenoid fossa, while changes to the anterior cranial base appeared to have a negligible influence (Hayashi, 2003). However, the position of the glenoid fossa may also be influenced by the coronal reorientation of the

petrous pyramids, as a result of the posterior cranial fossa adopting a deeper and wider shape with cranial growth (Nie, 2005). This change in the posterior cranial base and position of the glenoid fossa may also have a secondary influence on the geometry of the arch of the mandible, as the glenoid fossa is closely associated with the condyle of the mandible at the temporomandibular joint.

In contrast, the anterior cranial base significantly influences the growth of the facial region and as such plays an integrated role in co-ordinating craniofacial growth and development (Nie, 2005). The influence of the anterior cranial base on the growth of the craniofacial region is further evident through the additional passive influences exerted on the mandible via the attachment of the muscles of mastication to the anterior cranial base. These influences across the anterior cranial base are thought to be linked to the growth of the associated soft tissue structures *i.e.* tongue and muscles of mastication (Nie, 2005; Sperber *et al*., 2010). Subsequently, alterations to the morphology of the mandible during the prenatal and early postnatal stages of growth may be the result of the mandible accommodating the spatiotemporal demands of the growing tongue.

Thus, it was important in the present study to ascertain the nature of the growth influences imposed by the tongue and muscles of mastication on the morphology of the mandible, as these structures attach to both the anterior cranial base and the mandible. In the present study, both the length and breadth of the tongue appeared to increase significantly across consecutive age group comparisons. These increases in the dimensions of the tongue were well correlated with those observed across the mandible between 20 gestational weeks and 2 years of age. However, while these results highlighted the potential growth influences of the tongue on the mandible, they could not distinguish between the spatiotemporal (Coquerelle *et al*., 2013) and biomechanical (Liu *et al*., 2008a) nature of these influences.

During the early stages of development *i.e.* the middle of the 6th week of gestation, the intramembranous ossification of the mandible commences lateral to the buccal surface of the Meckel's cartilage in the embryonic first pharyngeal arch (Lee *et al*.,

2001). The initial site of ossification, the mandibular primary ossification centre, is located at approximately the middle portion of the future lingual surface of the mandible (Lee *et al*., 2001). It is from this site that the ossification of the mandible spreads in both an anterior and posterior direction during the 7th week of gestation (Lee *et al*., 2001). As ossification spreads, trabecular bone originating from the primary ossification centre advances in a buccal direction (Lee *et al*., 2001; Radlanski *et al*., 2003). It is at this stage of mandibular development that the lingual surface of the mandible is more advanced in its development when compared to the buccal surface. The development of the lingual surface is accompanied by the transition of the genioglossus muscle and the muscles of mastication, from their initial attachments on the Meckle's cartilage to their respective attachments on the lingual and buccal surfaces of the mandible, between the 7th and 9th weeks of gestation (Merida-Velasco *et al*., 1990; Lee *et al*., 2001). During the same period of development the tongue commences its descent in the future oral cavity, leading to the elevation of the palatal shelves (Humphrey, 1971). Shortly after the elevation of the palatal shelves, there is an increase in the size of the mandible without a corresponding increase in the dimensions of the maxilla (Humphrey, 1971). This disparity in growth between the mandible and maxilla during this period is suggestive of the arch of the mandible increasing in its dimensions to accommodate the spatial demands of the tongue (Swinehart, 1950; Humphrey, 1971; Lowe *et al*., 1985). The descent of the tongue as well as the translocation of its muscle attachments on to the body of the mandible is thought to induce significant spatial loading strains, across the lingual surface of the mandible, during its early stages of growth (Swinehart, 1950; Humphrey, 1971; Lowe *et al*., 1985; Lee *et al*., 2001).

The increases in the maximum length of the mandible and the length of the mandibular body observed in the present study during the late prenatal stages of growth, compliment the dimensional changes reported by other researchers during the earlier stages of growth (Bjork, 1955; Enlow & Harris, 1964; Enlow, 1966; Lee *et al*., 2001; Radlanski *et al*., 2003). Furthermore, in the present study, higher bone density values were observed across the lingual surface of the mandible when compared to the external surface. This disparity in bone density between the two surfaces of the mandible may be indicative of the advanced level of bone formation across the lingual surface, particularly during the prenatal period of growth. The

higher bone density observed across the lingual surface of the mandible compliments the suggested pattern of growth as proposed by the principle of 'V' (Enlow & Harris, 1964; Enlow, 1966). Thus, it may be suggested that the prenatal growth of the mandible is in response to the spatial demands of the tongue, following its descent in the oral cavity. In contrast, the changes in the dimensions and density of the mandible observed in the present study between 20 gestational weeks and 5 years of age, may be indicative of a combination of spatial and biomechanical changes in the loading conditions of the tongue and developing dentition.

The spatial and biomechanical demands of the tongue on the mandible have previously been investigated by testing the impact of a reduction in tongue volume on craniofacial growth and dental arch formation in a sample of Yucatan mini-pigs (Liu *et al*., 2008a). The glossectomy performed, was designed to reduce the volume of the tongue in length, width and thickness, while no additional changes were made to either the mandible or the maxilla (Liu *et al*., 2008a). There was a significant reduction observed over a period of four weeks in the height of the ramus, anterior mandibular length and dorsal width of the anterior mandible of the experimental sample when compared to the control sample (Liu *et al*., 2008a). The reduction in the arch dimensions of the mandible as a result of the reduction in the tongue volume was interpreted to be indicative of the withdrawal of the constant demands placed on the mandible by the developing tongue (Liu *et al*., 2008a). Thus, the proportional increase in the tongue and mandibular arch dimensions as observed in the present study, during the course of normal development and growth of the human mandible, is consistent with the proportional changes observed in the reduction experiment conducted by Liu *et al*. (2008a).

The effect of a change in the biomechanical loading conditions imposed by the tongue on the mandible during the late prenatal and early postnatal periods of growth is possibly illustrated by the change in the bone density of the lingual surface. While there was a degree of variability in the bone density of the mandible relative to each dental crypt within each age group in the present study, there appeared to be a general increase in the bone density of the lingual surface across the overall period of assessment. The general increase in the bone density of the lingual surface over

time is reflective of the age and level of maturity of the bone across this surface (Hernandez, 2008). In contrast, the variability in the bone density of the lingual surface across the prenatal and early postnatal periods is consistent with the increase in the level of biomechanical functioning of the tongue during its transition between sucking, suckling and mastication (Miller *et al*., 2003; Delaney & Arvedson, 2008; Liu *et al*., 2008a).

During the early prenatal stages of growth, the oral cavity adapts to accommodate forward thrusting movements by the developing tongue, which also makes contact with the lower border of the lingual surface of the mandible (Miller *et al*., 2003; Delaney & Arvedson, 2008). The oral cavity also adapts to accommodate increased levels of swallowing and suckling activity, as the laryngeal and associated pharyngeal spaces expand through a combination of horizontal and vertical growth (Coquerelle *et al*., 2013). The expansion of the pharyngeal spaces leads to the tongue being repositioned towards the posterior areas of the oral cavity and down into the oropharyngeal region. This produces a greater strain on the suprahyoid muscles in addition to the descent of the hyoid bone (Coquerelle *et al*., 2013). It is during the late prenatal stages of growth that the tongue, through a process of labial unrolling, flattens and adapts it's functioning from forward thrusting movements to more subtle anterior-posterior movements (Miller *et al*., 2003; Delaney & Arvedson, 2008; Coquerelle *et al*., 2013). This change in movement leads to a change in the level of strain imposed on the lingual surface of the mandible and thus a change in the bone density of this surface (Hernandez, 2008). This effect is illustrated by a significant decrease in the bone mineral content across the symphyseal region of the mandible, following a reduction in both the volume of the tongue and its associated biomechanical strain (Liu *et al*., 2008a). Furthermore the significantly lower bone density observed in the present study, across the symphyseal region of the mandible, is consistent with the reported changing level of strain imposed by the tongue on this area of the mandible during the prenatal and early postnatal stages of growth.

It should also be noted that the symphyseal region undergoes considerable morphological changes in the period extending from the 12th week of gestation to the first year of postnatal life. These changes may largely be attributed to the detachment

of the Meckle's cartilage from the mandible and the ventral symphyseal cartilages (remnants of Meckle's cartilage), which have commenced the process of endochondral ossification in the symphyseal region (Scheuer & Black, 2000; Radlanski *et al*., 2003). Endochondral ossification of the symphyseal cartilages continues until the latter half of the first year postnatally, when fusion of the hemi-mandibles in the symphyseal region is completed (Scheuer & Black, 2000; Hutchinson *et al*., 2012).

In the present study significant increases were observed in the length of the body of the mandible, the mental angle and in the mental interforaminal width between 20 gestational weeks and 3 years of age. The increases in the mental interforaminal width and the mental angle may be attributed to the changes in the symphyseal region of the mandible shortly after birth. However, the increase in the length of the body of the mandible is thought to be in response to the spatial demands of the developing dentition.

While the variations in the bone density of the lingual surface of the mandible have been attributed to changes in the loading conditions associated with the tongue, those variations observed across the external surface of the mandible appeared to be reflective of the level of dental development. The bone density of the alveolar and basal areas of the lingual surface of the mandible did not change as significantly with dental development when compared to the corresponding regions of the external surface of the mandible in the present study. Furthermore, while changes in the bone density of the alveolar region of the external surface corresponded to the associated level of dental development, variations in the bone density of the basal region were more subtle in nature. The variations in the bone density of the external surface relative to the level of dental development observed in the present study are consistent with the remodelling pattern sequence of human mandibular alveolar bone as observed by Radlanski *et al*. (2016).

The bone density in the area of the lateral incisor dental crypt in the present study was significantly higher than that of the canine and central incisor dental crypt areas

respectively. This difference in bone density may be attributed to the varying levels of dental development and tooth eruption across the three associated dental crypt regions. While the canine tooth generally only starts development after the lateral incisor, the central incisor tooth is thought to be more advanced in its development and degree of eruption, when compared to the lateral incisor (Alqahtani *et al*., 2010). Thus, the lower bone density observed in the present study in the area of the canine, may be attributed to the early stage of dental development of this tooth when compared to the more advanced development of the lateral and central incisor teeth respectively. However, the lower bone density of the central incisor dental crypt area in the present study may be the result of an increase in bone modelling owing to increased physiological tooth movement in the area (Wise & King, 2008), when compared to the lateral incisor dental crypt area.

Physiological tooth movement is considered to be a developmental process and is predominantly associated with bone modelling (Melsen, 1999; Dolce *et al*., 2002; Wise & King, 2008). The change in the occlusal state of the dentition, which accompanies physiological tooth movement, results in a modification in the cortical bone thickness and the bone density of the associated alveolar region (Choi *et al*., 2009). As there is an increase in the level of occlusion and the associated loading conditions with tooth eruption, there is a decrease in the bone density of the associated dental crypt regions (Melsen, 1999; Dolce *et al*., 2002; Hernandez, 2008; Wise & King, 2008; Choi *et al*., 2009). Thus, those dental crypts with teeth in the more advanced stages of eruption will reflect lower bone density values, when compared to the dental crypts with teeth in earlier stages of dental eruption or emergence. Subsequently the lower bone density observed across the alveolar bone of the anterior dental crypts in the present study may be a reflection of the advanced level of dental development and tooth eruption associated with these dental crypts. The changes in bone density observed across the external surface of the mandible in the present study were predominantly associated with the alveolar regions of the mandible. These observations are supportive of the level of dental development significantly affecting the bone density of the external surface of the mandible as previously reported (Melsen, 1999; Dolce *et al*., 2002; Hernandez, 2008; Wise & King, 2008; Choi *et al*., 2009).

Furthermore, the higher bone density observed in the present study in the region of the lateral incisor dental crypt, may also be attributed the lower levels of biomechanical activity associated with this region when compared to the symphyseal and the central incisor dental crypt regions. The lower bone density of central incisor and symphyseal regions is thought to be the result of a change in the morphology of the symphyseal region of the mandible (Scheuer & Black, 2000; Lee *et al*., 2001; Radlanski *et al*., 2003) as well as in the functioning of the tongue (Miller *et al*., 2003; Delaney & Arvedson, 2008). The fusion of the hemi-mandibles (Scheuer & Black, 2000) as well as the increased level of biomechanical loading associated with the tongue adapting to a change in function (Miller *et al*., 2003; Delaney & Arvedson, 2008) results in a high degree of bone modelling and remodelling in the symphyseal and central incisor dental crypt regions. This increase in bone modelling and remodelling results in the bone associated with these areas being lower in density, when compared to the more established bone of the lateral incisor dental crypt region (Hernandez, 2008). Thus, the higher bone density of the lateral incisor dental crypt region may be attributed to a high degree of bone modelling and remodelling across the symphyseal and central incisor dental crypt regions as well as increased levels of physiological tooth movement associated with the central incisor tooth.

The significant difference in bone density observed in the present study between the second deciduous molar and the first permanent molar dental crypt regions on the lingual surface of the mandible, may be the result of bone modelling and remodelling taking place due to a change in the orientation of the ramus. The ramus of the mandible undergoes a significant transition both in its orientation and morphology across the late prenatal and early postnatal periods of growth (Scheuer & Black, 2000). These changes are largely as a result of the increase in functional demand, as well as increased spatial demands from the developing dentition (Hutchinson *et al*., 2012). As the dentition advances in its development, the body of the mandible potentially increases in size in order to accommodate the increase in the size of the developing dentition. This demand results in increased bone modelling and remodelling, which leads to the ramus changing its orientation from a horizontal

disposition prenatally to a more vertical orientation postnatally (Scheuer & Black, 2000; Hutchinson *et al*., 2012).

While changes across the mandible, in particular the alveolar region, may be the result of the development and eruption of the dentition during the early stages of growth, the state of the dentition also appeared to have a significant influence on the morphology, during the later stages of adulthood. One of the most significant changes observed in the present study was the substantial reduction in the height of the alveolar region in the edentulous mandible when compared to the dentate mandible. The reduction in the alveolar height with tooth loss confirms the strong supportive role of the tooth and its associated structures in maintaining the surrounding alveolar bone of the tooth crypt. Thus, the absence of the tooth and its associated biomechanical loading within the dental crypt results in disuse atrophy (Ortman, 1962) of the supporting alveolar bone and subsequent residual ridge resorption (Atwood, 1979; Jahangiri *et al*., 1998).

A reduction in the length of the body of the mandible as well as an increase in the angle of the mandible and the mental angle respectively was also observed across the edentulous mandibles studied. The shorter length of the body of the mandible in the edentulous mandibles may be the result of the onset of disuse atrophy as a result of the absence of the dentition and a subsequent reduction in the associated biomechanical loading of the crypts (Ortman, 1962; Atwood, 1979; Cawood & Howell, 1988, 1991; Jahangiri *et al*., 1998). In addition, the reduction in the length of the mandibular body resulted in the ramus of the mandible assuming a more reclined position relative to the body of the mandible. The variation in the posture of the ramus relative to the body of the mandible with a change in the state of the dentition is consistent with the growth trajectory proposed by the principle of archial growth (Ricketts, 1972). In addition, the altered posture of the ramus relative to the body of the mandible further results in a change in the bony relationship between the mandible and the maxilla anteriorly. This change in the anterior relationship between the mandible and the maxilla with a change in the state of the dentition further compliments the concept of the growth of the mandible occurring about a centre of rotation (Bjork, 1955; 1969).

The directive role of the dentition in maintaining the morphology of the mandible throughout life is potentially illustrated by the collective findings of the present study. In the late prenatal and early postnatal stages of growth, the increases in the length of the body of the mandible, the maximum length of the mandible and the upright position of the ramus may all be attributed to influences arising from the development of the dentition. It thus appears that the development of the dentition is a significant driving factor in the early growth of the mandible. This is supported by the substantial decrease in the length of the body of the mandible as well as the obtuse angle in the position of the ramus with the onset of edentulism. In addition, the significantly reduced alveolar height further validates the role of the dentition in maintaining the morphology of the mandible. However, it is the biomechanical and spatial demands of the growing tongue that direct the growth of the mandible during the prenatal and early postnatal stage of growth.

While the influence of the muscles of mastication appear to be important in the moulding of the morphology of the mandible both, during perinatal stages of growth as well as across the transition between the various states of the dentition, further investigation into the biomechanical nature of the muscles are required. Thus, it is recommended that the patterns of change in the bone density of the mandible be assessed particularly across sites associated with the attachment of the muscles of mastication. In addition, changes in the volume of the muscles during growth may also serve to indicate when each muscle of mastication assumes its role in the overall functioning of the mandible during the early stages of mastication. This may further validate the nature of the biomechanical influences of the muscles of mastication on the moulding of the morphology of the mandible, particularly across the functionally complex period of growth assessed in this study. It is envisioned that the findings of this study will provide a stronger basis for interpretations into the effects of a change in the biomechanical environment on the morphology of the mandible. Furthermore it is anticipated that these findings may strengthen the quality of age assessment using the juvenile mandible within a forensic setting.

Considering the results of the various investigations conducted within the scope of this study the following may be concluded. The mandible, during the late prenatal

and postnatal stages of growth, appears to be significantly influenced by the spatial increases in the dimensions of the tongue. In addition, the growth of the mandible appears to be significantly influenced by the change in the level of the functioning of the tongue and as a result, by the subsequent change in the biomechanical loading conditions imposed on the mandible during this functional transition. While the tongue is thought to be a significant factor in the development and growth of the lingual surface of the mandible, the dentition appears to significantly guide the development and growth of the external surface of the mandible. In addition, the dentition also plays a crucial role in the general maintenance of the morphology of the mandible throughout life and its absence induces substantial changes in the morphology of the mandible. Thus, it may be concluded that the supporting tissues of the mandible *i.e.* the tongue and the dentition, may play a pivotal role in moulding and maintaining the morphology of the mandible throughout the life course of the individual.

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CHAPTER 7

APPENDICES

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Miss Erin Hutchinson

CLEARANCE CERTIFICATE	**M121145**
PROJECT	Aspects of Mandibular Development and Growth
INVESTIGATORS	Miss Erin Hutchinson.
DEPARTMENT	School of Anatomical Pathology
DATE CONSIDERED	30/11/2012
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 30/11/2012 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Prof Beverley Kramer

DECLARATION OF INVESTIGATOR(S)
To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

Human Research Ethics Committee (Medical)

Research Office Secretariat: Senate House Room SH 10005, 10th floor. Tel +27 (0)11-717-1252
Medical School Secretariat: Medical School Room 10M07, 10th Floor. Tel +27 (0)11-717-2700
Private Bag 3, Wits 2050, www.wits.ac.za. Fax +27 (0)11-717-1265

Ref: W-CJ-140604-1 25/01/2016

TO WHOM IT MAY CONCERN:

Waiver: This certifies that the following research does not require clearance from the Human Research Ethics Committee (Medical).

Investigator: School of Anatomical Sciences (Head: Prof M Steyn - Previously Prof T J M Daly, initial approval 04/06/2014 – recertified 27/01/2016).

Project title: Research on Cadaveric Material.

Reason: In terms of Chapter 8, sections 62-64 of the National Health Act No 61 of 2003 donated bodies and their tissues may be used for, among other purposes, health, and research. Use of such Material is subject only to permission from the responsible person in the School of Anatomical Sciences – the Head or person designed by the Head.

Professor Peter Cleaton-Jones

Chair: Human Research Ethics Committee (Medical)

UNIVERSITY OF THE WITWATERSRAND
OFFICE OF THE DEPUTY VICE CHANCELLOR RESEARCH
29 JAN 2016
RESEARCH OFFICE HREC (Medical)
JOHANNESBURG

Copy - HREC (Medical) Secretariat: Rhulani Mkansi, Zanele Ndlovu.

European Journal of Oral Sciences

Eur J Oral Sci 2014; 122: 181–189
DOI: 10.1111/eos.12126
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European Journal of Oral Sciences

Morphometric growth relationships of the immature human mandible and tongue

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Hutchinson EF, Kieser JA, Kramer B. Morphometric growth relationships of the immature human mandible and tongue.
Eur J Oral Sci 2014; 122: 181–189. © 2014 Eur J Oral Sci

The masticatory apparatus is a highly adaptive musculoskeletal complex comprising several relatively independent structural components, which assist in functions including feeding and breathing. We hypothesized that the tongue is elemental in the maintenance of normal ontogeny of the mandible and in its post-natal growth and development, and tested this using a morphometric approach. We assessed tongue and mandibular measurements in 174 (97 male) human cadavers. Landmark lingual and mandibular data were gathered individuals aged between 20 gestational weeks and 3 yr postnatal. In this analysis, geometric morphometrics assisted in visualizing the morphometrical growth changes in the mandible and tongue. A linear correlation in conjunction with principal component analysis further visualized the growth relationship between these structures. We found that the growth of the tongue and mandible were intrinsically linked in size and shape between 20 gestational weeks and 24 months postnatal. However, the mandible continued to change in shape and size into the 3rd yr of life, whereas the tongue only increased in size over this same period of time. These findings provide valuable insights into the allometric growth relationship between these structures, potentially assisting the clinician in predicting the behaviour of these structures in the assessment of malocclusions.

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Key words: development; mandible; principal component analysis; tongue

Accepted for publication February 2014

The masticatory apparatus is a highly adaptive musculoskeletal complex comprising several relatively independent structural and functional components (1, 2). This complex of structures collectively assist in the functions of feeding, phonation, swallowing, and breathing (3, 4). Understanding how the structure and function of the craniofacial elements, such as bone, muscle, and cartilage, change during late fetal development is a topic of considerable interest to biologists and clinicians alike (5–7). In recent times, molecular biology has provided much-needed knowledge regarding the mechanisms of development through the identification of genes and growth factors (5, 8–10). However, morphological studies have proven to be a valuable approach for understanding the relative roles of growth over time (11, 12). Morphological studies have placed great emphasis on the interactions of the various masticatory structures in older age-cohorts (13–17), leaving a significant knowledge gap in relation to the earlier stages of development. We have attempted to address this gap by investigating the static allometry of the tongue and mandible during the morphologically significant growth period of 20 gestational weeks to 3 yr of life. This period is particularly significant as it involves the transition from a prenatal to postnatal environment, which inherently introduces different biomechanical interactions associated with suckling, further tooth development and tooth eruption, and increasing degrees of mastication. Furthermore, the current study is innovative in its direct assessment of the relationship between the tongue and mandible and considers the effects of the muscles of mastication and the growth of the cranial base on the allometric growth relationship of the mandible and tongue.

The early morphological growth and development of the mandible as a central component of the masticatory apparatus has been extensively studied. Lee *et al.* (13) considered the sequential growth pattern of the human mandible and observed the radiating expansion of the mandible from the primary growth centre as early as the fifth week of development (13). The ossification of the mandible commences at the sixth week of development (11, 12) with insular cartilages observed between weeks 10 and 14 near the posterior region of Meckel's cartilage. The mandible alters its morphology to accommodate the development of the deciduous dentition and the increase in mastication between 30 wk of gestation and 3 yr of age (14, 15). However, these studies, although alluding to the potential influence of the tongue on the normal growth of the mandible, did not elaborate further on this fundamental relationship.

The relative roles and relationships of the masticatory apparatus within the developing oral cavity are of clinical importance in understanding the aetiology of

various cases of malocclusion. The emergence and eruption of the posterior permanent dentition, which in some cases may occur as early as 4.5 yr of age (16), presents the clinician with the possibility of a posterior cross-bite malocclusion (17). This type of malocclusion not only is considered as the most prevalent in relation to the deciduous dentition, but may result in facial asymmetry and temporomandibular disorders later in adulthood if left undiagnosed (17). One of the suggested diagnostic criteria for a posterior cross-bite malocclusion is a short lingual frenulum, which results in the tongue assuming a lower position within the oral cavity (17). This change in tongue posture leads to a disturbance in the dynamic equilibrium of the oral cavity and possibly manifests later as a narrow and shortened maxilla (17–19). Although there is a suspected link between the hard and soft tissues of the oral cavity in the aetiology of malocclusion, most of these investigations have been pursued in older age-cohorts.

In an early study, SWINEHART (18) summarized the relationship between the growth of the tongue relative to the mandible in patients of malocclusion and concluded that the tongue exerted considerable expansive forces on the mandibular arch during growth. However, SWINEHART (18) postulated that these forces only had a significant effect on the normal occlusion when the tongue had completed its descent in the oral cavity (18). LOWE *et al.* (19) further reinforced these hypotheses by investigating the role of the tongue in malocclusions in adults. He concluded that not only was the position of the tongue a significant variable in establishing a normal occlusion pattern, but that the degree of tongue protrusion relative to mandibular rotation was also significant (19). Whilst these studies addressed the potential role of the tongue in cases of malocclusion, they did not consider the normal prenatal and postnatal interactions between the involved components.

Prenatal and postnatal interactions between the tongue and the mandible are particularly significant in the understanding and the subsequent prevention and treatment of cases of maldevelopment. Developmental abnormalities associated with the masticatory complex vary considerably, from extremely rare cases of aglossia to the more common cases of bifid tongue (20–25). Whilst extensive literature exists describing the abnormalities of the masticatory apparatus, few studies have explained the relationship between the tongue and the mandible in any of the assessed patients. This would be important in understanding clinical conditions such as malocclusions and micrognathia. The possible association of microglossia with glossoptosis, and the possibility of micrognathia being outgrown later in life (20), raise the question of the influence of the tongue in the normal growth of the mandible. Furthermore, cases of aglossia and hypoglossia are accompanied by retarded development of the mandible and teeth (21, 25). This emphasizes the potentially significant role of the tongue in the general growth of the oral cavity.

Based on the results of earlier studies (14, 15, 18, 19, 21, 25), we hypothesized that the tongue is elemental not only in maintaining normal mandibular ontogeny, but also in its postnatal growth and development. To test this hypothesis, we utilized a morphometric approach. First, we used fetal cadavers to determine the changes in the maximum length and breadth dimensions of the tongue associated with growth. Second, we measured landmark data of the external surface of the mandible in individuals between 20 wk of gestation and 3 yr postnatally. In this analysis we used principal component analysis (PCA) to visualize the morphometrical changes in the mandible and tongue that are associated with growth. Additionally, a linear correlation analysis was used in conjunction with PCA to further visualize the growth relationship (i.e. the changes in size and shape) between the tongue and mandible within the sample.

Material and methods

Fetal and neonatal human cadavers were sourced from the Paediatric Collection, School of Anatomical Sciences, University of the Witwatersrand. The study sample consisted of complete human cadaveric remains of unknown provenance donated under the Human Tissue Act 65 of 1983. In addition, ethical clearance (M121145) was obtained from the Human Research Ethics Committee. The mandibles of 97 male and 77 female cadavers were assessed (Table S1). Specimens ranged in age from 20 wk of gestation to 3 yr postnatally. Ages were determined using the dental age standards proposed by ALQAHTANI *et al.* (16). In addition, standard body measurements (e.g. crown–rump length, toe–heel length, crown–heel length, and head circumference) were also utilized in the determination of age (26–28). The sample was subdivided into four groups: group 1 (20–30 wk of gestation), group 2 (31–40 wk of gestation), group 3 (0–12 months postnatal), and group 4 (12.5–36 months postnatal). Specimens were further classified, according to the state of the dentition, as dentulous, partially edentulous, or edentulous (Supporting Table S1) (15).

Individuals showing evidence of damage to the mandible or tongue, congenital abnormalities, soft- or bony-tissue pathologies, or surgical intervention, were excluded from the study.

Cadavers were fixed in 5% formalin. This solution has been shown to have no significant level of tissue shrinkage and therefore correction for shrinkage was not undertaken (29, 30).

Methods

Soft-tissue dissection was performed to expose the anterolateral surface as well as the inferior border of the mandible (Supporting Fig. S1). The tissues were reflected in order to preserve the overlying soft tissues for future studies.

In cadavers where the mouth could not be opened, a series of incisions was performed in order to maintain the position of the tongue relative to the mandible. The first incision was made along the capsule of the temporomandibular joint. The mandible was not removed from the specimen as the attachments of the lateral and medial pterygoid muscles were kept intact. There was no further dissection of the oral cavity.

An Immersion Microscribe G2 (Immersion, San Jose, CA, USA), with an accuracy of 0.38 mm, was used to record 21 homologous landmarks on the mandible (Fig. S2). These landmarks included the points necessary to calculate the linear measurements and to define the shape of

the mandible. Landmark points were digitized on the mandible in situ and were recorded in a pre-set sequence (clockwise direction).

Using each landmark, three coordinates (X, Y, and Z) were recorded and represent the horizontal, vertical, and depth planes, respectively. The three-dimensional coordinates were then imported into a MICROSOFT EXCEL worksheet and the Pythagorean formula was used to convert the data into linear distances:

$$= \sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2 + (z_1 - z_2)^2}$$

where x_1, y_1, z_1 and x_2, y_2, z_2 refer to the coordinates of the first and second landmarks respectively.

Eight standard anthropometric measurements, derived from DE VILLIERS (31), LOTH *et al.* (32), SCHEUER & BLACK (33), HESBY *et al.* (34), WHITE *et al.* (35), and HUTCHINSON *et al.* (15), were recorded using the previously digitized points (15, 31–35). In addition, the maximum bicondylar width was recorded according to a procedure specifically designed for the purposes of this study. From these measurements, morphological changes of the mandible and tongue in the sagittal and coronal planes were derived.

Measurements assessing changes in the sagittal plane included the mandibular body length (measured from the mental tubercle to the angle of the mandible) and the maximum length of the mandible (measured from the mental tubercle to the posterior tip of the mandibular condyle) (32). In the assessment of maximum length and mandibular body length measurements, the most anterior point on the mental symphysis cartilage ridge was used as the reference point in unfused mandibles. Additionally, the mandibular notch width (measured from the most posterior tip of the coronoid process to the most anterior tip of the condylar process) (31) and the maximum width of the mandible (measured between the most anterior point on the coronoid process and the most posterior point on the condyle of the mandible) (14, 33) were also assessed.

Measurements assessing changes in the coronal plane included the mandibular basilar bigonial width, the width of the mandibular arch between the mandibular angles (the point where posterior and inferior borders meet), as well as the mandibular basilar biantegonial width (the width of the mandibular arch from the bilateral points of the most superior point of the antegonial notch) (34). Additionally, the mental interforaminal distance was measured between the two mental foramina on the anterior surface of the mandible (31), the maximum bicondylar width was the distance measured between the most lateral points on the lateral surfaces of the mandibular condyles, and the mental angle, which was formed at the mental symphysis between the mandibular bodies of both hemi-mandibles (14), was also assessed.

The measurements used to calculate the mental angle included the mandibular body length of each side ($a + b$) as well as the mandibular basilar width bigonial (c). The cosine rule was then used for mental angle calculation, as the triangle formed by the measurements was not considered to be a right-angle triangle; thus, the Pythagorean equation could not be used.

The mental angle was calculated as follows:

$$\mathrm{CosC} = (a^2 + b^2 - c^2)/(2ab)$$

where a and b represent the left and right mandibular body lengths, respectively; c represents the mandibular basilar width bigonial, and C represents the calculated mental angle.

A sliding caliper (of 0.02-mm accuracy) was used for measuring the tongue. The tongue was extended to flatten out its natural curvature, conventionally induced by the confined space of the oral cavity. Three measurements were used to assess the dimensions of the tongue relative to the mandibular arch in both the sagittal and coronal planes respectively.

Measurements in the sagittal plane included the whole tongue length (measured from the tip of the tongue anteriorly to the posteriorly located median glossoepiglottic fold at the junction of the root of the tongue and epiglottis) and the anterior tongue length (measured from the tip of the tongue anteriorly to the opening of the foramen cecum). Measurements in the coronal plane included the maximum breadth of the tongue (measured just anterior to the laterally located palatoglossal arches). All tongue measurements were made on the dorsal surface of the tongue.

Data analysis

Descriptive statistics, including mean and SD, were calculated for all mandibular and soft-tissue (tongue) measurements. The distribution of the sample was tested for normality using the Shapiro-Wilk's test and further analyses were based on this result (36). All statistical analyses were conducted using the PAST (Paleontological Statistics) statistical program (37).

ANOVA was performed using the calculated geometric mean to assess variations in the dimensions of the tongue and mandible relative to age. The geometric mean assesses the total mean of all values associated with a specific individual and establishes the overall standing of an individual's size relative to the rest of the sample (38, 39). Between- and within-group variances were represented by the F-ratio, which indicated the degree of similarity in the means. A MANOVA was performed using the calculated Mosimann shape variables. The Mosimann shape variables were used as they alter the differences in scale between individuals by dividing each measurement value by the geometric mean, so that shape may be assessed relatively independently of size (38, 39). The use of parametric statistics in conjunction with the geometric mean and Mosimann shape variables was performed to correct for possible inferior distribution properties associated with these analyses (39).

The relationship between the tongue and the mandible was further assessed using a linear correlation coefficient. A significance level of alpha <0.05 was considered statistically significant and all correlation values of >0.7 were considered significant.

To visualize the differences in the size and shape of the mandible and tongue with growth, the geometric mean and Mosimann shape variables were independently subjected to a PCA using the PAST (Paleontological Statistics) statistical program (37).

Inter- and intra-observer errors were evaluated using 10 specimens and then tested using the Lin's concordance correlation coefficient of reproducibility, which provides information on the level of repeatability of the method and the degree of association between measurements (40).

Results

Analysis of the mean and SD of the measurements of the sample showed a general increase in the size of the mandible and tongue in all dimensions, except for

Table 1

Growth increases in the sagittal and coronal plane dimensions of the tongue and the mandible between 20 gestational weeks and 36 postnatal months of age

Variable	Group 1 20–30 wk (n = 41)	Group 2 30.5–40 wk (n = 60)	Group 3 0–12 months (n = 56)	Group 4 12.5–36 months (n = 17)
Maximum tongue length	25.07 ± 2.82	30.87 ± 4.84	33.44 ± 5.39	37.3 ± 3.74
Minimum tongue length	21.99 ± 2.56	27.63 ± 4.20	29.31 ± 5.46	32.83 ± 3.72
Tongue breadth	21.42 ± 2.12	25.75 ± 3.08	29.60 ± 4.31	35.2 ± 4.48
Mandibular basilar width bigonial	34.12 ± 3.39	39.25 ± 4.16	48.24 ± 5.83	58.42 ± 7.96
Mandibular basilar width biantegonial	30.21 ± 3.26	34.80 ± 3.73	43.34 ± 3.14	53.58 ± 7.51
Mental interforaminal distance	21.02 ± 2.43	24.84 ± 2.83	29.74 ± 3.14	34.11 ± 3.59
Mandibular body length	29.30 ± 2.82	34.57 ± 3.05	42.35 ± 4.46	51.91 ± 7.35
Maximum length of mandible	38.45 ± 3.09	45.18 ± 3.88	56.15 ± 6.98	72.31 ± 10.77
Maximum width of mandible	13.70 ± 1.93	15.64 ± 2.07	20.10 ± 3.72	25.88 ± 4.58
Mandibular notch width	6.67 ± 0.95	7.36 ± 1.24	9.70 ± 2.24	13.29 ± 2.69
Mental angle	71.42 ± 6.29	69.24 ± 6.15	69.45 ± 5.50	68.46 ± 2.98
Bicondylar width of mandible	45.20 ± 3.37	52.91 ± 4.62	62.43 ± 6.24	74.42 ± 9.87

Values are given as mean ± SD, in mm, for each age group. An overall increase was found for all tongue and mandible dimensions, except the mental angle.

the calculated mental angle, across groups 1–4 (Table 1). As no significant differences were observed between measurements on the left and right sides, the mean values of the right and left sides are given. The distribution of the sample data was observed to be normal.

The relationship between the tongue and the mandible was first tested, in terms of size, by both ANOVA and PCA. Both the tongue and the mandible showed statistically significant increases in size in all group comparisons (Table 2). This growth relationship was further emphasized by the high degree of overlap across the first three groups of the size-associated PC1 in both the mandible (Fig. 1A) and the tongue (Fig. 1B). The high degree of overlap indicated a consistent degree of growth in the lingual and mandibular dimensions across the prenatal, perinatal, and postnatal age ranges. Additionally, the growth relationship between the tongue and the mandible was further emphasized by the significant degree of overlap between groups 3 and 4 (Fig. 1A,B). Furthermore, the principal component box plots emphasized the effect of the significant size increases observed in the tongue and the mandible in the particular dimensions across the entire sample (Fig. 1A,B).

The mandible and the tongue were found to lengthen significantly along the sagittal plane, in all age groups (Fig. 1A,B). The mandibular width also increased slightly, indicating a widening in the mandibular notch area. The mandible was also observed to widen in the coronal plane where a significant increase in the maximum bicondylar width was observed over time (Fig. 1A). The posterior region of the mandible increased in width in the gonial region, where increases were observed in the mandibular basilar width bigonial and the biantegonial width. Tongue breadth increased at the same time (Fig. 1A,B). In the anterior region of the mandible, the mental angle and mental interforaminal distance showed a very small degree of widening (Fig. 1A).

The relationship between the tongue and mandible was then tested in terms of shape by using the

Table 2

Comparison between tongue and mandible measurements in groups 1–4

Variable	Mandible	Tongue
Tukey's pairwise comparison	Q/P*(same)	Q/P*(same)
Group 1 vs. Group 2	6.13 (9.2E−05)	7.79 (8E−06)
Group 1 vs. Group 3	16.96 (8E−06)	11.84 (8E−06)
Group 1 vs. Group 4	31.12 (8E−06)	18.32 (8E−06)
Group 2 vs. Group 3	10.84 (8E−06)	4.04 (0.02206)
Group 2 vs. Group 4	24.99 (8E−06)	10.53 (8E−06)
Group 3 vs. Group 4	14.16 (8E−06)	6.48 (3.4E−05)
Within-group variance (F-ratio)	150.5	52.9

Between-group comparison was performed by ANOVA using the calculated geometric mean values. P(same) values indicated whether mean values between groups were the same; and significant differences across all assessments were observed.
*Normal distribution of the sample was confirmed by the Shapiro–Wilk test.

Mosimann shape variables in both the MANOVA and PCA. Both the tongue and the mandible showed significant alterations in shape, across all age groups (Table 3). The lingual dimensions showed a statistically significant increase between groups 1 and 2, as well as between groups 2 and 3; however, no such increase in dimension of the shape of the tongue was observed between groups 3 and 4. This is in contrast to the mandible, which underwent significant increases in the dimensions of shape across all age groups (Table 3).

Significant increases in the dimensions of the shape of the mandible and tongue were observed by MANOVA, as a result of the significant degree of overlap in the tongue and mandible assessments across groups 1–3 of the PCA. A lesser degree of overlap was observed between groups 3 and 4 (Fig. 2A,B). Additionally, the mental angle exerted a significant degree of influence on the developing mandible (Fig. 2A,B).

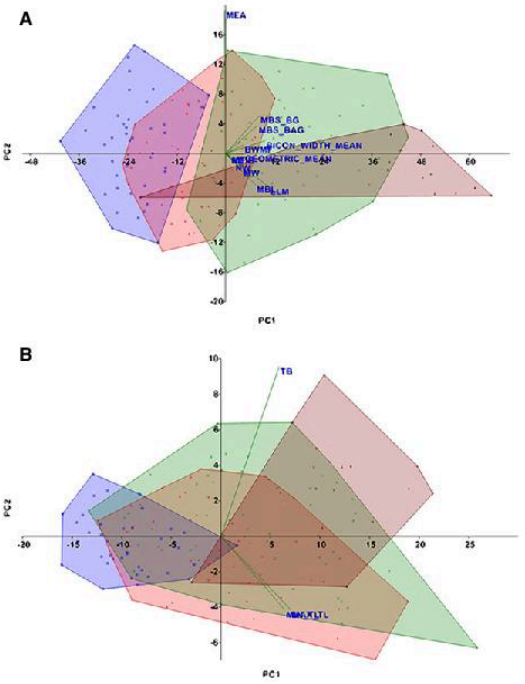


Fig. 1. Principal component analysis (PCA) assessing the level of size variance for the mandible (A) and the tongue (B), between each of the four groups: group 1 (blue: 20–30 gestational weeks), group 2 (red: 31–40 gestational weeks), group 3 (green: 0–12 months), and group 4 (brown: 12.5–36 months). Data are presented as box plots illustrating the effect of each measurement on PC1 and PC2. BICON_WIDTH, bicondylar width of mandible; BWMF, mental interforaminal distance; LLM, maximum length of the mandible; MAX_TL, maximum tongue length; MBL, mandibular body length; MBS_BG, mandibular basilar bigonial width; MBS_BAG, mandibular basilar biantegonial width; MBW, maximum bicondylar width; MEA, mental angle; MIN_TL, minimum tongue length; MW, maximum width of the mandible; NW, mandibular notch width; TB, tongue breadth.

The box plot of the mental angle indicated a major change in the shape of the mandible anteriorly. The overall lengthening of the mandible in the sagittal plane resulted in the ramus of the mandible assuming a more upright position, as observed in the decrease in the dimensions of the mandibular body length and the maximum length of the mandible. No major change was observed in the tongue length dimension over the same period of time. The decrease in the tongue breadth, and in the mandibular basilar width bigonial and biantegonial dimensions, indicated a flattening of the tongue in the coronal plane coupled with a small degree of eversion in the gonial region of the mandible (Fig. 2A,B). The significance of the overall relationship observed between the mandible and the tongue was further clarified in the linear correlation assessment (Table 4), with strong correlations being observed in tongue breadth and in the posteriorly assessed dimensions of the mandible.

Table 3

Comparison of tongue and mandible measurements in groups 1–4 Variable

	Mandible	Tongue
Group 1 vs. Group 2	1.34E−12	0.0275
Group 1 vs. Group 3	1.55E−24	0.0567
Group 1 vs. Group 4	2.85E−16	0.0025
Group 2 vs. Group 3	3.45E−12	0.0017
Group 2 vs. Group 4	1.98E−15	0.0009
Group 3 vs. Group 4	2.12E−06	0.1676

Between-group comparison was performed by multivariate analysis of variance (MANOVA) using the calculated Mosimann shape variable. Differences observed between groups 1–4 for the mandible were substantially more significant compared with those for the tongue. Comparisons between tongue groups 1 and 3 and 3 and 4 indicated differences that were not considered to be statistically significant in terms of shape.

The significant degree of correlation observed between the minimum and maximum tongue length and breadth indicated that this organ increased its length at a proportional rate to its breadth. The tongue breadth was also observed to widen at a very similar rate to the observed widening of the maximum bicondylar width. Furthermore, as the tongue lengthened, the posterior bicondylar region of the mandible widened, and a small widening was also observed in the mental interforaminal distance (Table 4).

Discussion

Recently, attention has focused on the molecular aspects of mandibular development (8, 9), and especially on the fundamental role of metalloproteinases in craniofacial matrix development (5). By contrast, few current studies have investigated morphological patterns in development and growth of the lingual–mandibular complex during the late trimester and early postpartum period. However, BARRON *et al.* (41) have recently produced interesting molecular data illustrating that the transcription factor, Hand2, plays a key role in mandibular arch development, which also initiates morphogenesis of the tongue mesenchyme. Previously, it was suggested that the tongue was elemental not only in maintaining normal mandibular ontogeny but also in its postnatal growth and development (4, 18, 19). To test our hypothesis that the tongue plays a crucial role not only in determining shape and attachment sites of individual jaw muscles but also in the evolution of complex bone architecture, we performed a series of comparative analyses using cadaveric human fetuses. We found that the tongue and the mandible were intrinsically linked (i.e. showed a positive correlation in the allometric growth relationship up to the second year of life). During the third year of life, no further significant changes took place in the morphology of the tongue, in contrast to the changes in the overall size and shape of the mandible. This contrast in growth patterns between the tongue and the mandible may be a result of

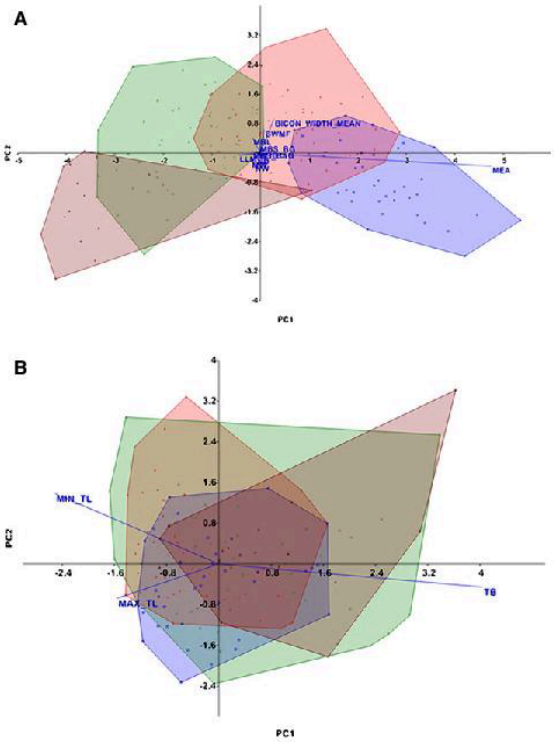


Fig. 2. Principal component analysis (PCA) assessing the level of shape variance for the mandible (A) and the tongue (B), between each of the four groups: group 1 (blue: 20–30 gestational weeks), group 2 (red: 31–40 gestational weeks), group 3 (green: 0–12 months), and group 4 (brown: 12.5–36 months). Box plots illustrating the effect of each measurement on PC1 and PC2. BWMF, mental interforaminal distance; LLM, maximum length of the mandible; MBL, mandibular body length; MBS_BG, mandibular basilar bigonial width; MBS_BAG, mandibular basilar biantegonial width; MBW, maximum bicondylar width; MEA, mental angle; MW, maximum width of the mandible; NW, mandibular notch width; TB, tongue breadth; MAX_TL, maximum tongue length; MIN_TL, minimum tongue length; BICON_WIDTH, bicondylar width of mandible.

increasing biomechanical and space demands on the mandible from the emerging deciduous dentition, as well as the successive developing permanent dentition during this time (42).

We used the geometric mean and Mosimann shape variables as tools to visualize the effects of variations in size and shape in the sample. By subjecting each of these variables to a PCA, with the main focus of each PCA being either size or shape, we effectively studied the influence of size and shape as independently as possible. This process of analysis further enabled us to visualize the influence of each of the assessed measurements on the size and shape changes of the sample with the aid of loading values obtained from the PCA. Significant age-related size increases were observed in both the mandible and the tongue in individuals between 20 wks of gestation and 3 yrs postnatally. The size increases were emphasized in our study by increases in the posterior region of the mandible, which may be associated with an increase in the width of the cranial base (18, 43) or the lateral expansion of the tongue, as shown in the present study. Mandibular growth and position relative to the posterior cranial base may be influenced by variations in the growth rate, length, and inclination of the posterior cranial base components (43, 44). The mandibular condyle, as a principal centre of growth of the mandible, through its association with the mandibular fossa of the temporal bone from 14 wk of development, will grow synchronously with the rapid lateral expansion of this region (43, 44). This is observed in the current study in the significant increase in the bicondylar width of the mandible. There is, at this stage, a strong correlation between the growth of the posterior cranial base components and the lateral expansion of the mandible. However, it should also be considered that there may be additional influences from the anterior cranial base, in terms of varying growth rates and their effect on muscle attachment sites (43, 45).

It has been suggested by Moss & Rankow (44) and Koski (2) that the significant increase in the bicondylar width of the mandible may result from muscular tension on the condyle of the mandible resulting from varying cranial base growth rates. The increased growth of the anterior cranial base when compared with the posterior cranial base may further influence the growth of the mandible through vectorial forces from the various attachments of the muscles of mastication (2, 43, 44).

Additionally, it appears that the lateral expansion of the growing tongue may influence the growth of the posterior regions of the mandible. This is evident in the highly correlated increased lateral expansion of the maximum breadth of the tongue with the mandibular gonial region seen here. Swineheart (18) postulated that it was the growing tongue, through its natural expansive forces, that increased the dimensions of the mandibular arch sufficiently to accommodate the dentition. Evidence of the tongue's natural expansive forces in normal development is emphasized by the significantly retarded transverse development of the mandibular arch in cases of aglossia (25).

As the current study delves into a particularly complex period of oral-cavity development, an appreciation of the preceding developmental events is necessary to fully understand the relationships observed within the current context. In the current study, the consistent widening of the posterior region of the mandible suggests a consistent lateral expansive force of the tongue descending into the oral cavity. Age, in this instance, should also be considered in evaluations of the tongue, as children are noted to have higher dorsal tongue heights when compared with adults (19). This is consistent with previous descriptions of growth where the tongue commences its descent only after the enlargement of the oral cavity, thus allowing the closure of the palatal shelves (3).

However, in addition to the expansive forces exerted by the growing tongue on the mandible, there are movement-related forces that also progressively exert their effects. During the eighth week of development,

Table 4

Linear correlation analysis between tongue and mandible measurements across groups 1–4

Variable	Maximum tongue length	Minimum tongue length	Tongue breadth
Mandibular body length	0.656	0.616	0.809
Maximum mandibular length	0.649	0.611	0.809
Mandibular width	0.575	0.539	0.724
Mandibular notch width	0.518	0.485	0.669
Mandibular basilar width bigonial	0.674	0.644	0.834
Mandibular basilar width biantegonial	0.662	0.637	0.834
Mental interforaminal distance	0.694	0.678	0.803
Mandibular bicondylar width	0.699	0.674	0.864
Mental angle	0.015	0.040	0.038
Maximum tongue length		0.962	0.806

Values >0.7 indicated a high degree of correlation. This was observed predominantly in the coronal plane measures. Alternatively, good to moderate correlations were observed in the sagittal plane measures.

the mouth has been found to open and close in response to reflex activity in the depressor and elevator muscle groups associated with the fetal mandible (3, 46, 47). These reflex activities produce low-magnitude and high-frequency strains, which have been shown to have a significant effect on the determination of bone structure (48). They further serve as precursors to the development of the skeletal attachment sites of the associated muscle groups (i.e. the condylar and coronoid processes) (46, 47, 49). These actions are subsequently followed by more powerful and complex suckling interactions between the tongue and the oral cavity (3, 46, 50, 51). Therefore, suckling during infancy is described as a combination of suction (negative pressure within the oral cavity) and expression (positive pressure exerted by the tongue), actions complicated by varying cycles of nutritive and non-nutritive suckling, which requires the independent functioning of both the anterior and posterior areas of the tongue (46). This suggests that there are functionally different demands on the anterior and posterior regions of the developing mandible with the onset of suckling and subsequently with the onset of mastication during the juvenile period of growth (3, 46, 47, 51). The influence of various types of suckling and the associated change in suckling dynamics is highly prevalent in the occurrence of malocclusions. In a recent study conducted by Melink *et al.* (17), it was concluded that infants who were bottle-fed adopted an incorrect tongue posture and function, which led to a change in the normal dynamics of the oral cavity (17). The change in oral-cavity dynamics through incorrect tongue posture and subsequent lengthening of the lingual frenulum, led to the development of a posterior cross-bite (17). In turn, this change resulted in a broadening of the mandible arch on the cross-bite side (17).

In the current study, significant lengthening in the sagittal plane of the mandible was noted. However, there was no significant widening in the interforaminal distance between the mental foramina. This suggests that the mandible maintains a constant growth rate anteriorly, despite considerable morphological changes resulting from early suckling. The relationship between the anterior region of the mandible and tongue has been reported to commence as early as the seventh week of development (13). The attachment of the genioglossus muscle, first observed on the inferior side of the anterior portion of Meckel's cartilage, reattaches to the trabeculae of the mandibular symphysis by the end of the eighth week (13). During the sixth week, the primary ossification centre of the mandible is first observed near the mental foramen (12, 13). The trabeculae of the mandible then extend radially towards the future mandibular symphysis, alveolar bone, mandibular body, and coronoid process (13). In a study of development in a porcine model, the radial extension of the trabeculae resulted in an increased level of mineralization in the body of the mandible compared with the condylar region (52). Thus, a higher turnover of bone, resulting from an increased growth rate in the condylar region of the mandible, is indicative of the direction of mandibular growth (52).

The high degree of mineralization in the body of the mandible in humans compared with pigs may also be related to the arrangement of muscle fibres associated with the muscles in the floor of the mouth (53). These closely reflect the size and orientation changes in the bony architecture of the mandible during growth (53). As the mandible grows, the mesenchymal tissue is stretched and indicates the muscle fibre arrangement in the direction of the imposed force (53). Furthermore, the dimensions of the mandibular arch increase to accommodate the growth of the tongue and the development and emergence of the deciduous dentition (14, 15).

In the current study the normal growth of the mandible and tongue is reflected as a synchronous relationship in which significant size increases in the length of the mandible and the tongue are observed. Liu *et al.* (54) analysed the effects of tongue reduction on mandibular growth and observed significant reductions in the anterior mandibular length, mandibular symphysis length, dorsal width of the anterior mandible, and mandibular dental arch at the canine teeth (54). These observations suggest that a reduction in the volume of the tongue during periods of growth would result in alterations in the craniofacial skeletal growth and dental arch formation (54). Salles *et al.* (25) similarly observed retardation in the growth of the mandible in a 14-yr-old patient diagnosed with aglossia. The alveolar arches had not developed transversally and there was significant retardation in the anterior growth of the mandible (25). There was also a significant reduction in the width of the mandibular arch, leading to a reduction in the space between the mandibular bodies (25). Retardation in the growth of the mandible was attributed to the absence of muscular stimulus between

the alveolar arches (25), usually provided by the tongue in the initial stages of suckling and later during mastication (3, 46). Additionally, similar observations were made in a case of hypoglossia, in which the patient presented with a smaller tongue as well as with a retruded lower lip and micrognathia (21, 25). Although the tongue was present, the significant reduction in tongue size was considered to result in severe retardation of the downward and forward growth of the mandible (21).

Although the mental angle was the only parameter that indicated a significant change in the shape of the mandible in our study during the time-period of assessment, it showed no significant change in the size. The change in the shape of the mental region may be caused by the tongue and the suprahyoid muscles (4). These muscular structures act as significant contributors to the positioning and orientation of the anterior teeth through bone remodeling, which ultimately shapes the prominence of the mental region (4). Furthermore, changes in the magnitude and frequency of strain produced by various muscle groups associated with speech production also play a significant role in shaping the mental region (48). High-frequency and low-magnitude forces associated with speech production, significant increases in the trabecular density, and bone volume may account for the significant postnatal development of this region (48). However, the significant change in the shape of the mental region may also be a result of the fusion of the hemi-mandibles and subsequent eruption of the deciduous dentition (14).

The increases in the posterior mandibular dimensions in the older specimens (i.e. those from cadavers at a later stage of development) indicated the possibility of the mandible increasing in width to accommodate the lateral expansion of the posterior cranial base (43). However, the consistent increase in the bigonial and biantegonial measurements does not preclude the mandible increasing in width to accommodate the descent of the growing tongue into the oral cavity (4, 18, 19). The consistent lengthening of the mandible and the tongue along the sagittal plane further reinforces the necessity for an increase in the mandibular arch to accommodate the growing and descending tongue (14, 18, 19, 21, 25). These findings provide insights into the allometric growth relationship between the tongue and the mandible, potentially assisting the clinician in predicting the behaviour of these structures in the assessment of malocclusions.

Acknowledgements – The authors would like to thank Professors Jan Koolstra, Roy Zent and Ambra Pozzi for their constructive comments and the Carnegie Foundation for funding parts of the project. Professor E. Libhaber and Mr J Hemingway are acknowledged for their statistical advice.

Conflicts of interest – The authors declare no conflicts of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. The age, sex and dental state of the specimens sourced.

Fig. S1. Planned incision lines A–E for dissection of a neonate.

Fig. S2. Diagrammatic view of the whole mandible from an anterior position indicating the 21 homologous landmarks.

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Licensed Content Publication	European Journal of Oral Sciences
Licensed Content Title	Morphometric growth relationships of the immature human mandible and tongue
Licensed Content Author	Erin F. Hutchinson,Jules A. Kieser,Beverley Kramer
Licensed Content Date	Apr 9, 2014
Pages	9
Type of use	Dissertation/Thesis
Requestor type	Author of this Wiley article
Format	Print and electronic
Portion	Full article
Will you be translating?	No
Title of your thesis / dissertation	An assessment of mandibular growth and development
Expected completion date	Dec 2016
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v1.10 Last updated September 2015

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European Journal of Oral Sciences

Eur J Oral Sci 2015; 123: 341–349
DOI: 10.1111/eos.12203
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European Journal of Oral Sciences

Importance of teeth in maintaining the morphology of the adult mandible in humans

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Hutchinson EF, Farella M, Kramer B. Importance of teeth in maintaining the morphology of the adult mandible in humans.
Eur J Oral Sci 2015; 123: 341–349. © 2015 Eur J Oral Sci

Edentulism can negatively affect both masticatory performance and dietary intake by altering the patterns of biomechanical stress and strain, which in turn modifies the morphology of the different regions of the mandible. The morphological changes in the mandible caused by these stressors are unknown when comparing mandibles across varying dentition states. This study investigated differences in the morphology of human mandibles across various states of the dentition. In total, 498 mandibles of individuals between 20 and 80 yr of age at death were sourced from the Raymond A. Dart Collection of Human Skeletons. These included fully dentate (n = 95), partially edentulous (n = 333), and fully edentulous (n = 70) mandibles. Twelve linear anthropometric measurements of the mandible were derived from digitized landmarks located on its external surface. Data analysis included geometric morphometrics and multivariate analyses. Compared with dentate and partially edentulous mandibles, fully edentulous mandibles showed a shorter alveolar height (−0.4 mm), a shorter mandibular body length (−4 mm), a larger gonial angle (+4°), a more obtuse mental angle (+4.7°), a broader bicondylar width (+3.8 mm), and a taller ramus height (+1.6 mm). Thus, edentulous states are associated with an altered mandibular morphology, which may result from a reduction in jaw function.

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Key words: dentition; edentulism; mandible; morphology

Accepted for publication July 2015

The influence of diet on the general performance of the masticatory system and mandibular morphology has been a focus in a variety of studies (1–3). The overall shift in food consistency from a hard diet to a soft diet is believed to cause a reduction in the size of the masticatory musculature as well as in the growth of the maxillomandibular complex in modern Nubians when compared with the earlier Mesolithic population (1). This reduction led to a compensatory decrease in the size of the teeth and the associated alveolar bone (1). Fukase & Suwa (2) observed a similar trend in the symphyseal region of prehistoric Jomon and modern Japanese populations with a shift from a hard diet to a soft diet type. The posterior region of the Jomon mandible (hard-diet consumers) was observed to have greater body height and thicker cortical bone in comparison with that of their modern counterparts (2), although this was not statistically significant. A progressive decline in the general dimensions of the mandible and size of the dentition with the adoption of a more refined diet was also observed in an assessment of the Tigara and Arikara population groups (3). Thus, it may be hypothesized that refinement in the diet could lead to a more gracile mandibular morphology, with a smaller dentition, as a result of a reduction in masticatory performance (1–4). However, although these studies documented a change in the morphology of the mandible in relation to diet, they did not investigate the potential effect of different dentition states (e.g. dentate compared with partial edentulism) on the overall morphology of the mandible.

The majority of studies investigating the effect of varying dentition states on the morphology of the mandible have focused on the relationship between alveolar bone resorption and the position of the mandibular canal (5–9). Cawood & Howell (6) found no significant changes in the morphology of the basal bone of edentulous adult mandibles, except in cases in which the bone was subjected to harmful load effects (e.g. the overloading of ill-fitting dentures). However, significant decreases in both the vertical and horizontal axes of the alveolar bone were observed following edentulism (6, 7). These significant changes in the dimensions of the alveolar bone may largely be attributed to the onset of disuse atrophy (10) and residual ridge resorption (11, 12) following tooth loss. Chrcanovic *et al.* (8) observed that the overall dimensions of the mandible were larger in dentulous individuals than in edentulous individuals. They concluded that with tooth loss, the resorption of the crest of the alveolar bone influenced the position of the mental foramen (8). In addition, Ozturk *et al.* (9), in their assessment of the

ramus height and general corpus dimensions of dentulous and partially edentulous adult mandibles, found that the number of teeth was the primary determinant of changes in the size and shape of the mandible.

Although the effect of each dentition state on the mandibular morphology has been previously documented (6–9), the influence of the varying tooth combinations on the general morphology of the mandible has not been investigated across all states in a particular sample of mandibles. Studies investigating the changes in the morphology of the mandible relative to a particular dentition state have also made use of small samples and undefined age ranges, which may act as confounders in the overall analysis of these samples. In addition, the majority of these studies have made limited use of geometric morphometrics and multivariate means of analysis, which effectively limits the degree of inferences that may be made regarding changes in the morphology of the mandible.

Considering the results of earlier studies in which morphological changes in the mandible were observed in isolation in the dentulous, partially edentulous, or edentulous states specifically (1–9), we hypothesized that the severity of edentulism would be significantly associated with the size and shape of the mandible, with a morphometric gradient from the fully dentate to the fully edentulous state.

Material and methods

Mandibles from early adulthood extending to the advanced stages of senescence were sourced from the Raymond A. Dart Collection of Human Skeletons, School of Anatomical Sciences, University of the Witwatersrand, South Africa. This collection of skeletons has been accumulated from 1924 to present. Four-hundred and ninety-eight mandibles were assessed, from 262 male and 236 female skeletons (Table 1).

Skeletal samples were obtained under the Human Tissue Act 65 of 1983, and ethical clearance (M121145) to utilize the sample was obtained from the Human Research Ethics Committee – medical.

The age of specimens ranged from 20 to 80 yr. The ages of the skeletal specimens were assessed based on the state of the ante-mortem dentition in addition to the recorded age at death of each individual. The sample was subdivided into three age groups: group 1, 20–40 yr; group 2, 41–60 yr; and group 3, 61–80 yr (Table 1). The state of the dentition of the sample was determined based on the condition of the dental crypts and was limited to the mandible. Individuals presenting with a full permanent dentition (third molars included) were classified as dentulous. Edentulous individuals were classified based on the complete/partial closure of the dental crypts and absence of the entire dentition (6, 7). In the case of individuals presenting with an incomplete dentition and/or intact dental crypts (post-mortem tooth loss), the mandible was classified as partially edentulous (Table 1). Individuals classified as partially edentulous presented with dentition sets that ranged from the presence of only one tooth to 15 mandibular teeth.

Individuals showing evidence of damage to the mandible, congenital abnormalities, bony tissue pathologies, or surgical intervention, were excluded from the study.

Table 1

Age, sex, and dental state of the specimens sourced from the School of Anatomical Sciences, University of the Witwatersrand

Age group	Sex	Dentulous	Partially edentulous	Edentulous	Total
Group 1	M	36	47	4	87
(n = 174)	F	24	61	2	87
20–40 yr					
Group 2	M	14	64	8	86
(n = 166)	F	11	58	11	80
41–60 yr					
Group 3	M	8	56	25	89
(n = 158)	F	2	47	20	69
61–80 yr					

F, female; M, male.

Evidence of previous orthodontic treatment, such as lingual retainers, also led to exclusion from the study.

Methodology

An Immersion MicroScribe G2 (Solution technologies, Oella, MD, USA), with an accuracy of 0.38 mm, was used to record 21 landmarks on the mandible (Fig. 1A). These landmarks included the points necessary to calculate the linear measurements and to define the shape of the mandible. To standardize the collection of landmark data, the dry mandibles were placed in a fixed position (Fig. 1). Landmarks were recorded in a pre-set sequence (clockwise direction) (Fig. 1).

Using each landmark, three coordinates (X, Y, and Z) were recorded and represent the horizontal, vertical, and depth planes, respectively. The three-dimensional coordinates were then imported into a Microsoft Excel worksheet and the Pythagorean formula was used to convert the data into linear distances:

$$\sqrt{((x_1 - x_2)^2 + (y_1 - y_2)^2 + (z_1 - z_2)^2}$$

where x_1, y_1, z_1 and x_2, y_2, z_2 refer to the coordinates of the first and second landmarks, respectively.

Twelve standard anthropometric measurements, derived from previous research (13–18), were recorded using digitized points. In addition, the measurements for the maximum bicondylar width (MBW) and the distance between the mental foramina and the superior border of the mandible (MF_SB) were specifically designed for the purposes of this study (Fig. 1). All measurements assessed morphological and size changes in the sagittal, coronal, and transverse planes of the mandible and are described in detail in Hutchinson *et al.* (19).

Furthermore, the measurements used to calculate the mental angle (MEA) included the length of the mandibular body of each side, as well as the mandibular basilar width bigonial. The gonial angle (MAN_ANG) was calculated using the height of the ramus, the length of the mandibular body, and maximum length of the mandible measurements for the left and right sides, respectively (Fig. 1). The cosine rule $\text{CosC} = (a^2 + b^2 - c^2)/(2ab)$ was then used to calculate the MEA and the MAN_ANG because the triangles formed by the measurements were not considered to be right-angled triangles; thus the Pythagorean equation could not be used.

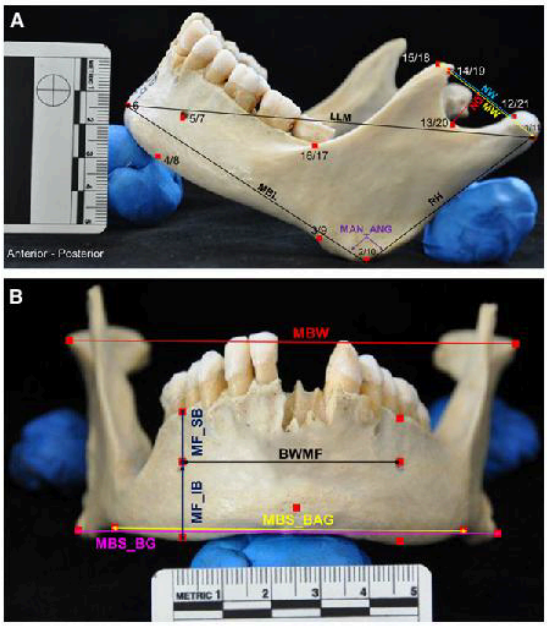


Fig. 1. (A) Lateral view of the left side of the mandible indicating the digitizing position used for osteological specimens (Raymond A. Dart Collection), as well as the measurements corresponding to the sagittal plane of assessment. The mental region is elevated and secured in place anteriorly by morphometric putty. The ramus of the mandible is secured posteriorly midway along the ramus to expose all necessary points for digitization. Twenty-one landmarks were recorded across each mandible (landmarks 1–5 and 17–21 were recorded on the right side of the mandible, and landmarks 7–16 were recorded on the left side of the mandible). (B) Anterior view of the mandible indicating the transverse and vertical measurements. BWMF, mental interforaminal distance; LLM, maximum length of the mandible; MBL, mandibular body length; MBS_BAG, mandibular basilar width biantegonial; MBS_BG, mandibular basilar width bigonial; MBW, maximum bicondylar width; MEA, mental angle; MF_IB, distance between the inferior border of the mandible and the mental foramen; MF_SB, distance between the mental foramina and the superior border of the mandible; MW, maximum width of the mandible; ND, depth of the mandibular notch; NW, width of the mandibular notch; RH, height of the ramus.

Statistical and morphometric analysis

Descriptive statistics, including means and SD, were calculated for all mandibular measurements. The Shapiro–Wilk test was used to assess the distribution of the sample, which further determined statistical analyses based on the distribution type (20). All statistical analyses were conducted using PAST (Paleontological Statistics, Paleontological Association, London, UK) (21) and SPSS v. 20 (IBM, Armonk, NY, USA) statistical programs.

A two-way ANOVA was conducted to evaluate the relationship between the dependent variables i.e. the anthropometric measurements and the independent variables, which included age and dentition state. The degree of interdependency between factors was also evaluated (20). In cases where a significant degree of interaction occurred between factors, a Tukey's honest significant difference (HSD) post-hoc test and pairwise comparisons were conducted to compare the independence of factors and the associated level of significance (20). Additionally, the relationship between size and shape was tested using a multivariate analysis of variance (MANOVA) (20). Furthermore a multivariate analysis of covariance (MANCOVA) was conducted in which the geometric mean was selected as the covariate (22). In general, the effects of age and dentition, and the interaction between age and dentition, were investigated further if variables between subjects were significant ($P < 0.05$).

To visualize the differences in the size and shape of the mandible with growth and varying state of the dentition, the sample data set was subjected to a generalized full Procrustes analysis and principal component analysis using PAST (21) and MORPHOLOGIKA 2 v. 2.5 (University College London, UK) (23, 24) respectively. A generalized full Procrustes analysis (23) was performed, which eliminates the influence of size, rotation, and translation of the mandible. Data were then organized according to dentition state (dentulous, partially edentulous, and edentulous) and the mean shape of each dentition state was assessed using wireframes.

Inter- and intra-observer errors were evaluated using repeated measurements on 10 random specimens that were then tested using the Lin's concordance correlation coefficient of reproducibility, which provides information on the level of repeatability of the method and the degree of association between measurements (25).

Results

A very high degree of repeatability (79–99%) was observed across the 14 measurements assessed in the current study. However, a moderate level of repeatability was also observed in the distance between the inferior border of the mandible and the mental foramen (60%).

The overall effect of age and the state of the dentition on all measurements assessed was first tested independently using a two-way ANOVA. Both age and the state of the dentition were observed to have an effect on the MBW ($P < 0.04$ and $P < 0.01$, respectively) and on the MAN_ANG ($P < 0.05$ and $P = 0.00$, respectively). Whilst the age of the individual had no further effect on the mandibular measurements, the state of the dentition influenced the geometric mean (GM) ($P < 0.01$), the mental interforaminal distance (BWMF) ($P < 0.05$), the MF_SB ($P = 0.00$), and the mandibular body length (MBL) ($P = 0.00$). In addition, the state of the dentition further influenced the mandibular width (MW) ($P < 0.01$), the height of the ramus (RH) ($P = 0.00$), and the MEA ($P = 0.00$) (Table 2).

The only differences in the overall size and shape (MANOVA) of the mandible associated with age, were found for MBW ($P < 0.036$) and MAN-ANG ($P < 0.046$), which were larger in the older age group (61–80 yr) than in the younger age groups (Table 3, Fig. 2). The differences in the position of the ramus became more obvious with the comparison between dentate and edentulous mandibles, in which the MBW ($P < 0.01$), the MAN-ANG ($P = 0.00$), and the RH ($P < 0.00$) were significantly larger in edentulous than in dentate mandibles (Table 3, Fig. 2).

The width of the mandible was significantly smaller ($P < 0.006$) in the edentulous mandibles than in the den-

Table 2

Two-way analysis of variance (ANOVA) investigating the between-group effects of age and dentition on all assessed measurements

Measurement	Age ($P < 0.05$)	Tukey's post-hoc test ($P < 0.05$)			Dentition ($P < 0.05$)	Tukey's post-hoc test ($P < 0.05$)		
		Group 1 vs. Group 2	Group 2 vs. Group 3	Group 1 vs. Group 3		Group 1 vs. Group 2	Group 2 vs. Group 3	Group 1 vs. Group 3
GM	0.69	–	–	–	0.01	0.00	0.48	0.00
MBS_BG	0.28	–	–	–	0.35	–	–	–
MBS_BAG	0.20	–	–	–	0.09	–	–	–
BWMF	0.11	–	–	–	0.05	0.00	0.09	0.00
MF_IB	0.13	–	–	–	0.56	–	–	–
MF_SB	0.20	–	–	–	0.00	0.00	0.00	0.00
MBL	0.24	–	–	–	0.00	0.00	0.16	0.01
LLM	0.14	–	–	–	0.37	–	–	–
MW	0.55	–	–	–	0.01	0.00	0.35	0.00
NW	0.41	–	–	–	0.06	–	–	–
ND	0.42	–	–	–	0.46	–	–	–
RH	0.24	–	–	–	0.00	0.13	0.92	0.02
MEA	0.86	–	–	–	0.00	0.00	0.02	0.00
MBW	0.04	0.00	0.22	0.00	0.01	0.00	0.52	0.00
MAN_ANG	0.05	0.16	0.87	0.05	0.00	0.00	0.00	0.06

BWMF, mental interforaminal distance; GM, geometric mean; LLM, maximum length of the mandible; MAN_ANG, gonial angle; MBL, mandibular body length; MBS_BAG, mandibular basilar width biantegonial; MBS_BG, mandibular basilar width bigonial; MBW, maximum bicondylar width; MEA, mental angle; MF_IB, distance between the inferior border of the mandible and the mental foramen; MF_SB, distance between the mental foramina and the superior border of the mandible; MW, maximum width of the mandible; ND, depth of the mandibular notch; NW, width of the mandibular notch; RH, height of the ramus; –, indicates absence of data.

tate and partially edentulous mandibles across the 41–80 yr age range ($P < 0.014$) (Tables 2 and 3). In addition to the overall size and shape variations observed in the rameal region of the mandible, the arch of the mandible was significantly shorter across the length of the mandibular body ($P < 0.000$) in the edentulous mandible in comparison with the dentate and partially edentulous mandibles (Table 3). The height of the mandibular body was also significantly reduced in the edentulous mandible. The MF_SB was significantly shorter ($P = 0.00$) in the alveolar region of edentulous mandibles, particularly when compared with the partially edentulous group (Tables 2 and 3). Furthermore, along the anterior mental region of the mandible, the influence of age and the absence of the dentition ($P < 0.001$) culminated in a significantly smaller BWMF ($P < 0.047$) (Tables 2 and 3, Fig. 2). No further significant changes in the BWMF were observed when comparing the partially edentulous and edentulous groups. In contrast, the MEA was significantly larger ($P = 0.000$) in the edentulous individuals, whereas that in the dentulous individuals, and particularly in the partially edentulous individuals, was small (Table 3, Fig. 2). The edentulous mandible was also observed to be significantly smaller than partially edentulous and dentate mandibles (GM, $P = 0.009$).

When comparing mandibles across age groups, the MAN_ANG of elderly individuals was significantly larger ($P < 0.046$) than that of individuals in the younger groups, and the body of the mandible was significantly longer in elderly individuals than in individuals of younger groups, both in the maximum length of the mandible (LLM) ($P < 0.004$) and in the MBL ($P < 0.031$). The mandibular arch was broader posterosuperiorly, with a significantly wider MBW ($P < 0.006$) in the elderly group (Table 4, Fig. 2).

When comparing mandibles across the dentate, partially edentulous, and edentulous dentition states, the significantly larger MAN_ANG ($P = 0.001$) in the edentulous group gave the ramus of the mandible a reclined appearance (Table 4). This gave the edentulous mandibular arch a longer appearance in the sagittal plane. This longer appearance was further emphasized by a significantly taller ramus ($P < 0.000$) and increased maximum length of the mandible (LLM) ($P = 0.000$) in the edentulous group (Fig. 2). A significantly shorter MBL ($P < 0.005$) and MF_SB ($P = 0.000$) resulted in the appearance of a vertically compressed mandibular body in the edentulous mandible (Table 4, Fig. 2). The compression of the body was further accentuated by a significantly wider MEA ($P = 0.000$), mandibular basilar width bigonial (MBS_BG) ($P < 0.001$), and mandibular basilar width biantegonial (MBS_BAG) ($P = 0.000$) across the transverse plane in the edentulous mandibles. In addition, the generally everted appearance of the ramus was further emphasized by a significantly larger MBW of the mandible ($P < 0.000$) and mandibular notch depth (ND) ($P < 0.012$), particularly when comparing the edentulous and partially edentulous groups (Fig. 2).

Discussion

The state of the dentition may influence the functional ability of the jaw (26, 27), and this, in turn, may affect the remodelling of mandibular bone (4). As a result of this interaction, we hypothesized that variation in the state of the dentition would have an effect on the mandibular morphology.

Table 3

Multivariate analysis of variance (MANOVA) indicating the pairwise comparisons for each of the age groups and dentition states associated with the individual measurements

Measurement	Age	Mean ± SD (mm)			Dentition ($P < 0.05$)				Mean ± SD (mm)		
	Univariate$F$ ($P < 0.05$)	Group 1 (20–40 yr) ($n = 174$)	Group 2 (41–60 yr) ($n = 166$)	Group 3 (61–80 yr) ($n = 158$)	Univariate$F$ ($P < 0.05$)	Dentate vs. Edentulous	Dentate vs. Partially edentulous	Partially edentulous vs. Edentulous	Dentate	Partially edentulous	Edentulous
GM	0.4 (0.69)	47.6 ± 2.4	47.7 ± 2.7	47.0 ± 2.7	4.7 (0.01)	0.00	0.09	0.02	47.9 ± 2.5	47.6 ± 2.6	46.1 ± 2.6
MBS_BG	1.3 (0.28)	89.1 ± 6.2	91.6 ± 6.8	91.7 ± 6.3	1.1 (0.35)	–	–	–	90.0 ± 6.2	90.6 ± 6.7	92.3 ± 6.3
MBS_BAG	1.6 (0.20)	81.7 ± 5.0	84.1 ± 4.9	83.9 ± 4.73	2.4 (0.09)	–	–	–	82.6 ± 5.1	83.1 ± 5.0	84.5 ± 4.5
BWMF	2.2 (0.11)	44.8 ± 3.0	44.8 ± 2.8	44.6 ± 3.1	3.1 (0.05)	0.06	0.02	0.79	45.5 ± 3.0	44.8 ± 2.9	43.5 ± 2.8
MF_SB	1.6 (0.20)	15.1 ± 2.5	13.9 ± 3.3	12.3 ± 3.5	69.2 (0.00)	0.00	0.00	0.00	13.4 ± 1.6	13.2 ± 1.6	13.0 ± 1.7
MF_IB	2.0 (0.13)	13.5 ± 1.6	13.0 ± 1.5	13.2 ± 1.6	0.6 (0.56)	–	–	–	15.4 ± 2.2	14.3 ± 2.8	9.1 ± 3.0
MBL	1.4 (0.24)	82.7 ± 5.1	84.3 ± 5.2	83.2 ± 5.3	8.5 (0.00)	0.00	0.00	0.12	84.5 ± 5.8	83.4 ± 5.1	81.5 ± 4.6
LLM	2.0 (0.14)	114.8 ± 5.8	117.6 ± 6.2	116.4 ± 5.8	1.0 (0.37)	–	–	–	115.8 ± 6.5	116.2 ± 5.9	117.0 ± 5.7
MW	0.6 (0.55)	38.9 ± 3.8	38.5 ± 3.9	38.0 ± 3.8	5.1 (0.01)	0.00	0.03	0.04	39.2 ± 4.0	38.6 ± 3.8	36.7 ± 3.4
NW	0.9 (0.41)	26.5 ± 3.3	27.0 ± 3.2	26.9 ± 3.3	2.9 (0.06)	–	–	–	27.1 ± 3.6	26.8 ± 3.2	26.3 ± 2.9
ND	0.9 (0.42)	12.0 ± 3.4	12.0 ± 3.4	11.5 ± 3.5	0.8 (0.49)	–	–	–	12.0 ± 3.6	11.7 ± 3.4	11.8 ± 3.1
RH	1.4 (0.24)	56.0 ± 5.1	57.1 ± 5.8	56.5 ± 5.8	5.9 (0.00)	0.01	0.67	0.00	56.5 ± 4.9	56.2 ± 5.6	58.1 ± 5.8
MBW*	3.3 (0.04)	104.7 ± 5.4	107.6 ± 6.0	108.7 ± 5.6	4.5 (0.01)	0.01	0.81	0.00	105.9 ± 6.3	106.7 ± 5.6	109.7 ± 6.0
MEA	0.1 (0.86)	65.2 ± 4.5	66.0 ± 5.0	67.1 ± 5.6	12.1 (0.00)	0.00	0.00	0.00	64.4 ± 4.0	65.9 ± 5.0	69.1 ± 5.5
MAN_ANG†	3.1 (0.05)	110.4 ± 4.9	111.4 ± 4.9	111.7 ± 5.2	8.5 (0.00)	0.01	0.00	0.96	108.9 ± 4.7	111.4 ± 4.9	112.9 ± 5.0

*Statistically significant differences in MBW were found for the group 1 vs. group 2 ($P < 0.01$) post-hoc comparison, within the age assessment.
†Statistically significant differences in MAN_ANG were found for the group 1 vs. group 2 ($P < 0.01$) post-hoc comparison, within the age assessment.
BWMF, mental interforaminal distance; GM, geometric mean; LLM, maximum length of the mandible; MAN_ANG, gonial angle; MBL, mandibular body length; MBS_BAG, mandibular basilar width biantegonial; MBS_BG, mandibular basilar width bigonial; MBW, maximum bicondylar width; MEA, mental angle; MF_IB, distance between the inferior border of the mandible and the mental foramen; MF_SB, distance between the mental foramina and the superior border of the mandible; MW, maximum width of the mandible; ND, depth of the mandibular notch; NW, width of the mandibular notch; RH, height of the ramus; –, indicates absence of data.

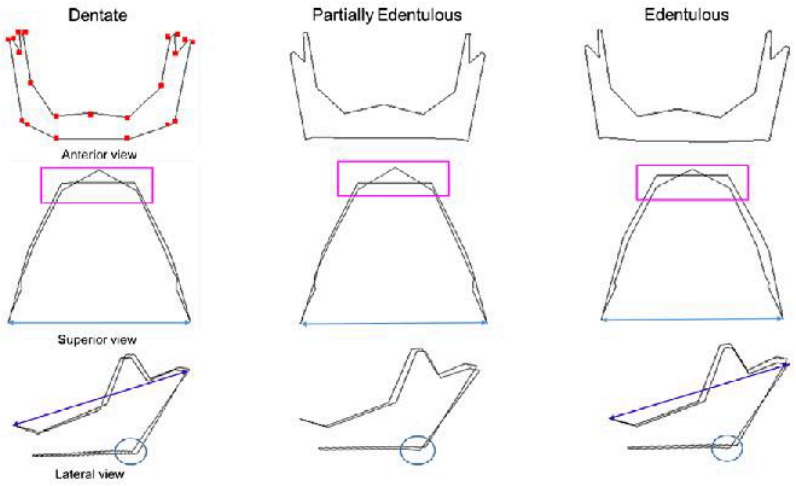


Fig. 2. Principal component analysis wireframes illustrating the superimposed superior and inferior surfaces (superior view) and left and right lateral surfaces (lateral view) and anterior view. A widening of the mandibular arch in the mental region (pink rectangle) and bigonial width (light blue line) in the superior view, as well as a reclining of the ramus indicated by an increase in the length of the mandible (dark blue line) and deepening of the antegonial notch (blue circle) in the lateral view, were all indicated across the assessed dentition states.

The overall dimensions of the edentulous mandibles were significantly smaller than those of mandibles where teeth were present, with the exception of the MEA and the MAN_ANG. Therefore, the presence of dentition appears to maintain an upright ramus and a morphologically distinct mental region.

The significantly lower height of the mandibular corpus observed in the edentulous mandible when compared with the dentate mandible, may be explained by alveolar bone resorption resulting from tooth loss (6), disuse atrophy (10), and residual ridge resorption (11, 12). Bone resorption and formation may persist throughout life at a reduced rate (11, 12, 28). The subsequent alteration in the morphology of the residual alveolar ridge affects the retention and functioning of dentures, which both rely on the quantity of bone, the

Table 4

Multivariate analysis of covariance (MANCOVA) indicating the pairwise comparisons for age and the dentition states associated with the individual measurements

Measurement	Age Univariate F ($P < 0.05$)	Dentition ($P < 0.05$) Univariate F ($P < 0.05$)	Dentate vs. Edentulous	Dentate vs. Partially edentulous	Partially edentulous vs. Edentulous
MBS_BG	2.6 (0.08)	7.0 (0.00)	0.00	0.17	0.00
MBS_BAG	2.7 (0.07)	10.6 (0.00)	0.00	0.24	0.00
BW_MF	2.2 (0.11)	1.9 (0.15)	–	–	–
MF_SB	1.3 (0.28)	72.7 (0.00)	0.00	0.01	0.00
MF_IB	1.9 (0.14)	1.9 (0.15)	–	–	–
MBL*	3.5 (0.03)	5.3 (0.01)	0.02	0.00	0.85
LLM†	5.5 (0.00)	9.8 (0.00)	0.00	0.60	0.00
MW	1.0 (0.38)	1.3 (0.26)	–	–	–
NW	2.5 (0.09)	1.3 (0.28)	–	–	–
ND	0.9 (0.41)	4.5 (0.01)	0.01	0.93	0.00
RH	1.1 (0.35)	19.4 (0.00)	0.00	0.46	0.00
MBW†	5.1 (0.01)	12.2 (0.00)	0.00	0.23	0.00
MEA	0.1 (0.87)	12.8 (0.00)	0.00	0.00	0.00
MAN_ANG‡	3.1 (0.05)	7.1 (0.00)	0.04	0.00	0.56

*Statistically significant difference in MBL was found for group 1 vs. group 3 ($P < 0.01$).
†Statistically significant differences in LLM and MBW were found for group 1 vs. group 2 ($P < 0.00$) and for group 1 vs. group 3 ($P < 0.01$).
‡Statistically significant difference in MAN_ANG was found for group 1 vs. group 2 ($P < 0.01$) post-hoc age comparison.
BWMF, mental interforaminal distance; LLM, maximum length of the mandible; MAN_ANG, gonial angle; MBL, mandibular body length; MBS_BAG, mandibular basilar width biantegonial; MBS_BG, mandibular basilar width bigonial; MBW, maximum bicondylar width; MEA, mental angle; MF_IB, distance between the inferior border of the mandible and the mental foramen; MF_SB, distance between the mental foramina and the superior border of the mandible; MW, maximum width of the mandible; ND, depth of the mandibular notch; NW, width of the mandibular notch; RH, height of the ramus; –, indicates absence of data.

architecture of the mandible (12), and the overall efficiency of the masticatory apparatus.

Tooth loss accompanied by the resorption of the residual alveolar ridge can influence the overall relationship between the maxilla and mandible, as well as muscle function and facial morphology (7). These changes may have occurred in our study sample because a significant decrease in the overall size, as well as in the length, of the mandibular body was observed in edentulous individuals when compared with their dentate counterparts. In addition, a significantly increased MBW and MEA were observed in the edentulous mandibles when compared with their dentulous counterparts. The shorter mandibular body and increased MBW and MEA may be attributed to bone resorption expedited by changes in bone density (29–31). The shortening and widening of the dental arch found in individuals ≥40 yr of age can be a result of resorption of the buccal cortex of incisor alveolar bone and of the lingual cortex of molar alveolar bone, which, in turn, may be facilitated by a decrease in bone density (32). In addition, the larger MBW of the mandible and the wider MEA, observed in our study, can be explained by an increase in the buccal cortical thickness of the mandible, accompanied by bone resorption lingually. The shorter mandibular body would also have an effect on the overall geometry of the mandibular arch.

In the current study, the LLM and the MAN_ANG were significantly larger in edentulous individuals when compared with their dentate counterparts. The ramus of the mandible in edentulous individuals also appears to be in a reclined position. The overall increase in the MAN_ANG with molar tooth loss (33) has been previously attributed to the so-called 'moulding effect' of the masticatory muscles on the mandibular morphology in an attempt to restore the occlusal plane and maintain optimal mastication (33). This effect on mandibular morphology is thought to be dependent on the functionality of the masticatory muscles (34). However, in the present study, as specimens were derived from a skeletal population, no inference can be made regarding the anatomy or level of activity of the muscles of mastication. In addition, as specimens presenting with pathologies were excluded from our study sample, the potential influence of systemic influences, such as osteoporosis and osteopenia, on the individual mandible has not been considered.

While a larger MAN_ANG is in agreement with previous studies assessing the transition between dentulism and edentulism (35–39), the larger RH is not consistent with the literature. The progressive loss of the natural dentition results in an alteration of occlusal relationships, rotations of the mandible, realignment of the mandibular corpus, and changes in the regional morphometry of the mandible (35). The overall change in the occlusal relationship further results in an altered pattern of the intensity and frequency of stress and strain imposed on the bone by the surrounding structures, which affect the balance between bone resorption and remodelling (40–42). This may assist in explaining why the RH in edentulous mandibles was larger than in their dentate counterparts. Indeed, this may be the result of condylar remodelling as a result of the different physiological demand associated with edentulism. Interestingly, as the loss of dentition proceeds, there is a reduction in the overall facial height and vertical dimensions of occlusion, resulting in mandibular prognathism (43–45).

The overall prognathism of the mandible with the onset of edentulism is further accentuated by the glenoid fossa adopting a more anterior position in edentulous individuals compared with its more posterior position in dentate individuals (44). Whilst there is a change in the antero-posterior position of the glenoid fossa with a reduction in the vertical facial dimensions, there is no further change in the lateral position of the glenoid fossa in edentulous individuals (45). However, the maximum bite force of the muscles of mastication is also influenced by a change in the vertical dimensions, with the elevator muscles having a greater mechanical advantage when the ramus is more vertical and the MAN_ANG more acute (46). In the current study, the ramus was observed to be significantly narrower in the edentulous mandible than in the mandibles of dentate and partially edentulous individuals. No additional significant differences were observed in the dimensions of the mandibular notch across the various dentition states. The narrowness of the ramus with edentulism may be attributed to bone resorption resulting from a decrease in bite force associated with tooth loss and denture use (38, 40–42).

The present study has a number of methodological limitations that should be considered. The study design is cross-sectional and not longitudinal. This effectively limits any causal inferences regarding changes in morphology with age. Furthermore, there is a potential confounding effect of secular trends. The concept of a secular trend is described within the context of an overall increase in the dimensions of the body over a substantial period of time and is widely discussed within the scope of growth-related studies (47–49). In addition, the occurrence of secular trends has previously been ascribed to either an individual's genetic potential or their socio-economic status (50). However, the genetic admixture and magnitude of trends across various socio-economic settings within the South African population have essentially reduced the potential for secular trends within this population group (47).

The strengths of this study include a relatively large number of observations, the use of geometric morphometrics to describe shape templates, and the use of multivariate data analysis. As a result, the multivariate analysis used in the current study allowed for the investigation of the individual influences of age and the state of the dentition as potential confounders on the sample.

In conclusion, mandibular edentulism is associated with site-specific effects on mandibular size and shape. The morphological differences that were found between the various states of the dentition may be ascribed to the altered functional activities of the jaw that accompany the onset of edentulism.

Acknowledgements – The authors dedicate this article to Professor Jules A. Kieser, BSc, BDS, PhD, DSc (1950–2014), who was Professor in the Sir John Walsh Research Institute, Faculty of Dentistry, University of Otago, Dunedin, New Zealand for many years. Jules was an outstanding and inspiring teacher, a phenomenal supervisor, and a forensic scientist of immense stature. At the time of his death, he was actively engaged in the planning and data analysis for this paper. Professor E. Libhaber and Mr J. Hemingway are acknowledged for their statistical advice. In addition, this work is based on research supported by the National Research Foundation (Grant UID#91645). Opinions expressed and conclusions arrived at are those of the authors and are not necessarily to be attributed to the NRF.

Conflicts of interest – The authors declare no conflicts of interest.

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Licensed Content Title	Importance of teeth in maintaining the morphology of the adult mandible in humans
Licensed Content Author	Erin F. Hutchinson,Mauro Farella,Beverley Kramer
Licensed Content Date	Aug 19, 2015
Pages	9
Type of use	Dissertation/Thesis
Requestor type	Author of this Wiley article
Format	Print and electronic
Portion	Full article
Will you be translating?	No
Title of your thesis / dissertation	An assessment of mandibular growth and development
Expected completion date	Dec 2016
Expected size (number of pages)	300
Requestor Location	Erin Hutchinson 7 York Road, Parktown Johannesburg, South Africa 2193 Attn: Erin Hutchinson
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Journal of Anatomy

J. Anat. (2017) doi: 10.1111/joa.12591

Variations in bone density across the body of the immature human mandible

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Abstract

During growth the mandible accommodates increases in biomechanical loading resulting from changes in the function of structures of the oral cavity. Biomechanical loads are thought to play an intricate and vital role in the modelling and remodelling of bone, with site-specific effects on bone mineral density. It is anticipated that the effects of this loading on bone mineral density are intensified during the functional transition from prenatal to postnatal stages. The aim of this study was thus to evaluate changes in bone mineral density across the body of the immature human mandible during the early stages of dental development. The study sample included 45 human mandibles, subdivided into three age groups: prenatal (30 gestational weeks to birth; n = 15); early postnatal (birth to 12 months; n = 18); and late postnatal (1–5 years; n = 12). Mandibles were scanned using X-ray micro-computed tomography. Eight landmarks were selected along the buccal/labial and lingual surfaces of each dental crypt for evaluation of the bone mineral density. Bone mineral density values were calculated using a reference standard and analysed using multivariate statistics. The bone mineral density of the lingual surface was found to be significantly higher ($P \leq 0.000$) than that of the buccal/labial surface. Furthermore, bone mineral density in the alveolar region of the buccal/labial surface of the deciduous central incisor ($P \leq 0.001$), the deciduous first molar ($P \leq 0.013$) and lingual alveolar area of the deciduous second molar ($P \leq 0.032$) were significantly greater in the early postnatal period than in the prenatal period. While changes in bone mineral density across the lingual surface were consistent with the progression of development and the biomechanical demand of the tongue as previously demonstrated, changes observed across the buccal/labial surface of the mandible appeared to accompany the advancing dental development. Thus, changes in bone mineral density across the mandible appear to be reflective of the stage of dental development and the level of biomechanical loading.

Key words: embryology; functional morphology; growth/development; imaging; jaw biomechanics; paediatrics.

Introduction

During the transition from the prenatal to postnatal stages, the mandible and the masticatory apparatus adapt to significant variations in the biomechanical forces that are associated with the development, emergence and eruption of the dentition (Herring, 1985; Mavropoulos et al. 2005; Sato et al. 2005). Loading conditions acting on the mandible are thought to play a marked and influential role in the modelling and remodelling of bone (Herring, 1985; van Eijden, 2000; Koolstra, 2002), with site-specific effects on the bone mineral density (van Eijden, 2000; Ruffoni et al. 2007; Hernandez, 2008; Roschger et al. 2008; Cioffi et al. 2010; Hichijo et al. 2015). As such, it may be expected that the effects of increased biomechanical loading on bone mineral density are particularly evident during the functional transition from the prenatal to the postnatal stages of growth, within the immature mandible.

During fetal growth, as the upper digestive tract adapts to increased functional demands and as various mouth-opening reflexes and perinatal oral sensorimotor functions commence, the functionality of the tongue and associated oral cavity musculature is intensified (Miller et al. 2003; Delaney & Arvedson, 2008; Coquerelle et al. 2013). This

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Accepted for publication *30 November 2016*

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surge in the functionality of the oral cavity musculature results in increased strain on the muscle attachment sites as well as escalation in early mandibular movements, which are thought to be initiated by tongue movements (Lee et al. 2001; Coquerelle et al. 2013). During the early postnatal period, the functioning of the masticatory apparatus becomes more sophisticated due to the development, emergence and eruption of the dentition as well as changes in tongue posture. These changes are thought to result in variations in the loading conditions imposed on the mandible. This may lead to a possible change in the bone mineral density of the associated areas of the mandible (Miller et al. 2003; Delaney & Arvedson, 2008; Coquerelle et al. 2013).

Studies assessing variations in mandibular bone mineral density have contrasted hard and soft diet types to assess the impact of forces arising from the muscles of mastication on their respective mandibular attachment sites (Mavropoulos et al. 2004, 2005). However, the influences of increased biomechanical forces resulting from the dentition have not been investigated (Mavropoulos et al. 2004, 2005). Furthermore, while these studies focused on the volumetric density changes, i.e. the amount of mineralised bone associated with bone density, they did not specifically assess changes in the degree of mineralisation of the mineral component of bone density (Mavropoulos et al. 2004, 2005; Roschger et al. 2008). Volumetric density changes serve as valuable indicators of the biomechanical potential of bone, while bone mineral density distributions provide an indication of its strength, stiffness and toughness (Ruffoni et al. 2007; Hernandez, 2008; Roschger et al. 2008; Cioffi et al. 2010; Hichijo et al. 2015). Furthermore, bone mineral density distributions assist in predicting the pattern of biomechanical force and strain across a developing bone, through the identification of areas of bone presenting with low levels of mineralisation and a higher degree of bone modelling and remodelling (Roschger et al. 2008; Cioffi et al. 2010; Hichijo et al. 2015).

Furthermore, studies assessing the prenatal growth of the mandible have limited their focus to the embryonic or early fetal periods of development (Merida Velasco et al. 1990; Lee et al. 2001; Radlanski et al. 2002, 2003, 2004). While the mandible undergoes a significant degree of development during these periods, it is the transition across functional states from the fetal to the early postnatal periods that significantly influences the mandibular morphology (Miller et al. 2003; Delaney & Arvedson, 2008; Coquerelle et al. 2013; Hutchinson et al. 2014). In addition, while studies have assessed the changes in the mandibular morphology during this functionally complex period, there is a paucity of information on how the bone mineral density varies, particularly over periods of dental development and eruption. As such, it was hypothesised that the pattern of bone mineral density of the immature human mandible would be consistent with the levels of biomechanical loading of the bone and the dentition, during perinatal stages of growth. Thus, the aim of this study was to evaluate changes in bone mineral density across the body of the immature mandible during the early stages of dental development. While imaging modalities such as dual-energy X-ray provide a basis for studying volumetric density changes, modalities such as micro-computed tomography (CT) provide detailed information about the bone mineral density distributions and as such the degree of mineralisation of bone (Ruffoni et al. 2007; Roschger et al. 2008). Thus, the study made use of micro-CT scanning to investigate changes in the bone mineral density distribution across areas of the body of the immature mandible associated with the tongue and developing dentition.

Materials and methods

Forty-five fetal and neonatal mandibles were utilised, of which 28 were sourced from fresh cadaveric tissue of unknown provenance from the Johannesburg Forensic Paediatric Collection, Division of Forensic Medicine and Pathology. Soft tissue dissection was performed to extract the mandibles from the fresh cadaveric remains. The remaining 17 mandibles were from skeletonised individuals of the Raymond A. Dart Collection of Human Skeletons, School of Anatomical Sciences. Identical protocols were followed in processing the individual remains from each collection. Use of the skeletonised mandibles is permissible under the Human Tissue Act 65 of 1983, while ethical clearance for the study was obtained from the University of the Witwatersrand's Human Ethics Research Committee (M121145).

Specimens ranged in age from 30 gestational weeks to 5 years postnatal. The ages of all specimens were estimated by means of small dental radiographs (two sets: anterior view showing the anterior dental crypts and associated dentition; and a lateral view showing the posterior dental crypts and the associated developing dentition) obtained with a NOMAD portable X-ray unit (Aribex, Charlotte, NC, USA) and dental development standards previously proposed by Alqahtani et al. (2010). The sample was subdivided into three groups: prenatal (30 gestational weeks to birth, $n = 15$); early postnatal (birth to 12 months, $n = 18$); and late postnatal (1–5 years, $n = 12$). The sample was subdivided according to these chronological intervals as the mandible is thought to undergo significant changes during these designated periods with the progression of oral and masticatory function. A visual examination was conducted across the study sample, and individuals showing obvious evidence of congenital abnormalities, surgical intervention or general damage to the mandible were excluded from the study. As such, 15 specimens were excluded from the total study sample ($n = 60$), and only 45 mandibles were found to be suitable for further analysis.

All mandibles were scanned using a Nikon XTH 225L micro-focus CT X-ray unit (Nikon Metrology, Leuven, Belgium), located at the MIXRAD laboratory at the South African Nuclear Energy Corporation, Pelindaba. Scanning parameters were set to 100 kV/100 μA and 100 μm as a means of standardising the scanning conditions across the sample. A 0.1 mm thick aluminium filter was used to approximate a homogeneous X-ray beam spectrum by removing the lower energy photons. Specimens were securely mounted in a polystyrene mould, with the body of the mandible vertically oriented. In addition a small plastic peg was used as a density reference sample owing to its uniform material composition. The

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reference sample was placed within the polystyrene mould for each scan to further assist in standardising scanning conditions across scans. The mounted specimens were then placed on to a rotating sample manipulator, which facilitated scanning at 360 °. One-thousand projection images were obtained in the 360 ° that resulted in a good-quality scan with optimised time. The scanning setup was optimised for highest spatial resolution by setting the sample with maximum magnification (magnification is a property of a cone beam). Consequently a resolution of between 0.023 and 0.050 μm was obtained. The scans were then reconstructed using NIKON CTPRO software (Nikon Metrology).

Following three-dimensional reconstruction, all the scans were imported into VGStudio Max V2.2 (Volume Graphics GmbH, Heidelberg, Germany) as volume files for further analysis. All imported volume files were then subjected to a surface determination analysis to allow for clear determination of surface landmarks. The orientation of the micro-CT slices of the mandible was then standardised by aligning each slice with an established transverse plane. This alignment allowed for further analysis of the dental crypt in a horizontal cross-section. The transverse reference plane was established by selecting a minimum of three reference points (fiducial markers) along the inferior region of the mandible (Fig. 1). These points, located between the antegonial notch and the dental crypt of the canine (Fig. 1), assisted in standardising the alignment of the micro-CT slices with the transverse plane. All slices of the mandible were then aligned to these reference points and the transverse reference plane was established parallel to the inferior region of the mandible.

After the micro-CT slices of the mandible were aligned with the established transverse plane, the mesiodistal mid-point of each individual dental crypt was determined (Fig. 2A). All dental crypts were included in the study assessment, with the exception of the M2 molar crypt that was only present in one individual. The maximum mesiodistal diameter used to determine the mid-point of each dental crypt was established tangential to the buccal/labial surface (Fig. 2A). The mid-point of the maximum mesiodistal diameter of the dental crypt was then located and a section was taken at this point (Fig. 2A). A single mid-point section was used for the anterior dental crypts as they were not as long as the posterior dental crypts (Fig. 2A). In the case of the posterior dentition, the maximum mesiodistal diameter was subdivided into three sections (distal, midpoint and mesial; Fig. 2A).

To further study the mineral density of the lingual and buccal/labial surfaces of the mandible relative to each dental crypt, the mandible was again subjected to a surface determination analysis (Fig. 2A). The surface determination analysis defines the surface of the mandible in three-dimensional space, which then allows for the selection of points or areas of interest. Following the surface determination analysis, all micro-CT slices were then re-aligned to the sagittal plane at the midpoint of the mesiodistal diameter of each dental crypt and subjected to further cross-sectional analysis (Fig. 2B). The re-alignment of the micro-CT slices from the transverse plane to the sagittal plane provided the opportunity to study changes in the bone mineral density along the superior, middle and inferior points of the lingual and buccal/labial surfaces, respectively (Fig. 2B). This process was repeated for all subsequent dental crypts.

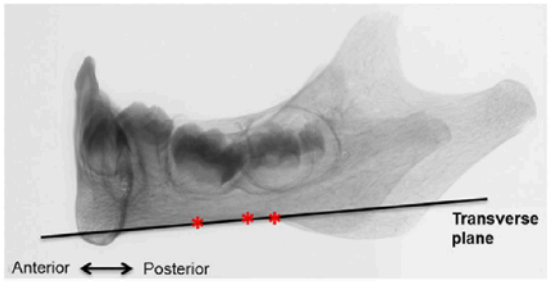


Fig. 1 Lateral view of the immature mandible indicating the reference (fiducial) (*) points and transverse plane used in the alignment of the micro-CT slices.

Measurements

To assess the bone mineral density on both the buccal/labial and lingual surfaces of each dental crypt, a total of eight points were selected along these surfaces (Fig. 2B) on the basis of the functionality and stage of dental development (Jager et al. 1990; Park et al. 2008; Hsu et al. 2011; Chang et al. 2012). The size of each assessment point was adapted to accommodate the change in the size of the body of the immature mandible. Thus, the area of each assessment point included a radius of 0.4 mm and a depth of 1 mm. The depth of each assessment point included multiple slices through an area. Superior, middle and inferior points were located in the alveolar region, and a midpoint within the basal region of each surface.

The superior points of the lingual and buccal/labial surfaces were located at the level of the opening of the dental crypt (a), which corresponded to the bony crests of each surface (Fig. 2B). The middle points of the lingual and buccal/labial surfaces were located at the level of the maximum width of the dental crypt (c), which was associated with the midpoint of the dental crypt height (b). The dental crypt height was measured between the most inferior point of the dental crypt and a point directly perpendicular to it, which was located at the level of the opening of the dental crypt (Fig. 2B). The inferior points on both the lingual and buccal/labial surfaces were aligned with the inferior border of the dental crypt (d) (Fig. 2B). The lingual and buccal/labial surface midpoints were located at the midpoint of the basal region (f) indicated at the midpoint of the basal bone height (e). The basal bone height was measured from the most inferior point located along the inferior border of the mandible to a perpendicular point located along the inferior border of the dental crypt (Fig. 2B).

Data analysis

The grey value assessed at each point was used as an estimate of bone mineral density. This value was standardised using the grey value of the reference material included in each scan, i.e. the grey value of each assessment point was subdivided by the grey value of reference. This resulted in the data for each assessment point being presented as ratios or absolute values, which allowed for further statistical analysis and comparisons of the bone mineral density distribution values across the sample.

Descriptive statistics, including means and standard deviations, were calculated for all the density ratios (as absolute density values cannot be used with polychromatic X-ray beams) associated with each dental crypt. A Shapiro–Wilk's test assessed the distribution of the sample, which further determined statistical analyses based on the distribution type. All statistical analyses were conducted using SPSS v.23 (IBM, Armonk, NY, USA) and PAST (Paleontological Statistics) statistical programs (Paleontological Association, London, UK; Hammer et al. 2001).

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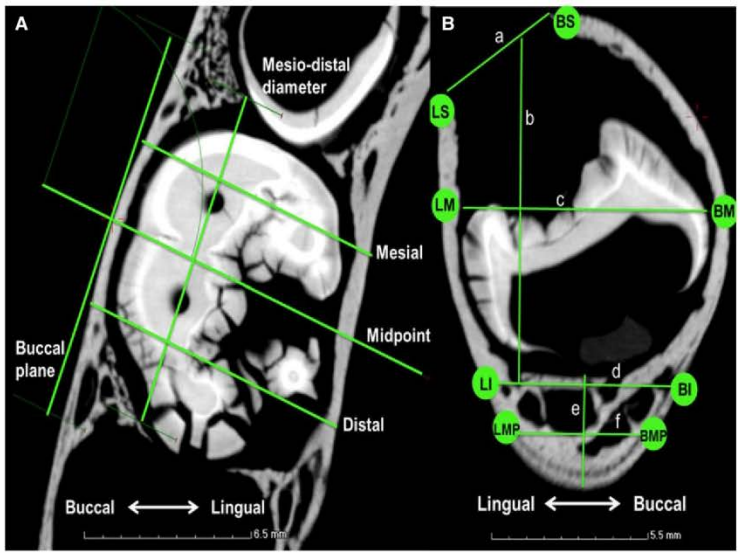


Fig. 2 (A) Superior view of a transverse section of the left second deciduous molar crypt. The figure indicated the position at which coronal sections were taken at the mesial, midpoint and distal positions of the dental crypt. (B) Lateral view of a midpoint coronal section of the left deciduous molar crypt indicating the four buccal and four lingual density points (BS, buccal superior; BM, buccal middle; BI, buccal inferior; BMP, buccal basal midpoint; LS, lingual superior; LM, lingual middle; LI, lingual inferior; LMP, lingual basal midpoint).

Considering the parametric distribution of the data, an analysis of covariance (ANCOVA) was first conducted to evaluate the influence of age on the bone mineral density distribution patterns of the buccal/labial and lingual surfaces, respectively (Zar, 1999). Furthermore, a principal component analysis was conducted to visualise the level of interaction between the buccal/labial and lingual surfaces of the mandible and their associated alveolar and basal areas across the prenatal, early postnatal and late postnatal groups.

In the cases where a significant degree of interaction was observed between the buccal/labial and lingual surfaces, a multivariate analysis of variance (MANOVA) was conducted to evaluate the level of interaction between the independent factors, i.e. age group and tooth type, and the dependent factors, i.e. mineral density ratios associated with each surface (Zar, 1999). A Tukey's honest significant difference *post hoc* test and pairwise comparisons were also conducted. A significance level of $\alpha < 0.05$ was considered statistically significant. Inter- and intra-observer errors were evaluated using 10 specimens and then tested using Lin's concordance correlation co-efficient of reproducibility, which provides information on the level of repeatability of the method and the degree of association between measurements (Allan, 1982).

Results

A high level of repeatability (77.3–99.9%) was observed across all measurements within the study. As no significant differences were observed between the superior, middle and inferior mineral density landmarks of the alveolar areas of each surface, these values were averaged to provide a value for the general alveolar area. Furthermore, as no significant differences were observed between the left and right measurements, these values were also averaged. While the age group had no effect on variations in bone mineral density across the alveolar and basal areas of the

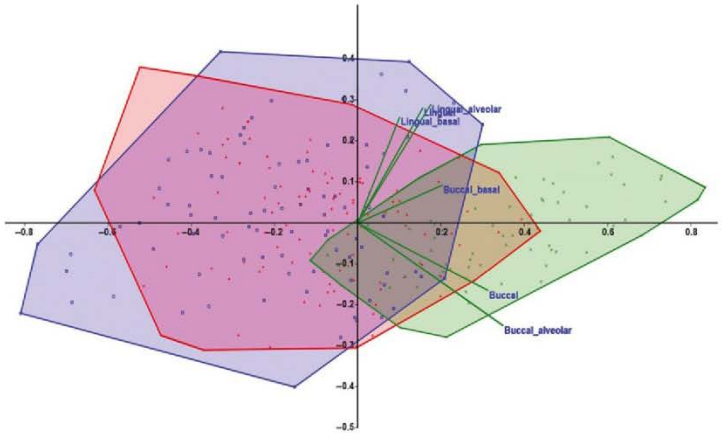


Fig. 3 Principal component analysis indicating the level of correlation between lingual and buccal surface measurements across the prenatal, early postnatal and late postnatal periods of growth (prenatal: blue; early postnatal: red; late postnatal: green).

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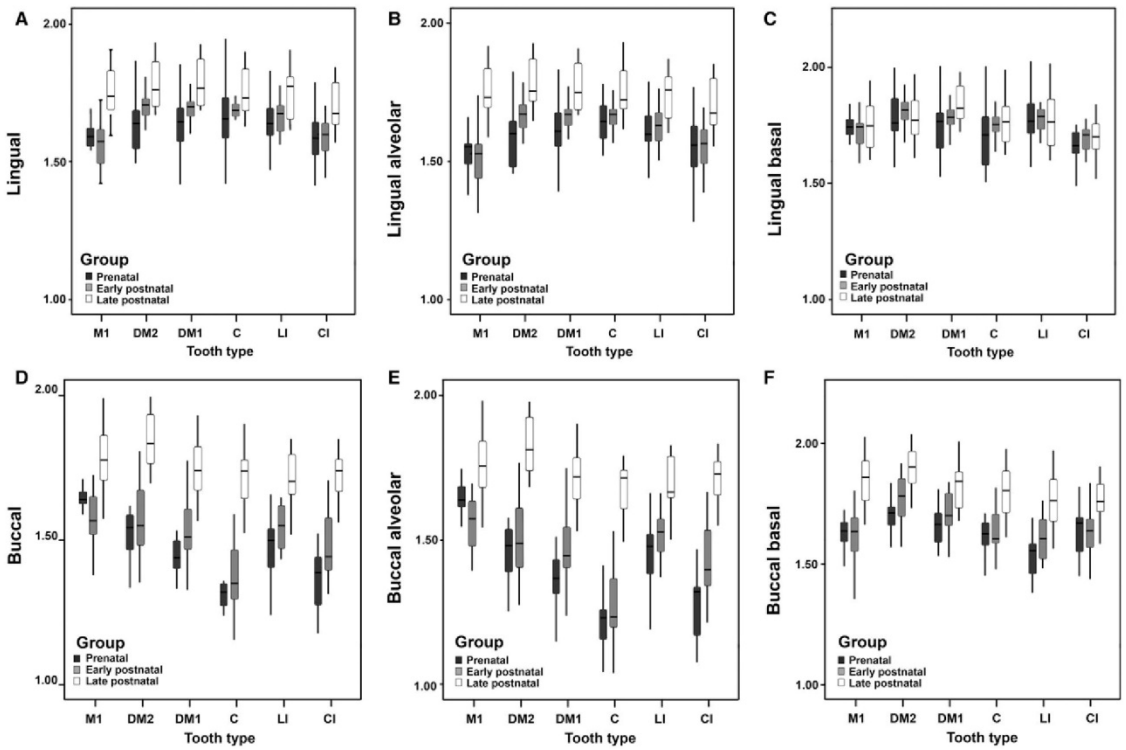


Fig. 4 Box-whisker plots indicated differences in the bone mineral density values across the general lingual (A), lingual alveolar (B), lingual basal (C), general buccal/labial (D), buccal/labial alveolar (E) and buccal/labial basal (F) surfaces of the mandible during the prenatal (30 gestational weeks to birth), early postnatal (birth to 1 year) and late postnatal (1–5 years) periods. Density differences between each of the dental crypts are also indicated across all assessed age groups for each surface (CI, central incisor; LI, lateral incisor; C, canine; DM1, first deciduous molar; DM2, second deciduous molar; M1, first permanent molar).

lingual surface ($P < 0.153$), both areas of the buccal/labial surface were well correlated with changes in age ($P < 0.001$; Fig. 3).

The buccal/labial surface vs. the lingual surface of the mandible

Bone mineral density values across the lingual surface were significantly higher ($P < 0.001$) than those of the buccal/labial surface across all dental crypts, with the exception of the first permanent molar crypt in the prenatal and early postnatal periods (Fig. 4A,D; Table 1). Furthermore, in the late postnatal period, bone mineral density values across the lingual surface were significantly higher ($P < 0.001$) across the lateral incisor crypt, canine crypt and first deciduous molar dental crypt when compared with the buccal surface (Fig. 4A,D; Table 1). In the early postnatal period, bone mineral density along the lingual surface in the area of the lateral incisor crypt (4.4%, $P < 0.015$) was significantly greater than that of the central incisor crypt, and bone mineral density of the second deciduous molar crypt (8.2%, $P < 0.001$) was significantly greater than that of the first permanent molar crypt (Fig. 4A; Table 1). Bone mineral density across the lingual surface of the second deciduous molar crypt in the early postnatal period was significantly greater (3.7%, $P < 0.046$) than that of the prenatal period (Fig. 4A; Table 2). In addition, bone mineral density along the lingual surface was significantly greater in the late postnatal period (CI: 7.5%, $P < 0.001$; LI: 5.4%, $P < 0.013$; C: 4.8%, $P < 0.029$; DM1: 5.9%, $P < 0.005$; DM2: 4.7%, $P < 0.023$; M1: 12.2%, $P < 0.001$) when compared with the early postnatal period (Fig. 4A; Table 2).

In the prenatal and early postnatal periods, bone mineral density along the buccal/labial surface of the mandible, in the area of the lateral incisor crypt, was significantly greater than that of the central incisor crypt (8.1%, $P < 0.004$) and canine crypt (9.5%, $P < 0.001$) areas, respectively (Fig. 4D; Table 1). In addition, bone mineral density in the area of the first deciduous molar crypt was significantly greater (7.0%, $P < 0.001$) than that of the canine crypt area (Fig. 4D; Table 1). In the late postnatal period, only bone mineral density in the area of the second deciduous molar crypt was significantly greater (5.1%, $P < 0.026$) than that of the first deciduous molar crypt (Fig. 4D; Table 1). Along the general

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Table 1 Multivariate analysis of variance (MANOVA) indicating the pairwise comparisons of density ratios between dental crypts for each of the assessed mandibular surfaces within each age group.

	Mean ± SD						Pairwise comparisons ($P < 0.05$)					
	Central incisor (CI)	Lateral Incisor (LI)	Canine (C)	Deciduous molar 1 (DM1)	Deciduous molar 2 (DM2)	Molar 1 (M1)	Univariate F ($P < 0.05$)	CI vs. LI	LI vs. C	C vs. DM1	DM1 vs. DM2	DM2 vs. M1
Group 1 (n = 15): 30 gw to birth												
Buccal	1.36 ± 0.11	1.47 ± 0.11	1.33 ± 0.10	1.43 ± 0.09	1.51 ± 0.09	1.64 ± 0.05	15.580 (0.001)	0.004	0.001	0.009	0.028	0.002
Lingual	1.58 ± 0.12	1.65 ± 0.14	1.67 ± 0.14	1.63 ± 0.12	1.64 ± 0.11	1.59 ± 0.07	2.072 (0.069)	0.047	0.470	0.236	0.802	0.166
Buccal alveolar	1.27 ± 0.13	1.45 ± 0.12	1.24 ± 0.13	1.36 ± 0.10	1.45 ± 0.11	1.64 ± 0.06	21.703 (0.001)	0.001	0.001	0.004	0.029	0.001
Lingual alveolar	1.55 ± 0.13	1.60 ± 0.15	1.66 ± 0.14	1.60 ± 0.12	1.59 ± 0.11	1.54 ± 0.08	2.653 (0.023)	0.125	0.124	0.084	0.940	0.173
Buccal basal	1.64 ± 0.11	1.53 ± 0.09	1.62 ± 0.11	1.65 ± 0.11	1.70 ± 0.07	1.63 ± 0.07	4.468 (0.001)	0.005	0.016	0.459	0.153	0.077
Lingual basal	1.67 ± 0.11	1.78 ± 0.13	1.71 ± 0.15	1.73 ± 0.14	1.78 ± 0.12	1.73 ± 0.09	2.557 (0.028)	0.004	0.060	0.477	0.240	0.248
Group 2 (n = 18): birth to 1 year												
Buccal	1.48 ± 0.11	1.54 ± 0.07	1.38 ± 0.12	1.52 ± 0.14	1.58 ± 0.12	1.57 ± 0.09	9.654 (0.001)	0.054	0.001	0.001	0.124	0.936
Lingual	1.59 ± 0.08	1.66 ± 0.06	1.68 ± 0.06	1.69 ± 0.06	1.70 ± 0.05	1.56 ± 0.08	7.550 (0.00)	0.015	0.506	0.800	0.689	0.001
Buccal alveolar	1.43 ± 0.13	1.52 ± 0.08	1.29 ± 0.15	1.46 ± 0.16	1.51 ± 0.13	1.56 ± 0.09	12.335 (0.001)	0.015	0.001	0.001	0.192	0.171
Lingual alveolar	1.55 ± 0.09	1.63 ± 0.07	1.66 ± 0.06	1.66 ± 0.06	1.67 ± 0.06	1.50 ± 0.10	8.622 (0.001)	0.029	0.270	0.992	0.874	0.001
Buccal basal	1.63 ± 0.10	1.61 ± 0.09	1.63 ± 0.10	1.71 ± 0.09	1.77 ± 0.10	1.60 ± 0.14	8.258 (0.001)	0.582	0.535	0.013	0.076	0.001
Lingual basal	1.69 ± 0.08	1.78 ± 0.05	1.75 ± 0.06	1.78 ± 0.06	1.82 ± 0.06	1.72 ± 0.07	3.795 (0.002)	0.009	0.420	0.321	0.300	0.005
Group 3 (n = 12): 1–5 years												
Buccal	1.73 ± 0.08	1.71 ± 0.10	1.71 ± 0.11	1.75 ± 0.10	1.84 ± 0.10	1.78 ± 0.12	2.804 (0.001)	0.768	0.978	0.418	0.026	0.150
Lingual	1.71 ± 0.09	1.75 ± 0.09	1.76 ± 0.09	1.79 ± 0.09	1.78 ± 0.09	1.75 ± 0.10	1.199 (0.310)	0.263	0.779	0.446	0.878	0.422
Buccal alveolar	1.72 ± 0.08	1.70 ± 0.10	1.69 ± 0.12	1.72 ± 0.10	1.82 ± 0.10	1.76 ± 0.12	2.291 (0.046)	0.671	0.830	0.487	0.029	0.177
Lingual alveolar	1.71 ± 0.10	1.74 ± 0.08	1.76 ± 0.09	1.77 ± 0.09	1.78 ± 0.09	1.75 ± 0.10	0.827 (0.531)	0.415	0.730	0.690	0.772	0.445
Buccal Basal	1.76 ± 0.09	1.77 ± 0.12	1.80 ± 0.12	1.83 ± 0.10	1.90 ± 0.10	1.85 ± 0.11	3.176 (0.008)	0.797	0.535	0.368	0.121	0.221
Lingual basal	1.70 ± 0.09	1.77 ± 0.12	1.77 ± 0.11	1.84 ± 0.09	1.78 ± 0.11	1.75 ± 0.10	2.580 (0.027)	0.074	0.979	0.092	0.147	0.457

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Table 2 Multivariate analysis of variance (MANOVA) indicating the pairwise comparisons of density ratios between age groups for each of the assessed mandibular surfaces across all associated dental crypts.

	Buccal				Lingual				Buccal alveolar				Lingual alveolar				Buccal basal				Lingual basal			
	Univariate F ($P < 0.05$)	Group 1 vs. Group 2	Group 1 vs. Group 3	Group 2 vs. Group 3	Univariate F ($P < 0.05$)	Group 1 vs. Group 2	Group 1 vs. Group 3	Group 2 vs. Group 3	Univariate F ($P < 0.05$)	Group 1 vs. Group 2	Group 1 vs. Group 3	Group 2 vs. Group 3	Univariate F ($P < 0.05$)	Group 1 vs. Group 2	Group 1 vs. Group 3	Group 2 vs. Group 3	Univariate F ($P < 0.05$)	Group 1 vs. Group 2	Group 1 vs. Group 3	Group 2 vs. Group 3	Univariate F ($P < 0.05$)	Group 1 vs. Group 2	Group 1 vs. Group 3	Group 2 vs. Group 3
Central incisor	42.745 (0.001)	0.002	0.001	0.001	7.818 (0.001)	0.777	0.001	0.001	49.797 (0.001)	0.001	0.001	0.001	11.433 (0.001)	0.850	0.001	0.001	6.913 (0.001)	0.784	0.001	0.001	0.290 (0.749)	0.615	0.763	0.763
Lateral incisor	19.081 (0.001)	0.045	0.001	0.001	4.635 (0.011)	0.599	0.013	0.013	15.304 (0.001)	0.081	0.001	0.001	7.536 (0.001)	0.498	0.002	0.002	18.648 (0.001)	0.032	0.001	0.001	0.010 (0.990)	0.961	0.919	0.919
Canine	53.430 (0.001)	0.215	0.001	0.029	3.525 (0.031)	0.687	0.029	0.029	58.439 (0.001)	0.159	0.001	0.001	4.170 (0.017)	0.902	0.011	0.011	12.201 (0.001)	0.828	0.001	0.001	1.468 (0.232)	0.248	0.517	0.517
Deciduous molar 1	32.518 (0.001)	0.011	0.001	0.005	9.774 (0.001)	0.060	0.005	0.005	33.544 (0.001)	0.013	0.001	0.001	10.794 (0.001)	0.055	0.003	0.003	10.980 (0.001)	0.069	0.002	0.002	3.868 (0.022)	0.175	0.108	0.108
Deciduous molar 2	37.138 (0.001)	0.087	0.001	0.023	8.039 (0.001)	0.046	0.023	0.023	38.724 (0.001)	0.150	0.001	0.001	12.586 (0.001)	0.032	0.002	0.002	12.339 (0.001)	0.044	0.001	0.001	0.743 (0.477)	0.263	0.364	0.364
Permanent molar 1	14.666 (0.001)	0.088	0.001	0.001	16.731 (0.001)	0.393	0.001	0.001	10.244 (0.001)	0.070	0.001	0.001	24.709 (0.001)	0.321	0.001	0.001	22.230 (0.001)	0.467	0.001	0.001	0.307 (0.736)	0.802	0.463	0.463

Group 1: 30 gestational weeks to birth; Group 2: birth to 1 year; Group 3: 1–5 years.

buccal/labial surface of the mandible, bone mineral density was significantly greater in the early postnatal period (CI: 8.8%, $P < 0.002$; LI: 4.8%, $P < 0.045$; DM1: 6.3%, $P < 0.011$) when compared with the prenatal period (Fig. 4D; Table 2). In addition, bone mineral density along the buccal/labial surface was significantly greater in the late postnatal period (CI: 16.9%, $P < 0.001$; LI: 11.0%, $P < 0.001$; C: 23.9%, $P < 0.001$; DM1: 15.1%, $P < 0.001$; DM2: 16.5%, $P < 0.001$; M1: 13.4%, $P < 0.001$) when compared with the early postnatal period (Fig. 4D; Table 2).

Alveolar region

In the early postnatal period, bone mineral density across the lingual surface was significantly greater in the area of the lateral incisor crypt (5.2%, $P < 0.029$) and the second deciduous molar crypt (10.2%, $P < 0.001$) when compared with the central incisor crypt and the first permanent molar crypt, respectively (Fig. 4B; Table 1). Bone mineral density in the area of the second deciduous molar crypt was significantly greater (5.0%, $P < 0.032$) in the early postnatal period than the prenatal period (Fig. 4B). In addition, bone mineral density along the lingual surface was significantly greater in the late postnatal period (CI: 10.3%, $P < 0.001$; LI: 6.7%, $P < 0.002$; C: 6.0%, $P < 0.011$; DM1: 6.6%, $P < 0.003$; DM2: 6.6%, $P < 0.002$; M1: 16.7%, $P < 0.001$) when compared with the early postnatal period (Fig. 4B; Table 2).

Along the buccal/labial surface of the mandible, bone mineral density variations observed across the alveolar region followed a similar pattern of change to that previously observed along the general buccal/labial surface of the mandible (Fig. 4D,E; Table 1). Along the buccal/labial surface of the mandible, bone mineral density was significantly greater in the early postnatal period (CI: 12.6%, $P < 0.001$; DM1: 7.4%, $P < 0.013$) when compared with the prenatal period (Fig. 4E; Table 2). In addition, bone mineral density along the buccal/labial surface was significantly greater in the late postnatal period (CI: 20.3%, $P < 0.001$; LI: 11.8%, $P < 0.001$; C: 31.0%, $P < 0.001$; DM1: 17.8%, $P < 0.001$; DM2: 20.5%, $P < 0.001$; M1: 12.8%, $P < 0.001$) when compared with the early postnatal period (Fig. 4E; Table 2).

Basal region

In the prenatal period, bone mineral density across the lingual surface was significantly greater in the area of the lateral incisor crypt (6.6%, $P < 0.004$) when compared with the central incisor crypt area (Fig. 4C; Table 1). In the early postnatal period, bone mineral density in the areas of the lateral incisor crypt (5.3%, $P < 0.009$) and second deciduous molar crypt (5.5%, $P < 0.005$) were significantly greater when compared with the central incisor crypt and first permanent molar crypt areas, respectively (Fig. 4C; Table 1). Bone mineral density across the buccal/labial surface, in the

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areas of the central incisor crypt (6.7%, $P < 0.005$) and canine crypt (5.9%, $P < 0.016$), was significantly greater than that of the lateral incisor crypt area within the prenatal period (Fig. 4F; Table 1). In the early postnatal period, bone mineral density in the areas of the first (4.9%, $P < 0.013$) and second (9.6%, $P < 0.001$) deciduous molar crypts were significantly greater when compared with the canine crypt and first permanent molar crypt areas, respectively (Fig. 4F; Table 1).

Along the buccal/labial surface of the mandible, bone mineral density was significantly greater in the early postnatal period (LI: 5.2%, $P < 0.032$; DM2: 4.1%, $P < 0.044$) when compared with the prenatal period (Fig. 4F; Table 2). In addition, bone mineral density along the buccal/labial surface was significantly greater in the late postnatal period (CI: 8.0%, $P < 0.001$; LI: 9.9%, $P < 0.001$; C: 10.4%, $P < 0.001$; DM1: 7.0%, $P < 0.002$; DM2: 7.3%, $P < 0.001$; M1: 15.6%, $P < 0.001$) when compared with the early postnatal period (Fig. 4F; Table 2).

Discussion

The transition between the late prenatal and early postnatal periods of growth represents a functionally complex period. It is during this period that the growth of the mandible and the associated masticatory apparatus is synchronised with the development of functions such as sucking, suckling and mastication, which are required for successful nutritional intake (Miller et al. 2003; Delaney & Arvedson, 2008). As the oral cavity structures adapt to accommodate the change in function, it is expected that bone mineral density will be significantly affected by both changes in the state of the dentition as well as the functionality of the masticatory apparatus during the late prenatal and early postnatal periods of growth. This change in bone mineral density distribution illustrates the impact of functional changes on the developing mandible during its early period of growth. The change may also be significant when considering that many of the bone disorders, for example osteogenesis imperfecta, affect children (Roschger et al. 2008). Furthermore, the bone mineral density distribution across the cortical bone of the mandible observed in this study provides valuable clinical insights into the impact of biomechanical changes arising from dental development on the mandibular bone during growth (Roschger et al. 2008).

While this study offers valuable insights into the growth and development of the mandible during a particularly complex period of growth, there were some limitations in the sample that should be noted. The cross-sectional nature of the study ultimately limits the level of causal inferences regarding changes in age or function. In addition, part of the sample was sourced from a forensic setting, thus detailed demographic data for each specimen were not available. Furthermore, the nutritional status and any underlying metabolic diseases of the study sample are not known and, as such, inferences concerning the effects of any metabolic disturbances on the structure and morphology of the mandible fall outside the scope of this study. Thus, the study made use of ratios or absolute values rather than Hounsfield's units, as a means to describe a pattern of change in bone mineral density vs. quantifying the bone density of an area. While the present study assessed changes in bone mineral density across the body of the immature mandible, it did not specifically assess changes in the amount of mineralised bone tissue or change in the bone volume. Thus, the depth of inferences regarding changes in bone density across the immature mandible is limited to bone mineral density distribution.

Considering that the development and growth of the oral cavity soft tissues precedes that of the developing dentition, the findings of the current study will be discussed in accordance with this timeline. In general, bone mineral density across the lingual surface was observed to be significantly higher than the buccal/labial surface across the prenatal and early postnatal periods. This disparity in bone mineral density between the surfaces of the mandible may be attributed to the early development of the mandible. During the 6th week of gestation, the intramembranous ossification of the mandible commences relative to the facial surface of Meckel's cartilage (Lee et al. 2001). Trabecular bone originating from the primary growth centre of the mandible advances in a buccal direction (Lee et al. 2001; Radlanski et al. 2003). Thus, the bone of the lingual surface is further advanced in its growth as well as degree of mineralization when compared with the buccal/labial surface of the mandible.

As the morphology of the mandible is thought to change with the development of the associated masticatory structures and an increase in masticatory activity, variations in bone mineral density within the alveolar and basal areas of the lingual and buccal/labial surfaces of the mandible were investigated further. The higher bone mineral density of the lateral incisor crypt region, particularly during the prenatal and early postnatal periods of growth, may be attributed to changes in both the morphology of the symphyseal region of the mandible (Scheuer & Black, 2000; Lee et al. 2001; Radlanski et al. 2003; Hutchinson et al. 2014) as well as in the functioning of the tongue (Miller et al. 2003; Delaney & Arvedson, 2008).

During the 12th week of gestation, the ventral symphyseal cartilages commence the process of endochondral ossification (Scheuer & Black, 2000; Radlanski et al. 2003). This process, which includes increased bone modelling and remodelling, continues until fusion of the hemi-mandibles is completed (Scheuer & Black, 2000; Hutchinson et al. 2012, 2014). Thus, bone in the symphyseal region and associated central incisor crypt region is younger and as such lower in density (Hernandez, 2008) when compared with the more established bone of the lateral incisor region. The lower density bone of the central incisor crypt area and the

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associated symphyseal region of the mandible may also be attributed to a high level of biomechanical activity originating from the tongue. The level of biomechanical demand is linked to the level of functional activity, which is associated with a variation in the degree of strain imposed on the lingual surface of the mandible. In a study investigating the effects of a reduction in the volume of the tongue on craniofacial growth, Liu et al. (2008a,b) observed a reduction in the symphysis width and anterior dental arch width as well as a significant reduction in the bone mineral density (Liu et al. 2008a,b). The observations made in these investigations were based on a porcine sample, making direct inferences to the human difficult. However, Hutchinson et al. (2014) observed similar patterns in the relationship between the growth of the tongue and the mandible in prenatal and early postnatal human samples.

The significant difference observed in bone mineral density between the second deciduous molar and the first permanent molar crypt regions along the lingual surface may be a result of a change in the orientation of the ramus. The ramus of the mandible undergoes a significant transition both in its orientation and morphology across the late prenatal and early postnatal periods of growth as a result of the increase in functional and spatial demands from the developing dentition (Hutchinson et al. 2012, 2014). These demands result in increased bone modelling and remodelling, which leads to the ramus transitioning from a horizontal disposition prenatally to a more vertical orientation postnatally (Scheuer & Black, 2000; Hutchinson et al. 2012, 2014). Furthermore, there is also a change internally within the body of the mandible, as the development and growth of the mandibular canal is thought to be synchronised with the dentition (Hutchinson et al. 2016).

The differences in bone mineral density across the buccal/labial surface of the mandible may be reflective of the stage of dental development. The bone mineral density in the area of the lateral incisor crypt was significantly higher than that of the canine and central incisor crypt regions, which may be attributed to the level of dental development across the three dental crypt regions. While the canine tooth only starts development after the lateral incisor, the central incisor tooth is thought to be more advanced in its growth and development when compared with the lateral incisor (Alqahtani et al. 2010). The lower bone mineral density in the area of the canine may be attributed to the early stage of dental development, while the lower bone mineral density of the central incisor crypt area may be the result of an increase in bone modelling owing to increased physiological tooth movement in the area (Melsen, 1999; Dolce et al. 2002; Wise & King, 2008). The effect of the level of tooth development may also account for the progressively higher bone mineral density found across successional crypts in a posterior direction in the current study.

In conclusion, variations in the bone mineral density associated with the lingual surface of the mandible were observed to be more reflective of the level of development of the tongue and muscles of mastication as well as a change in the level of biomechanical demand of these structures on the lingual surface. In contrast, the variations in the bone mineral density associated with the buccal/labial surface of the mandible are more reflective of the state of dental development and associated stage of physiological tooth movement. As such, changes in bone mineral density across the mandible appear to be reflective of the stage of dental development and the level of biomechanical loading consistent with the transition from the fetal to early postnatal stages of growth.

Acknowledgements

This work was supported by the Seed Funding Initiative, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg (Grant Number: 0012518421101512l105). The authors dedicate this article to Professor Jules A. Kieser, BSc, BDS, PhD, DSc (1950–2014), who was Professor in the Sir John Walsh Research Institute, Faculty of Dentistry, University of Otago, Dunedin, New Zealand, for many years. Jules was an outstanding and inspiring teacher, a phenomenal supervisor, and a forensic scientist of immense stature. At the time of his death, he was actively engaged in the planning and data analysis for this paper. Professor E. Liebhaber is acknowledged for her statistical advice. The authors declare no conflict of interest.

Author contributions

E.F. Hutchinson contributed to the conception, design, data acquisition, analysis and interpretation, as well as drafted and critically revised the manuscript. M. Farella contributed to the conception, design and data interpretation, as well as drafted and critically revised the manuscript. J. Hoffmann contributed to the data acquisition and analysis, as well as critically revised the manuscript. B. Kramer contributed to the conception, design and data interpretation, as well as drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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December 8, 2016

Beverley.kramer@wits.ac.za

To whom it may concern:

Supervisor of and co-author with Ms. Erin Hutchinson

I, Beverley Kramer, agree that Ms Erin Hutchinson (0504795W) may use the articles entitled; "***Variations in bone density across the body of the immature mandible", "Importance of teeth in maintaining the morphology of the adult mandible in humans" and "Morphometric growth relationships of the immature human mandible and tongue"***, on which I am a co-author, for her Ph.D. Ms Hutchinson contributed to the conception, design, data acquisition and analysis. She also drafted and revised each manuscript.

Yours sincerely,



Beverley Kramer PhD., Hon FAS
Professor of Anatomy
Assistant Dean: Research and Postgraduate Support

UNIVERSITY of OTAGO

Te Whare Wānanga o Otāgo

NEW ZEALAND

29 November 2016

To whom it may concern:

This is to confirm that the undersigned has no objections that Erin Hutchinson will use the publications, which I have co-authored, as part her PhD thesis.

Yours sincerely,

Prof Mauro Farella
Associate Dean for Postgraduate Studies



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To whom it might be concerned:

Collaborator of and co-author with Me. Erin Hutchinson

I, Jakobus Hoffmann, agree that Ms Erin Hutchinson (0504795W) may use the article entitled;" ***Variations in bone density across the body of the immature mandible'***, on which I am a co-author, for her Ph.D. Ms Erin Hutchinson contributed to the conception, design, data acquisition and analysis. She also drafted and revised the manuscript.

Yours sincerely

Mr. Jakobus Hoffman
Instrument Scientist: Radiography /Tomography
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MORPHOLOGY AND FUNCTION:

ASPECTS OF MANDIBULAR DEVELOPMENT AND GROWTH AT DIFFERENT STAGES ACROSS THE LIFESPAN

Erin Frances Hutchinson

Original published work submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy Johannesburg, South Africa, 2016

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CHAPTER 8

THESIS

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