GROWTH MODIFICATION OF THE TEMPOROMANDIBULAR JOINT BY FUNCTIONAL APPLIANCES: A HISTOMORPHOMETRIC STUDY USING SHEEP

A thesis submitted for degree of Doctor of Philosophy
by

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To my wife, Yu-mei Guo

Declaration

This work contains no material which has been accepted for the award of any degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Summary

In order to investigate growth modifications of the temporomandibular joint (TMJ) during dentofacial orthopaedic treatment, various functional appliances have been used to prompt the mandible into a protrusive position in various animal experimental models. The general purpose of this project was (i) to test the effectiveness of a functional appliance specially designed for sheep; (ii) to clarify whether or not forward mandibular displacement in sheep is associated with faster and/or redirected condylar growth; (iii) to evaluate the sheep as a model for dentofacial orthopaedic research by comparing the similarities of mandibular condylar growth in sheep and humans; (iv) to detail the position of the mandible during forward mandibular posturing and the effects of mandibular forward displacement on modelling and remodelling of the mandibular condyle. The specific purpose of this project was to reveal whether functional appliance treatment increases the quantity of bone formed during the treatment, or changes the distribution of the bone, or both.

Eight, 4-month old, castrated male Merino sheep were randomly assigned to experimental or control groups with 4 in each group. Cast functional appliances were fabricated for the animals in the experimental group. The treatment period was 15 weeks. Calcein (day 1) tetracycline (13 weeks) and alizarin red S (3 days before sacrifice) fluorochromes were administered to all animals. Dental casts, endosseous implant markers and cephalograms were used to analyse the 3-D displacement of the mandible. Undecalcified mid-sagittal sections of TMJ were used to evaluate the tissue responses induced by the appliances. Dynamic parameters of bone formation, static indices of

bone-forming and resorbing activity as well as structural indices of trabecular bone were estimated using histomorphometry. The trabecular bone was sampled from two regions: (i) a "subchondral region" (determined by 2nd and 3rd labels), believed to comprise bone newly-formed during the experimental period; and (ii) a "central region" (labelled by all the three fluorochromes), believed to comprise bone which existed before the experiment. The cortical bone was divided into anterior and posterior regions for analysis. The weight of the animals was measured monthly to monitor their growth. Metacarpus growth was also evaluated.

During the experimental period, the animals were found to maintain their weight within the normal range and grew normally. The appliance was found to displace the mandible to a downward and forward position with a net condylar displacement of 2.4 mm. The observed adaptive responses in the TMJ induced by the appliances included; the condylar process was less tapered and rounder in the experimental group than in the controls, and anteriorly thickened condylar cartilage and a thickened compact bone layer along the anterior surface of the posterior wall of the glenoid fossa. The mandibular condylar growth vector in sheep was found to be in a postero-superior direction. Condylar growth in the control sheep during the experimental period varied from 8.8 to 11.9 mm, with the mean being 10.6 mm, which is quantitatively similar to two years of condylar growth in human adolescents. In the experimental sheep, the condylar growth varied from 8.5 to 13.3 mm, with the mean being 11.4 mm. When metacarpal growth and weight gain were taken into consideration using multivariant analysis, the

coefficients for growth in the postero-superior and posterior direction were found to be high, with adjusted r^2 as 0.84 and 0.82 respectively. The induced condylar growth was estimated to be largest in the posterior direction (2.3 mm), which is also similar to previous reports in humans. Regional differences in adaptive response within the mandibular condyle were found in this study. In the experimental group, bone volume fraction (BV/TV) of the subchondral regions decreased, although the specific bone surface and bone formation rates increased. This low BV/TV was associated with decreased trabecular thickness and increased trabecular separation. In the central region of the experimental group's condyle, BV/TV was unchanged. However, an increased osteoid surface (OS/BS) was defined when the eroded surface (ES/BS) was taken into consideration.

The sheep were found to cope well with the experimental procedures and the appliance used in this study has been effective in inducing adaptive responses in the TMJ. Consequently, it is believed that the sheep is an appropriate animal model for quantitative histological analysis of the responses to functional appliance treatment.

The first null hypothesis, that functional appliance treatment has no effect on bone matrix mineralisation was rejected. The second null hypothesis, functional appliance treatment has no effect on the mineralisation lag time, was rejected. The results indicated that the treatment effects of functional appliances involve reorganisation of the TMJ through bone modelling and remodelling. An important mechanism of functional appliance treatment is,

therefore, suggested to be a change in the distribution of bone rather than an increase in the quantity of bone. Posterior rotation of the principle tensile strain angle (*Et*) suggested an posteriorly altered direction of the condylar growth. Increased new bone formation in the glenoid fossa suggested an anterior re-positioning of the temporomandibular joint.

Publications Related to This Thesis

Referred Journals:

1 **MA B**., SAMPSON W., FAZZALARI N., WILSON D. and WIEBKIN O., Induced mandibular condylar growth in a sheep model after functional appliance treatment, *Australian Orthodontic Journal* 2001; 17(2): 81-8.

2 **MA B**., SAMPSON W., FAZZALARI N., WILSON D. and WIEBKIN O., Experimental forward mandibular displacement in sheep, *Archives of Oral Biology* 2002; 47(1): 75-84.

3 **MA B**., SAMPSON W., WILSON D. WIEBKIN O. and FAZZALARI N., A histomorphometric study of adaptive responses of cancellous bone in the sheep mandibular condyle following experimental forward mandibular displacement, *Archives of Oral Biology* 2002; In press.

4 **MA B**., SAMPSON W., WILSON D. WIEBKIN O. and FAZZALARI N., Increased mandibular condylar length is associated with re-distribution of the bone matrix in experimental functional appliance treated sheep, *Journal of Dental Research* 2002; Submitted for publication.

Short or Abstract Publications:

5 **MA B**., SAMPSON W., FAZZALARI N., WILSON D. and WIEBKIN O., Adaptive responses in mandibular condylar cartilage following mandibular displacement in sheep (abstract), Matrix Biology Society of Australia and New Zealand Silver Jubilee Meeting; 2001 Oct 4-7; Canberra, Australia.

6 **MA B**., FAZZALARI N., SAMPSON W.J., WILSON D.F. and WIEBKIN O., Changes in condylar cancellous bone volume fraction (BV/TV) and turnover in sheep following forward mandibular displacement (abstract), Australian

and New Zealand Bone and Mineral Society 11th Annual Scientific Meeting; 2001 Oct 7-10; Auckland, New Zealand.

7 **MA B**., SAMPSON W.J., FAZZALARI N., WIEBKIN O., and WILSON D.F. The bone adaptation in the mandibular condyle of functional appliance treated sheep (poster), 18th Australian Society of Orthodontics Congress; 2002 March 15-19; Perth, Australia.

Foreword

A critical aspect in the enlargement of the face is mandibular growth. Most of the research that addresses the correction of Class II malocclusion using functional appliances has focussed on the contribution of the mandibular growth to the orthodontic correction (Woodside, 1998). Knowing the mechanism of modified mandibular growth by functional appliances has, therefore, great clinical relevance.

The distance between condylion (Co), the most posterosuperior point of the mandibular condylar head, and pogonion (Pog), the most anterior point of the chin, has been usually used as an overall measurement to reflect the length of the mandible (Ghafari et al., 1998). During embryonal and early postnatal development, the mandibular condyle functions as a growth site. Its elongation contributes to the development of the mandible and the formation of the temporomandibular joint (TMJ). Increased mandibular condylar growth has been suggested as leading to an increased mandibular length after treatment with experimental functional appliances in rodents (Petrovic et al., 1975) and in non-human primates (McNamara & Bryan, 1987). Modified cartilage and bone growth in the mandibular condyle as well as the changed bone deposition in the glenoid fossa were suggested as also leading to modified TMJ development (Charlier et al., 1969; McNamara & Carlson, 1979; Woodside, 1987).

In clinical practice, an increased mandibular length is variously reported in patients with Class II malocclusion following functional appliance treatment (Aelbers & Dermaut, 1996). The bone remodelling in the TMJ following

functional appliance treatment is also not consistent (Ruf & Pancherz, 1999; Chintakanon et al., 2000 (b)). These clinical findings are highly relative since, obviously, only the patients who present such skeletal changes; namely, the increased mandibular length or TMJ remodelling; are those who definitively need functional appliance treatment for the correction of skeletal Class II malocclusion. Because the dental changes in the correction of Class II malocclusion can be achieved by many types of more effective fixed appliances, functional appliance treatment becomes merely optional. Therefore, it is ideal to select only those patients who are likely to have favourable skeletal changes before the commencement of treatment. Unfortunately, no reliable method is available so far to help with the patient selection.

In order to develop a patient selection method, we initially need to detect the skeletal changes, then to identify the characteristics of the patients who have these skeletal changes. The prediction for an individual can then be made by evaluating if that particular individual presents those characteristics. The increased mandibular length and the TMJ remodelling are currently detected using medical imaging techniques; e.g. computer tomogram (CT), magnetic resonance imaging (MRI), orthopantomogram (OPG). The information has only been accumulated from case reports or case series with a relatively small sample size and no untreated control group. The lack of information on the morphological characteristics of the mandible and the TMJ during growth in normal subjects, plus ethical issues in collecting such data limits the potential of using medical imaging techniques to help patient selection.

The purpose of this project was to investigate the mechanisms associated with functional appliance treatment, so as to develop a theory to guide clinical practice. In particular, to test the rational of an approach to detect the skeletal changes induced by the functional appliance treatment, which might be further developed to help with the patient selection. The theory, which can be used to detect the skeletal changes induced by the functional appliance treatment, is presented hypothetically in Figure 1A.

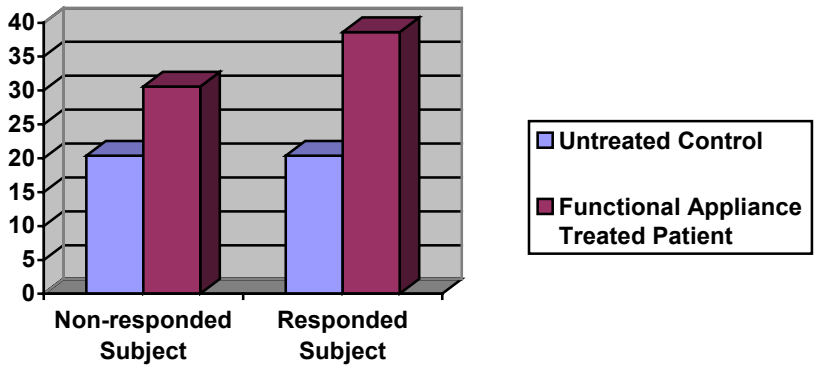


Figure 1A: Illustration of the hypothesised theory which can be used to detect the skeletal changes induced by the functional appliance treatment. The numerical index measures the overall bone formation activity of the individual. In the untreated controls, the bone formation activity depends on the bone growth, bone modelling and remodelling activities. In the functional appliance treated subjects, the bone remodelling in the alveolar bone results a higher bone formation activity irrespective of the bone responses from the mandibular growth or TMJ development. This results in a higher bone formation activity compared with the untreated controls. If there are some responses from the mandibular growth or TMJ development, an additional bone formation activity is presented. Therefore, subjects with the highest bone formation activity can be concluded to present responses from the mandibular growth or TMJ development following the functional appliance treatment.

The present project investigates a very basic question: “Does functional appliance treatment increase the quantity of bone formed during the treatment, or does the treatment change the distribution of bone? Or both?” Based on the answer to this question, it can be further clarified which part of bone formation (bone matrix formation vs. bone matrix mineralisation) can be

used to detect the skeletal changes induced by the functional appliance treatment.

In *Chapter 1*, the importance of the TMJ in functional appliance treatment is highlighted and the formulation of research questions and the demand for a theory are introduced.

At the initial stage of developing the theory guiding clinical practice, an animal experiment was designed to mimic functional appliance treatment in humans. In *Chapter 2*, in order to select an appropriate method to measure the changes induced by the functional appliances, current methods are reviewed and appropriate methods are selected. Detailed materials and methods are also introduced.

In *Chapter 3*, sheep as an animal model for experimental functional appliance treatment are evaluated. In this chapter, a novel appliance that produces mandibular displacement in sheep is evaluated. Findings related to TMJ adaptation are described. The sheep as an animal model is also compared with other animal models previously used in dentofacial orthopaedic research.

In *Chapter 4*, mandibular condylar growth modified by the experimental functional appliance in sheep is quantitatively evaluated and compared with reported findings in human subjects with particular reference to the increased mandibular condylar length.

In *Chapter 5*, the questions whether or not forward mandibular displacement can influence trabecular bone structure and bone formation-resorption dynamics in the mandibular condyle, and whether regional differences exist are investigated.

In *Chapter 6*, the normality of the distribution of the histomorphometric data is assessed. This assessment helps selection of the appropriate statistical procedure.

In *Chapter 7*, the bone structure of the mandibular condyle during functional appliance treatment in the sheep model is characterised, so that the mechanical environment of the TMJ modified by functional appliances can be explored. This is also helpful to establish the cause-effect relationship between the treatment and the morphological changes in the TMJ.

In *Chapter 8*, the histological variables found to be different between the control and the experimental groups in previous chapters, are examined using Pearson Correlation analysis with respect to the mandibular position. Change in the mandibular position following the functional appliance treatment is the most frequently used measurement for clinical evaluation. The analysis in this chapter links this most frequently used measurement with the histological changes observed in the TMJ.

In *Chapter 9*, answers to the study questions related to developing the theory guiding clinical practice on functional appliance treatment are introduced.

Based on the research questions answered in this study, a theory is formulated. The skeletal changes in mandible or TMJ are also characterised from a biological viewpoint.

It has taken about 5 years to bring the project to this stage. I started to discuss this project with Professor Wayne Sampson and A/Professor Nick Fazzalari in 1997. The selection of an animal model that might provide sufficient statistical differentiation resulted in animal experimentation commencing in 1999. Three and a half years later, we have a clearer idea about where this project might go. Thus, to focus on clinical relevance, in the *Epilogue*, future directions are suggested for the development of the method to detect the skeletal changes induced by the functional appliance treatment. The un-answered study questions are also discussed.

Chapter 1
Literature Review

Part I: Growth Modification of the Temporomandibular Joint in Dentofacial Orthopaedics

1.1. Functional Appliances in Dentofacial Orthopaedics: An Overview

The purpose of this section is to establish the importance of the temporomandibular joint (TMJ) in the treatment of Class II malocclusion and its relevance to clinical performance.

1.1.1. Dentofacial orthopaedic treatment principles

Class II malocclusion constitutes up to 25% of the malocclusions found in children 14-18 years old (Goldstein & Stanton, 1936; Massler & Franke, 1951; Ast et al., 1965). In skeletal Class II malocclusion, the treatments include; some form of dentofacial orthopaedic treatment to correct the skeletal discrepancy, a surgical approach to correct the skeletal discrepancy or dento-alveolar camouflage of the underlying skeletal discrepancy. Among them, dentofacial orthopaedic treatment using functional appliances is thought to be less invasive compared with the surgical approach and it aims to treat the skeletal basis of the problem compared with treatment to create dento-alveolar camouflage. Therefore, the treatment of skeletal Class II malocclusion using dentofacial orthopaedic appliances has attracted the attention of many practitioners and research groups around the world.

The principles involved in the clinical management of problems requiring dentofacial orthopaedic treatment currently focus on the neuromuscular responses to altering unfavourable posturing of the mandible. Removal of the

restraining muscle forces conditions the patients' musculature to sustain an altered, favourable forward mandibular posture (Hicks, 1994). Consequently, alter action of the mandibular position from a muscle-controlled forward position to a structure-controlled forward position is achieved by the growth and remodelling of the TMJ (Chintakanon et al., 2000 (a)).

1.1.2. Aetiological of skeletal Class II malocclusion

The aetiology of a skeletal malocclusion is usually thought to be the result of variations in the size, form, or position of the jaw (Rothstein & Yoon-Tarlie, 2000). Compared with normal Class I subjects, Class II patients have a smaller mandible which is often positioned posteriorly. To the contrary, Class III patients have a larger mandible which is often positioned anteriorly (Kerr et al., 1994). The form of the mandible has a combined effect with the size and the position of the mandible. Patients with an open bite show a larger gonion angle but those with a deep bite usually show a smaller gonion angle (Blair, 1954; Guyer et al., 1986).

Dibbets (1996) has presented a contradictory opinion regarding mandibular size as an aetiological factor. He has reported no systematic difference in the mandibular size between Class I, Class II or Class III patterns. One explanation is that a variety of operational definitions for the Angle classification that have been applied in different studies. For example, in some studies, the classification has been determined from dental casts. In other studies the classification is determined according to the clinical examination.

Another explanation evokes the age differences between the study populations among different studies. From the literature, it can be argued that any difference in mandibular size which might exist between Class I, Class II or Class III emerges later in development and, therefore, these differences are more likely to be found in adult samples (Jacobson et al, 1974).

For clinical efficacy, the aetiology of a malocclusion needs to be determined at an early age. Since the size of the mandible has not been found to differ among different types of occlusion in young subjects, mandibular size is not considered to be the primary aetiological factor. Any difference in mandibular size among different occlusions in adults is now thought to be the result of unfavourable facial growth at an earlier age. Unfavourable facial growth causes the mandible to grow in an unfavourable direction, causing unfavourable mandibular form. Also the unfavourable facial growth leads to the unfavourable position of the mandible.

From a growth point of view, the cranial-base has also been suggested to be a primary aetiological factor in malocclusion (de Coster, 1953; Stamrud, 1959, Scott, 1967). Growth after birth occurs at the spheno-occipital synchondrosis, the speno-ethmoidal sutures and fronto-ethmoidal sutures. Neural growth of the cranial base has been shown to be complete by about 7 to 8 years of age. Growth at the sutures is complete by the end of the first decade, whereas growth at the synchondrosis continues until 12 to 16 years of age.

Ford (1956) has shown that, in humans, the cranial base angle, as measured by basion-sella-nasion, opens from 135° to 149° between the 10th and 40th weeks of foetal life. George (1978) has shown that this angle closes from birth to 5 years 9 months from about 142° to 130° but that little change is noted after 1 year 9 months. From 5 to 15 years it has been shown by Kerr (1978) that if correction for movement of nasion is made, the cranial bass angle does not, on average, change significantly.

The consensus of these reports is that the cranial-base region has considerable influence both upon total facial prognathism and the establishment of the anteroposterior relationship of the upper to lower jaw (Hopkin et al, 1968). Kerr and Hirst (1987) have shown that for subjects who develop Class I and Class II occlusions by 15 years of age, the cranial-base angle at 5 years is an accurate predictor of the ultimate occlusal type in 73% of cases.

Clear evidence from these studies reinforces the view that cranial-base length is related to occlusal type; i.e. Class II malocclusion tends to have the longest cranial base and Class III occlusion the shortest. The mandibular condyle tends to be located further back in relation to the pterygomaxillary vertical (PMV), frontomaxillary nasal suture (FMN) and nasion (N) in Class II, division 1 subjects and furthest forward in Class III subjects. But these differences between Class II, division 2 and Class II division 1 subjects do not achieve statistical significance (Kerr & Adams, 1988).

Based on all these studies, the aetiological factors of malocclusion can be described as follows: Class II malocclusion is developed on a longer cranial-base and the mandibular condyle is posteriorly located. Class III malocclusion is developed on a shorter cranial-base and the mandibular condyle is anteriorly located. Accordingly, the form of the mandible develops during growth.

1.1.3. Dentofacial orthopaedics and the TMJ

In the diagnosis of Class II malocclusion, one needs to assess the relative position of maxilla and mandible to the cranial-base, including their vertical relationships (Owen, 1984). With the aetiology of Class II malocclusion in mind, particularly Class II malocclusion in some patients is the result of a posteriorly located mandible residing on a longer cranial-base, one treatment option for Class II malocclusion beckons for the anterior re-location of the mandible. The re-location of the mandible requires re-organization of the TMJ.

The adaptation in the TMJ after functional appliance treatment has been substaitially documented in clinical case-reports (Bakke & Paulsen, 1989; Paulsen et al., 1995; Paulsen et al., 1998; Pancherz, 2000). After functional appliance treatment, the changes in the TMJ, such as new bone formation, have been detected by different methods in different age groups. Viewed from X-ray imaging, new bone formation has been observed as “double contours”. On orthopantomogram (OPG), double contours on the posterior aspect of the condyle have been described in one 16 years old male patient by Bakke and Paulsen (1989). Similar double contours viewed from OPG

images showed double contours of the condylar heads as well as on the cranio-posterior part of the ramus in a 14 years old male subject (Figure 1.1). Corresponding CT-scans taken at the same time also showed double contours in the fossa articularis and at the posterior part of the capitulum (Figure 1.2) (Paulsen et al., 1995).

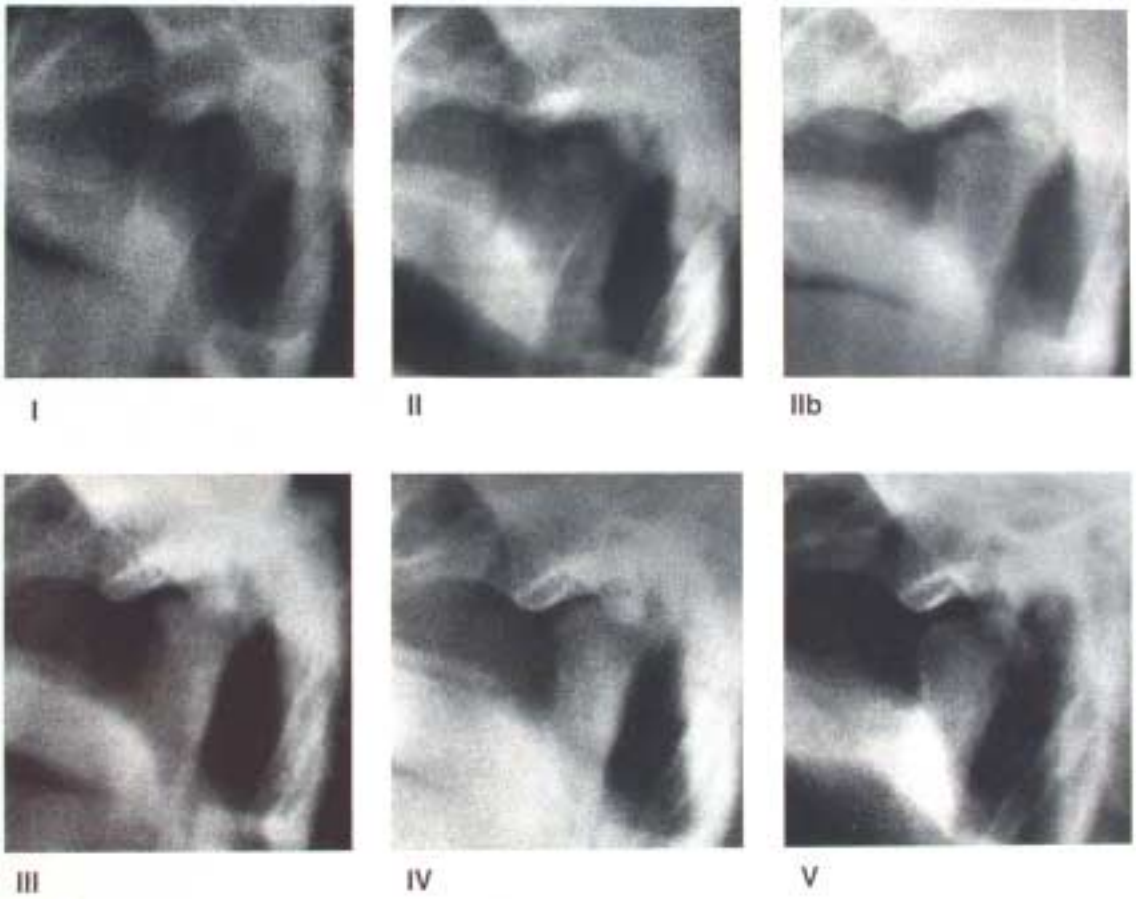


Figure 1.1. Left condyle from OPG images showing double contours of the condylar heads as well as on the cranio-posterior part of the ramus. The images were taken (I) before, (II) during Herbst treatment (after Herbst extension), (IIb) before Herbst removal, (III) after treatment (after Herbst removal), (IV) post-treatment, and (V) control. Note: development of double cortical layers (II) of the cranio-posterior part of the condylar process during treatment (3 months), change of morphology (II-III), and later remodelling of the condylar head (IV-V) (Paulsen et al., 1995).

Figure 1.2. CT-scanning of TMJ 3 months after insertion of Herbst appliances. The CT-scans were obtained depicting 2-mm contiguous axial slices parallel to the most concave aspect of the two fossa articulates (A). New bone formation was revealed as a double contour in the fossa articularis (B: right side, C: left side) and on the posterior part of the condylar head (D: right side, E: left side) (Paulsen et al., 1995).

Viewed from magnetic resonance images (MRIs), new bone formation was observed as increased signal density on the proton density-weighted MRIs. This increased signal density was observed in five of 15 subjects (4 girls and 11 boys with average age of 13.5 years old). The amount of new bone formation in the glenoid fossa was smaller than that of the condyle (Ruf & Pancherz, 1998). Similar results have been reported by the same group (Ruf & Pancherz, 1999) in a larger study population with 25 adolescents (11.4-15.7 years old) and 14 young adults (13.6-19.8 years old). In those studies, the adolescent or young adults referred to the skeletal age which was determined according the methods described by Hägg and Taranger (1980). Indeed, increased signal density on the proton density-weighted MRIs has been observed in the patients as old as 20s (Figure 1.3) (Pancherz, 2000).

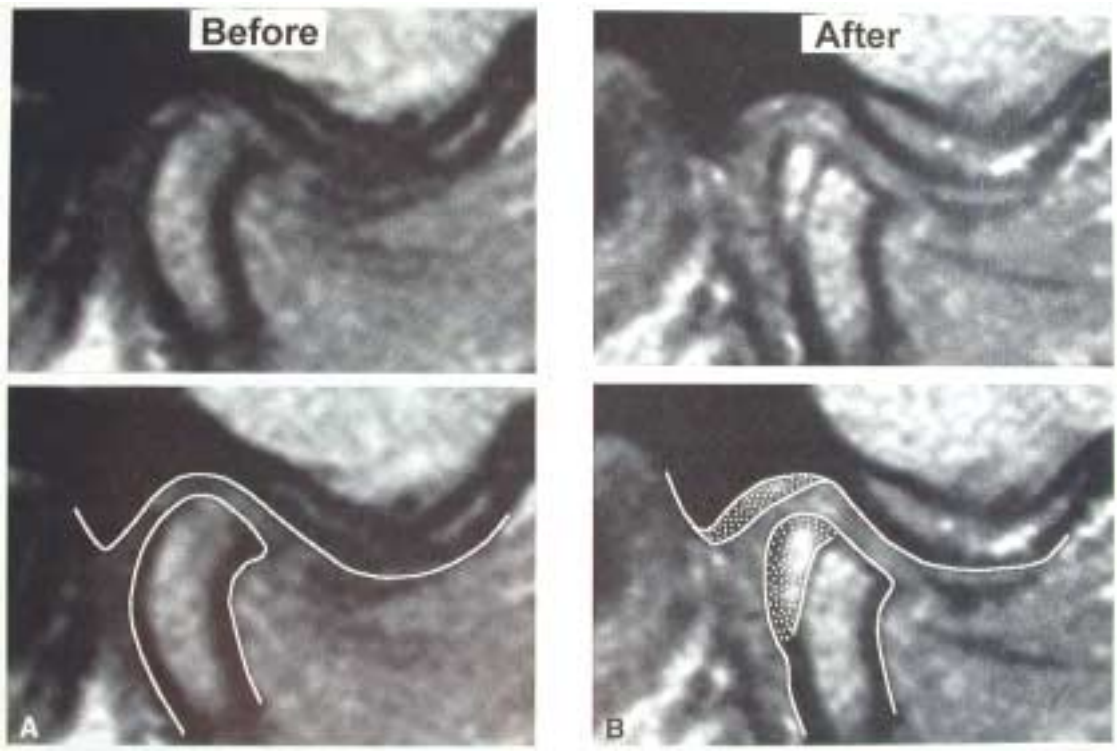


Figure 1.3. Twenty-year-old male patient with a Class II Division 1 malocclusion treated with the Herbst appliance for 10 months. Protron density weighted parasagittal MRIs from the right TMJ with corresponding tracings are shown (A) before treatment and (B) after treatment. Outline of condyle and glenoid fossa and appositional changes are marked (Pancherz, 2000).

Furthermore, bone scintigraphy with ^{99m}Tc-HDP which is a direct measure of bone metabolic activity (Gray et al., 1994) has indicated a significant increase in the bone metabolic activity of the TMJ after functional appliance treatment in a 13 years old female patient (Figure 1.4) (Paulsen et al., 1998).

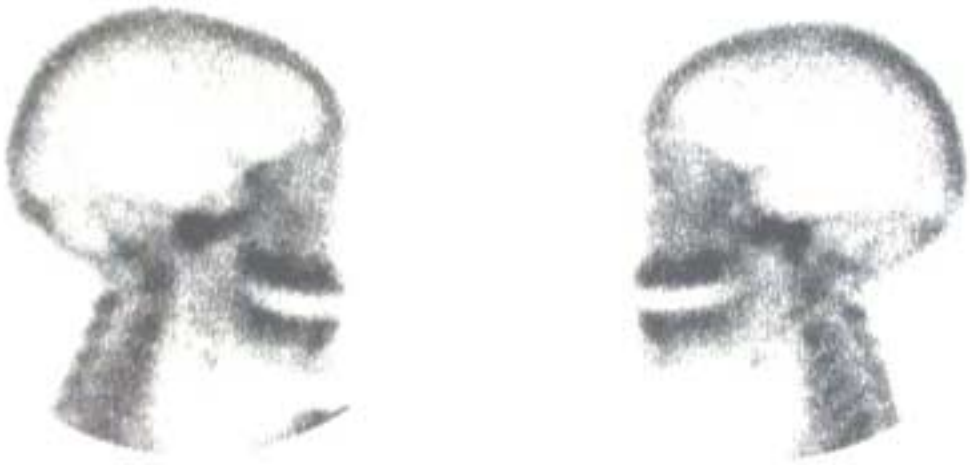


Figure 1.4. Bone scintigraphy with ^{99m}Tc-HDP of TMJ regions of the skull shows different metabolic activity between the left and right sides (Paulsen et al., 1998).

To the above authors' best knowledge, all the responses in the TMJ resulted from the Herbst appliance treatment. The bone responses in the TMJ have not been reported with the use of other kinds of functional appliances. Differing from many other functional appliances, the objectives of the functional components of the Herbst appliance are designed to constantly advance the mandible to a forward position. Nevertheless, one group of important mechanisms involved with functional appliance treatment implicates bony changes in an adapted TMJ.

1.2. Clinical Evidence of the Growth Modification Effects on the TMJ Collected from Large Clinical Trials

The purpose of this section is to identify the changes induced by functional appliance treatment in humans, so that experimental procedures can be designed to best mimic the functional appliance treatment regimens.

1.2.1. Changes in maxillofacial morphology

In analysing oral maxillofacial morphological changes, five randomised controlled trials (RCT) on the treatment of Class II malocclusion using functional appliances are deemed to be relevant. Two of them were conducted in the United Kingdom (Lund & Sandler, 1998; Illing et al., 1998). Two of them were conducted in the United States (Tulloch et al., 1997; Keeling et al., 1998). One of them was conducted in Australia (Chintakanon et al., 2000(b)).

The RCT, conducted by Lund and Sandler (1998), observed 63 Class II patients. Those patients were randomly assigned into 2 groups: Clark Twin-Block treatment group (36 patients) and untreated control group (27 patients). At the completion of the study, the skeletal changes were found as a forward growth/repositioning of the mandible (2.4 mm, measured at Ar-Pog), an increased ANB angle, an increased lower anterior facial height and mostly unaffected maxilla.

The RCT, conducted by Illing and co-workers (1998), observed 78 Class II patients. Fifty-eight of the patients were treated either by a bionator, a twin block or by a bass appliance. Twenty patients were selected as untreated controls. At the completion of the study, patients in both the Bionator and Twin Block group showed an increased in the distance of articulare to pogonion (Ar-Pog) as 3.9±2.7 mm and 3.7±2.1 mm respectively, an anterior movement of pogonion and B point as well as an increased lower face height. The above authors claimed the increased Ar-Pog represented increased mandibular length. However, the distance Ar-Pog does not show full mandibular length. A change in the amount and direction of the condylar growth does not necessarily create the same change of the point Ar (Aelbers & Dermaut, 1996).

The RCT, conducted by Tulloch and co-workers (1997), observed 107 mild to severe Class II patients. Those patients were randomly assigned into 3 groups: headgear treatment group, bionator treatment group and an untreated control group. At the completion of the study, comparison between groups showed that treatment with headgear and the functional appliance reduced the severity of the skeletal discrepancy. When the amount of skeletal change (measured as the ANB angle) in the three groups was compared, the two treatment groups had statistically significant reductions in ANB angle. When the amount of skeletal change was measured as SNA angle, headgear treatment group has shown a decreased SNA angle compared with both untreated controls and bionator treatment group. The SNA angle did not differ between the bionator treatment group and the untreated controls. These

results indicated headgear treatment reduced ANB angle through the restriction of the maxillary growth whereas bionator treatment reduced ANB angle through an anterior re-location of the mandible.

The RCT, conducted by Keeling and co-workers (1998), observed 249 Class II patients. Those patients were also randomly assigned into 3 groups: bionator treatment group, headgear/biteplane treatment group and an untreated control group. At the completion of the study, the skeletal changes were found to be largely attributable to the increased mandibular growth in both the headgear and bionator treatment groups. The skeletal changes remained stable 1 year after the treatment.

The RCT, conducted by Chintakanon and co-workers (2000 (b)) at the University of Adelaide, South Australia, observed 40 Class II patients using MRI within an entire sample of 71 Class II patients. Those patients were assigned into 2 groups: Clark Twin-block treatment group and an untreated control group. At the completion of the study, the condylar axial angle was measured on the MRI. The comparison between groups showed that the treatment group had a stable axial angle whereas the control group had a reduced axial angle. This result suggested that the treatment induced a posteriorly rotated condylar growth response.

In summary, these studies have indicated that functional appliances help to correct the Class II malocclusion either through relocating the mandible

forward, increasing the growth of the mandible or inducing a re-direction in growth of the mandible.

1.2.2. Changed morphology of the TMJ

Changes in the TMJ due to functional appliance treatment have also been reported more specifically to involve increased condylar growth and altered shape of the condyle. Following the case reports, reviewed earlier in this chapter, Pancherz and co-workers conducted quantitative measurements of the condylar growth in 40 Class II, division 1 malocclusion patients aged 10.4 ± 1.3. The condylar growth was measured by superimposing cephalograms on the relative stable reference bone structures of the mandible. The authors interpret the positional change of the condyle to be equivalent to effective condylar growth, which is an expression of condylar remodelling, glenoid fossa remodelling and changed condylar position in the fossa. After treatment, the condyle grew on average 9.6 mm vertically and 2.1 mm horizontally backward. This growth was larger than the condylar growth observed in a normal population over an equivalent period (Ruf et al, 2001).

1.2.3. Changes in masticatory function

In addition to the findings from the study of Chintakanon et al. (2000 (b)) where 71 Class II patients were assigned into Clark Twin-block treatment group and an untreated control group, the maximum protrusive force was also measured. The comparison between groups showed that in the treatment group, the protrusive force did not change over time. In the control group, the maximum protrusive force increased over time. This result suggested that the

treatment shortened the protrusive muscles and consequently slowed down the increase of muscle forces observed in the control group (Chintakanon et al., 2000 (a)).

1.2.4. The quality of dentofacial orthopaedic clinical management and related questions

The treatment of skeletal Class II malocclusion is largely based, and relies on the accumulation of clinical experience but the treatment planning is still in the state of “half art, half science”. Since considerable investment of time and money has been put into pursuing the correction of dental malocclusion, the resultant quality of its clinical management must be measured in terms of *efficacy, effectiveness* and *efficiency* of dentofacial orthopaedic treatment. *Efficacy* of the treatment deals with the appropriate time to start the intervention, *effectiveness* of the treatment deals with the outcome of treatment objectives and e*fficiency* of the treatment deals with optimal time frame for the treatment objectives can best be achieved.

Regarding the first issue of “when to start the treatment”, the answer remains equivocal. Clinicians now agree that to start dentofacial orthopaedic treatment early in specific cases of mixed dentition is effective and desirable. However, the evidence is equally compelling that such an approach is not indicated in many cases for which later, single-phase treatment in permanent dentition is more efficacious. Therefore, clinicians must decide, on a case-by-case basis, when to provide appropriate dentofacial orthopaedic treatment (Kluemper et al., 2000).

For a second issue “can the treatment be effective”, the answer is whether the treatment can produce changes to a clinically significant level. Knowledge is available on the changes induced by the functional appliances and on the factors influencing these changes. However, it is still unclear whether the cause-effect relationship of functional appliance treatment closely matches the induced changes when the various presumed influential factors are taken into account.

For a third issue “how long is required for the treatment”, the answer also depends on accumulated knowledge about the cause-effect relationship of functional appliance treatment and the induced changes with the influential factors taken into account. In orthodontics, it is generally accepted that teeth, which have been moved orthodontically, will drift back to their original position. Therefore, a retention phase is usually designed to minimise this tendency (Fricker, 1998). In dentofacial orthopaedic treatment, based on the clinical experience, it is very likely that jaws, which have been moved orthopaedically, will also drift back to their original position and a retention period might also be necessary. The planning for the retention phase as a part of the dentofacial orthopaedic treatment requires knowledge of the biology of the jaw response to the treatment. Unfortunately, this information is currently unclear.

Since all the treatment planning is made on an individual by individual basis, as listed above, a fourth issue arose “should we use population norms to guide the treatment for each individual”. The best answer to these questions comes from understanding the mechanisms of the treatment. The

understanding needs to start with the biology of the major components involved in the treatment, then finally to the functional appliance mode of action.

Part II: The Biology of Growth Modification in the TMJ

1.3. Biological Basis of the Mandibular Condylar Growth

Since functional appliance treatment is thought to achieve some of the corrections through adaptive responses in the TMJ and mandibular condyle is thought to be taking important roles during the growth and the development of the TMJ, this section will introduce insight into growth of the mandibular condyle.

1.3.1. Microanatomy of the mandibular condyle: the tissue and cellular components

On the basis of gross anatomy, the normal mandibular condyle can be divided into two parts; the head and the neck. Different connective tissue components are recognised in the mandibular condyle. These include investing connective tissue membranes, cartilage and bone which build up the structure of the mandibular condyle and hemopoietic tissue which is contained in the mandibular condyle. The covering of the condyle is the perichondrium, an avascular fibrous layer on the condylar head and which thins at the periphery of the condyle to blend with the periosteum of the mandible on the condylar neck (Seipp, 1964). The periosteum is a special, vascular, fibroblastic

connective tissue. It consists of two main layers: a fibrous limiting membrane and a cellular layer comprising osteogenic cells.

Examination of sections of the mandibular condyle shows the condylar head has a darkly stained peripheral region referred to as the "perichondrium" and a broader area referred to as the "hyaline cartilage". Below these are the trabeculae of the so called endochondral bone which connect with the trabeculae in the condylar neck. As illustrated in Figure 1. 5 (Luder et al., 1988), under the avascular fibrous articular layer, the perichondrum can be further divided into polymorphic and flattened cell layers. The polymorphic and flattened cell layers largely consist of differentiating cells. The term pre-chondroblasts and chondroblasts respectively have been almost uniquely adopted in the TMJ literature. They are also known as the pre-chondroblast zone and the chondroblast zone. The hyaline cartilage region is composed of hypertrophic chondrocytes and has been divided into an upper layer which contains unmineralised extra-cellular matrix and a lower layer containing mineralised extra-cellular matrix.

Characteristic features of all bones can be seen in the sections of condylar neck beneath the periosteum. These include a dense outer sheet of cortical bone and an inner part representing of a marrow cavity. In growing children and adolescents, the marrow cavity is filled with red bone marrow (hemopoietic tissue). Red bone marrow contains bone marrow stroma and an extensive vascular system. The marrow cavity is interrupted throughout the length of the mandible by a network of trabecular bone particular under the

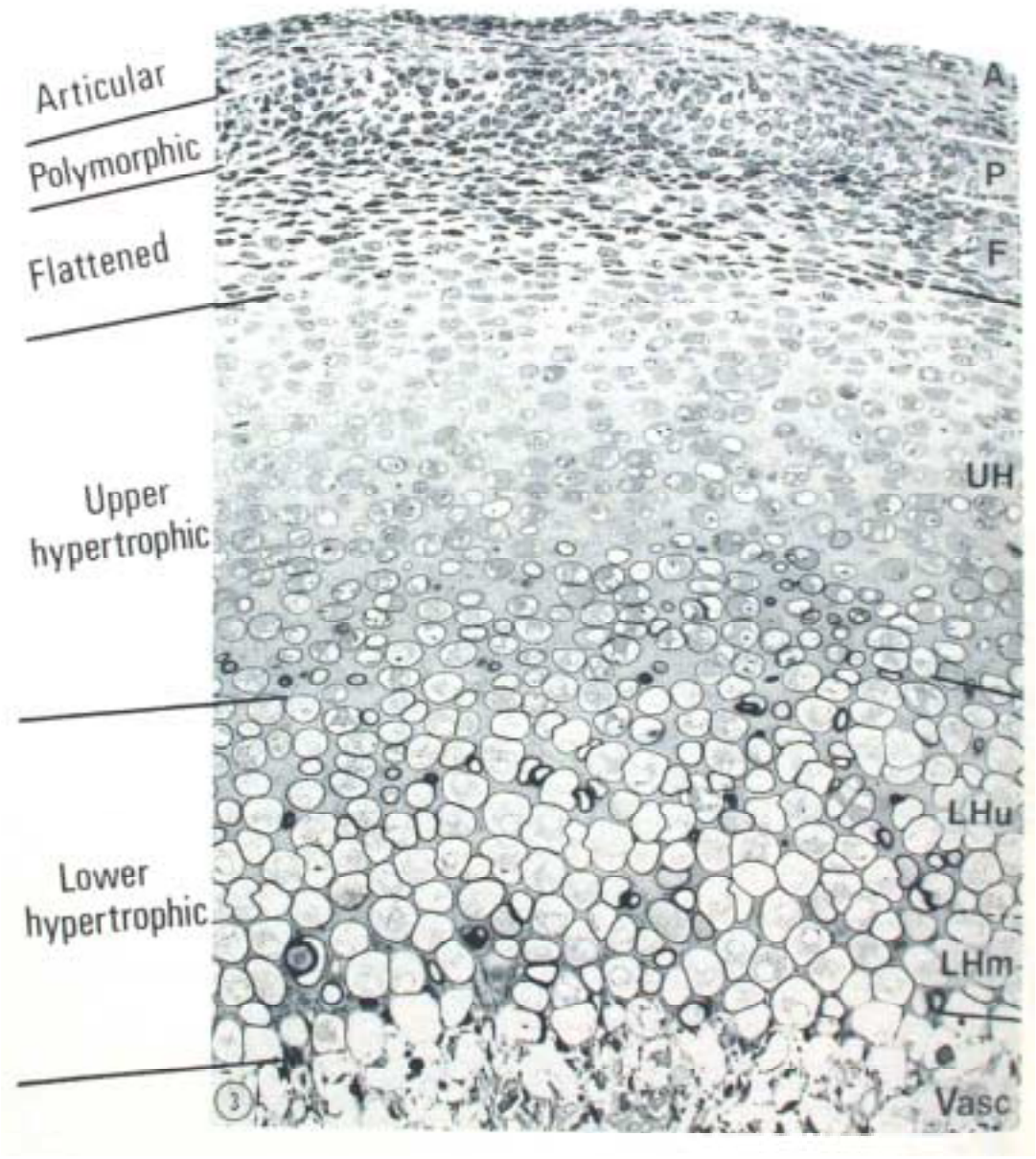


Figure 1.5. Layers of the mandibular condylar cartilage. Each layer is indicated by its abbreviated name at left and corresponding abbreviation at right. Under the un-mineralised portion of the lower hypertrophic cell layer (LHu), the mineralised portion of the lower hypertrophic cell layer (LHm) borders on the zone of vascular invasion (Vasc). Toluidine blue stain. ×260 (Luder et al., 1988).

condylar cartilage (Ten Cate, 1989). The inner surface of the condylar neck is lined by endosteum, which is similar to periosteum but not as well defined (Krause & Cutts, 1981). The endosteum is a single, cellular layer that surrounds the bone marrow tissue and the trabeculae. Sometimes Haversian envelopes, which contain Haversian capillaries are present only in cortical bone and are defined as a special type of endosteum. These cover the Haversian canals (Wlodarski, 1988).

In the bony structure of the mandibular condyle, including cortical bone and trabecular bone, three types of bone cells are described, each of which have specified functions. They are; the osteoblast, which forms bone; the osteocyte which, together with inactive osteoblasts maintains bone; and the osteoclast which resorbs bone (Krause & Cutts, 1981).

Both osteoblasts and osteoclasts are derived from partially differentiated progenitor cells that reside in the bone marrow. Osteoblasts belong to the mesenchymal lineage of the marrow stroma, and osteoclasts belong to the hematopoietic lineage of the bone marrow stroma (Ten Cate, 1989). Bone marrow stroma contains a unique cell population, referred to as marrow stromal cells, capable of differentiating along multiple progenitor cell lineages (Bergman et al., 1996; Phinney et al., 1999). In mammalian bone marrow, three kinds of marrow stromal cells are recognised, they are: hematopotetic support cells, osteogenic stromal cells and adipocytes. They are all differentiated from stromal stem cells. Alternative models for stromal stem cell differentiation have been suggested by Glimble et al. (1996).

i. Stromal stem cells are distinct from the committed lineages: adipocytes, hematopoietic supporting cells, and osteoblasts;
ii. Functional overlap exists between the stromal, hematopoietic-supporting, and osteoblast lineages;
iii. Functional overlap exists between the stromal, hematopoietic-supporting, and adipocyte lineage;
iv. Functional overlap exists between the stromal, adipocyte and osteoblast lineage;
v. Functional overlap exists between the stromal stem cells and all lineages

1.3.2. Endochondral ossification

In growing subjects, bone formation in the condyle is recognised both under the periosteum and under the cartilage where blood vessels are present (Ten Cate, 1989). The bone formation within the condylar head under perichondrium and hyaline cartilage is believed to be "endochondral ossification". This growth pattern involves the continuous replacement of pre-existing condylar cartilage (Kraus & Cutts, 1981).

The process of endochondral ossification can be interpreted as two steps (see Figure 1.5); the first step being avascular cartilage differentiating to hypertrophic cartilage and the second, where hypertrophic cartilage undergoes erosion and vascularisation leading to bone deposition (Carleraro et al., 1997). The details of the first step can be described as follows.

i. The polymorphic cell layer and the flattened cell layer are sites of mitotic activity, giving rise to cells which undergo endochondral differentiation. Special pressure resulting from cell population and matrix production cause the articular and polymorphic cell layers to be displaced further and further away.
ii. In the upper hypertrophic layer, cells markedly increase their volume and increase their secretary activity, until cells reach the lower hypertrophic layer where their secretary activity gradually decreases and matrix mineralisation proceeds. The cells are eventually lost when their lacunae are invaded by blood vessels (Luder & Schroeder, 1992).

Based on morphological observations, the detail of the second step of endochondral ossification has been described as follows.

i. Degeneration and removal of the un-mineralised transverse septal cartilage by rough-surfaced endoplasmic reticulum (RER)-rich mononuclear cells concomitant with capillary vessel invasion into newly opened lacunar channel.
ii. Phagocytosis of the cartilage fragments of the longitudinal septa by RER-rich mononuclear cells.
iii. Bone matrix deposition over persisting septal cartilage by osteoblasts, and
iv. Degradation of bone and calcified cartilage by differentiated osteoclasts in the lower potion of the provisional ossification zone (Sasaki et al., 1996; Lewinson & Silbermann, 1992).

The cellular mechanism of this process is now recognised as: endothelial cell migration and invasion which lead to blood vessels growing into the hypertrophic cartilage and eroding it to produce a scaffold on which osteoblasts settle to produce woven bone (Alini et al., 1996). During this process the growth of blood vessels in the subchondral region is always in advance of the differentiation of osteoblasts from the mesenchymal cells. As the growth in length proceeds, the subchondral mesenchyme cells are left behind, to lie between blood vessels and surviving island of cartilage matrix on which bone deposition occurs (Brookes & Revell, 1998).

The detailed mechanism of the regulation of endothelial cells during blood vessel invasion (angiogenesis) is still not completely understood. Angiogenesis is a pivotal event in endochondral ossification. Signalling molecules have been found to be important in the regulation of angiogenesis. A chemo-attractant, non-mitogenic molecule for bovine hypertrophic chondrocytes was reported to have the ability to induce angiogenesis in the rabbit cornea model *in vivo* by Alini et al. (1996). Transferrins from a number of different sources, including hypertrophic chondrocytes, have been reported to stimulate endothelial cell migration and invasion during angiogenesis (Carleraro et al., 1997). Now, researchers are trying to define one or several signalling molecules which have control over angiogenesis. With numerous cross-reactions among those molecules the process of research is going very slowly.

To date, most of the studies on the control of angiogenesis by signalling molecules have been conducted on long bones rather than mandibular condyle. It is not clear if a changed functional environment, e.g., compressive force, can directly or indirectly influence angiogenesis during endochondral ossification. However, Leung et al. (2001) suggested that the angiogenesis is enhanced in the mandibular condyle following experimental functional appliance treatment. The details will be introduced in Section 1.7.1 of this Chapter.

1.3.3. Intramembranous ossification

Periosteum has the ability to form bone. The bone formation under periosteum is referred to as "intramembranous ossification". In this kind of bone formation, bone first forms in the connective tissue bed, where osteoblasts differentiate from mesenchymal cells and start to produce bone matrix. As more and more bone matrix is deposited, the cells and their processes become trapped in the matrix and are then identified as osteocytes. The initial matrix produced by osteoblasts consists of extracellular matrix and collagen fibres and being un-mineralised, is called osteoid. The osteoid then becomes mineralised to form bone (Krause & Cutts, 1984).

Growth of the mandibular condyle is achieved by the proliferation and differentiation of cells both in cartilage and bone; in particular, chondroblasts and osteoblasts. In the following part of this review, the association between mechanical force (including compressive force and tensile force) and chondroblast and osteoblast proliferation and differentiation will be reviewed.

1.4. Experimental Evidence of the Growth Modification Effects in the TMJ

The purpose of this section is to summarise the adaptive responses in the TMJ during experimental functional appliance treatment.

1.4.1. TMJ tissue responses to functional appliance treatment in animal studies: the tissue level

The adaptive responses in the TMJ to functional appliance treatment have been investigated at the tissue level based on experiments performed on non-human primates. In an experiment which recruited 14 functional appliance treated and 14 control male monkeys (*Macaca mulatta*) observed for various periods of up to 24 weeks, the thickness of the mandibular condylar cartilage was found to be significantly increased in the posterior region and the posterosuperior region. The increase was the most pronounced in the pre-chondroblast and chondroblast zones (McNamara & Carlson, 1979).

In subsequent studies conducted by the same researchers, subchondral bone was reported to be different both in bone deposition and in trabecular orientation. Increased bone deposition in hyperpropulsor treated animals was found in the posterior border of the ramus and in the anterior surface of the post-glenoid spine (Hinton & McNamara, 1984).

In an experiment using 11 functional appliance treated and 12 control male monkeys (*Macaca mulatta*), evaluated over a period up to 144 weeks, the

mandibular length was found to be significantly increased after 48 weeks of functional appliance treatment. By the end of the study, the mandibular length was 5 to 6 mm greater in the functional appliance treated monkeys, which represented a 7% increase in mandibular length. The condylar-ramus-occlusal (CRO) angle was also found to be different in the functional appliance treated monkeys showing a posterior rotation of the mandibular condylar growth (McNamara & Bryan, 1987). In this study, adaptations in the TMJ were concluded to be an increased mandibular length and an opened CRO angle. Whether these were an adaptation of the condylar cartilage, or condylar bone, or both, was not specified. The authors also suggested that the histologic adaptive responses described in monkeys closely resemble the changes observed radiographically in human subjects who had condylar fractures involving dislocation of the condyle from the fossae (Thomson et al., 1964; Lindahl & Hollender, 1977; Hinton & McNamara, 1984). But the correlation between these adaptive responses and TMJ growth and development was not discussed.

1.4.2. TMJ tissue responses to functional appliance treatment in animal studies: the cellular level

Biological responses in the TMJ to functional appliance treatment have been studied at the cellular level based on experiments performed on rats. In an experiment using 96 male Sprague-Dawley rats over a period up to 4 weeks, the articular zone of the mandibular condyle was found to be thicker and the cells were rounder. The thickening was also found in the pre-chondroblast zone with increased cell divisions as well as in the chondroblast zone. The

orientation of the condyle was also found to be changed. The authors concluded that the functional appliances brought about an additional growth of the condylar cartilage by stimulating the cells in the pre-chondroblast layer (Charlier et al., 1969).

In an experiment involving 96 male rats (strain not specified) over a period up to 4 weeks, lengthening of the mandible was found to be correlated with the number of [^{3}H]-thymidine labelled cells in the condylar cartilage. The authors concluded that the lengthening of the mandible corresponded to the increased condylar growth rate measured as the increased number of [^{3}H]-thymidine labelled cells in the condylar cartilage (Petrovic et al., 1977).

Petrovic and co-workers (1981) used 120 male Sprague-Dawley rats with the experimental period up to 76 days, and found the lengthening of the mandible correlated with increased condylar cartilage growth rate and the lateral pterygoid muscle (LPM) activity. The authors concluded that the lengthening of the mandible corresponded to the increased condylar growth rate and that these changes were the result of increased LPM activity.

In summary, Petrovic and co-workers hypothesised that a functional appliance primarily induces an amplification in “skeletoblast” (half differentiated mesenchymal cells with the potential to further differentiate into either chondroblasts or osteoblasts) and mitotic activity of the cells in pre-chondroblast zone. This leads to an acceleration in differentiation of “skeletoblasts” into these pre-chondroblasts, which resulted in an increase in

the transformation of pre-chondroblasts into functional chondroblasts and an acceleration in chondroblast hypertropy and endochondral bone growth. Simultaneously, there is an increase in the subperiosteal growth rate at the posterior border of the ramus. The authors also pointed out that the bone mineralisation and bone turnover increased in the mandible as a whole (Petrovic et al., 1990).

1.5. Re-evaluation of the Experimental Evidence for Growth Modification Effects in the Condylar Cartilage

With advances in human knowledge, the same observation sometimes can be given different meanings. The purpose of this section is to re-visit the early findings made by McNamara and co-workers as well as the findings made by Petrovic and co-workers, and to re-evaluate the conclusions made by those authors. Based on the summary made by Petrovic and co-workers described in the previous paragraph, six hypotheses were specified and they are discussed in this section and the following sections.

1.5.1. Hypothesis No. 1. Functional appliance treatment increases condylar growth through inducing cartilage cell proliferation and cartilage matrix production

Here, only cells of the cartilage are discussed, the cells in other condylar tissue will be discussed in the following sections. According to Petrovic and co-workers (1990), functional appliances amplify mitotic activity in the pre-chondroblast zone, resulting in an increase in the transformation of pre-

chondroblasts into functional chondroblasts, which means more "pre-chondroblasts" differentiate into "chondroblasts" and increase endochondral bone growth. This hypothesis was re-evaluated based on the question of whether or not the total number of pre-chondroblast can be increased.

Histo-chemical and immuno-chemical examinations have been used to investigate the responses of the mandibular condylar cartilage to functional appliance treatment during growth. Since pre-chondroblast and chondroblast zones are the zones of cell division, a mitotic index (the number of mitotic cells per 100 or 1000 cell count) is used as a measurement of the cell proliferation in the mandibular condylar cartilage. Another measurement for mitotic activity is the intensity of immuno-stained cAMP or cGMP (products formed during cell proliferation). The number of [^{3}H]-thymindine labelled cells is a measurement of both cell proliferation and differentiation. Unlike the immuno-staining of cAMP and cGMP, [^{3}H]-thymindine label is retained throughout the whole life-span of cartilage cells. In serial studies conducted by Petrovic and co-workers, the mitotic index and [^{3}H]-thymindine labeling were found to increase in the thick regions of the pre-chondroblast and chondroblast zones (Petrovic et al., 1966; Charlier et al., 1969; Petrovic et al., 1975; 1981).

In contrast to the explanation given by Petrovic and co-workers that the increased number of [^{3}H]-thymindine labelled pre-chondroblasts indicates an increased cell proliferation, others suggest that increased labelled pre-chondroblasts observed from a histologic section can be the result of either

more cell proliferation from mesenchymal cells into pre-chondroblasts or the result of slowed cell differentiation of pre-chondroblasts into chondroblasts. Indeed, increased [^{3}H]-thymindine labelled pre-chondroblasts observed from a histologic section does not necessarily mean an increased pre-chondroblast proliferation.

The answer to whether or not the mechanical environment can affect condylar cartilage growth has long been controversial. Some researchers believe that cell division within the condyle could occur independently of the functional environment. Environment could only influence the differentiation of pre-chondroblasts (Meikle, 1973 (a) (b); Copray et al, 1985 (a) (b) (c) (d)). *In vitro* study using organ culture systems showed that the actual onset of hypertrophy, (i.e., the enlargement of a mature chondroblast due to increased hydration), did not seem to be influenced by experimental compressive force (Copray et al, 1985 (b)). However, in a later *in vitro* cell culture system, static compressive force was found to increase the DNA synthesis of cartilage cells. This result suggested that compressive force might stimulate chondrocytes into both proliferation and differentiation (Yamamoto et al., 1991).

In vivo studies have been designed using the same [^{3}H]-thymindine labelling techniques to investigate the impact of compressive force on cartilaginous cell proliferation and differentiation. By feeding animals with a soft diet, the bite force was presumed to be reduced and compressive force over the condylar surface was presumed to be reduced accordingly. After four weeks of this treatment, increased length and height of the mandible was observed. The

migration of [^{3}H]-thymindine labelled cells in the cell layer beneath the pre-chondroblast zone increased indicating accelerated differentiation of mesenchymal cells to pre-chondroblasts (Kantomaa et al., 1994). This result indicates that reduced compressive force increases the rate of cell differentiation in the mandibular condylar cartilage.

Unilateral bite raising appliances have been designed to increase compressive force on the treated side. Four weeks after this treatment, increased [^{3}H]-thymindine labelled cells were found together with increased cAMP, cGMP staining intensity in the pre-chondroblast zone. This result was explained as slowed differentiation of cells in the pre-chondroblast zone. Increased labelled cells as well as increased cAMP and cGMP staining intensity in the hypertrophic zone indicated slowed differentiation of hypertrophic chondrocytes (Lindsay, 1977; Ehrlich et al., 1980). This result indicates that increased compressive force reduces the rate of cell differentiation in the mandibular condylar cartilage.

The finding that a reduced compressive force increased the rate of cell differentiation and increased compressive force reduces the rate of cell differentiation can also be observed in different areas of the mandibular condylar cartilage within one condyle. The mandibular condylar cartilage was divided into 7 regions (Figure 1.6). Thirty-day old rats were forced to keep their mouth open for 6 hours a day for 10 days. When the mouth is opened, the compressive force in the anterior regions (Region I, II, III) of the mandibular condylar cartilage is thought to be reduced and the compressive

force on the mandibular condylar cartilage is thought to be increased in the posterior regions (Region IV, V, VI, VII). In treated animals, [^{3}H]-thymindine labelled cells were found to be decreased in the anterior regions and increased in the posterosuperior regions. Decreased labelling in the anterior regions suggests acceleration in the progression of cells out of the proliferation pool. Increased labelling in the posterosuperior regions of the condyles suggests retardation in the progression of cells out of the proliferation pool. (Kantomaa & Pirttinirmi, 1996).

Based on these studies, one could explain the changed cartilage thickness in rats to be due to compressive force affects the mandibular condylar cartilage growth by influencing the rate of pre-chondroblast differentiation. During functional appliance treatment, the altered condylar-fossa relationship is thought to be increases the compressive force in the posterior part of the condyle and decrease it anteriorly. This changed compressive force leads to accelerated pre-chondroblast differentiation in the anterior region and slowed pre-chondroblast differentiation in the posterior region. This change leads to increased cartilage thickness posteriorly. There is not sufficient evidence to indicate that any increased total number of pre-chondroblasts following functional appliance treatment. In addition, the study period of all the above studies were relatively short compared with the relative growth period of rats. The study period varied from 10 days (Kantomaa & Pirttinirmi, 1996) to 21 weeks (Lindsay, 1977). They did not measure the differentiation rate of cartilage cells in the pre-treatment period or follow the responses for the sufficiently long post–treatment period.

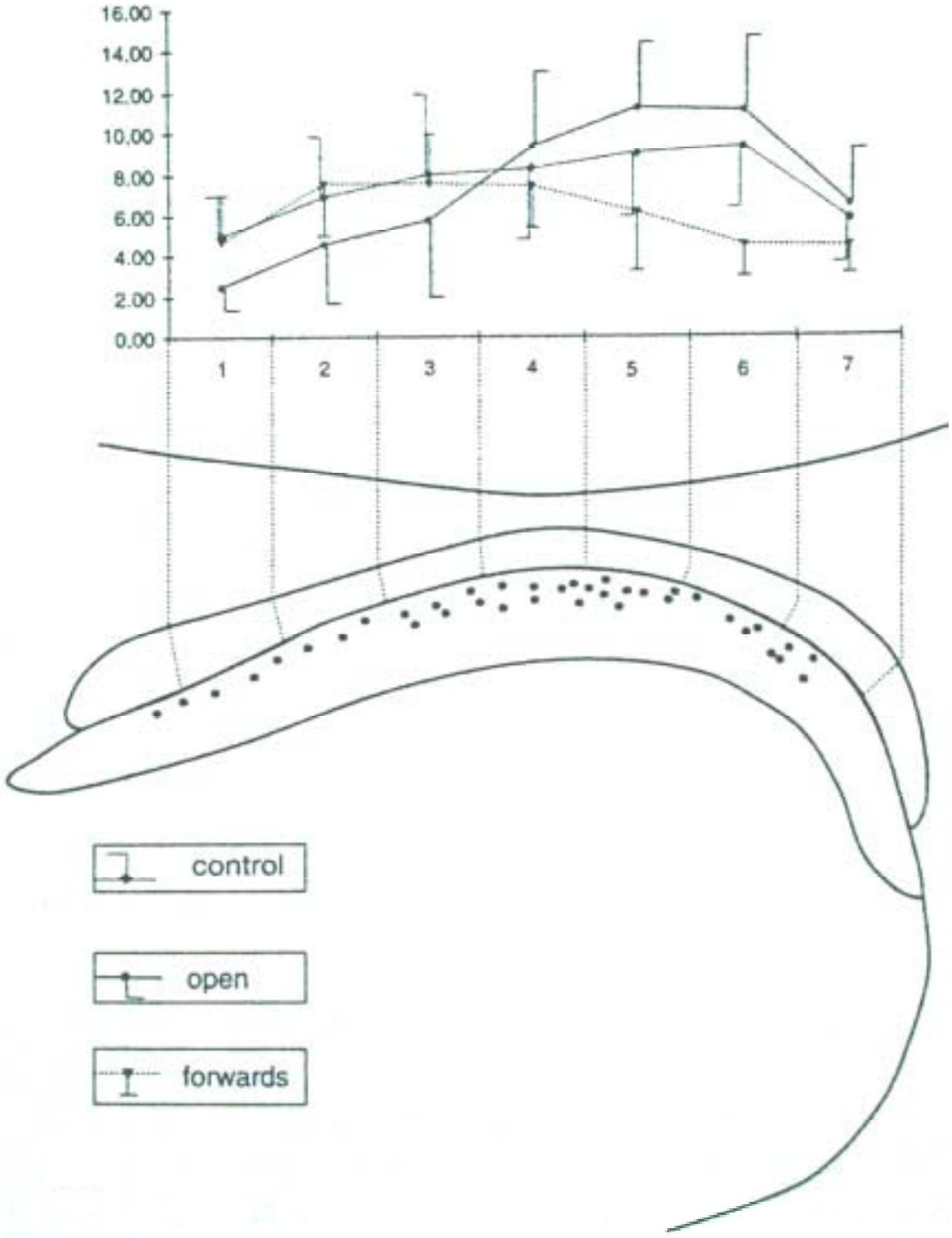


Figure 1.6. Schematic representation of the differences in [^{3}H]-thymindine incorporation in the mandibular condylar cartilage between the experimental and control rats. In the animals with the mandible in an open position (open), increased labelling in the posterosuperior region of the condyles suggests retardation in the progression of cells out of the proliferation pool. Decreased labelling in the anterior region suggests acceleration in the progression of cells out of the proliferation pool. These results were obtained from the comparsion with the animals in the group had their mandibles maintained with phosphate cement in a protracted position (forwards) and untreated controls (control) (Kantomaa & Pirttiniemi, 1996).

1.5.2. Hypothesis No. 2. Functional appliance treatment increases condylar growth by increasing both the life-span of cartilage cells and deposition of cartilage matrix during endochondral ossification

Following on from the previous topic, the life-span of cartilage cells is now discussed. Based on [^{3}H]-thymidine labelling techniques, the total time of cartilage cell differentiation from pre-chondroblast to hypertrophic chondrocyte has been analysed in 20-day old rats. It was concluded that this time period is about 8 days. Differentiating cells spent 2.9 days in the polymorphic cell layer (as pre-chondroblasts); 1.8 days in the flattened cell layer (as chondroblasts); 2.3 days in the upper hypertrophic cell layer (as chondrocytes) surrounded by un-mineralised matrix and 1.1 days in the lower hypertrophic cell layer (as chondrocytes) surrounded by mineralised extracellular matrix (Luder et al., 1988). Three-week old or 4 week old rats were usually used to study the effect of functional appliance treatment on condylar cartilage growth. The evidence for the similarity between life-spans of the cartilage cells in 20-day old rats and in 4-week old rats is compelling.

The hypothesis that functional appliances increase the life-span of cartilage cells which in turn leads to more cartilage matrix production and increased condylar growth, is an alternative explanation for the increased number of [^{3}H]-thymindine labelled pre-chondroblasts. Increased labelled pre-chondroblasts observed from a histologic section can be a result of slowed cell differentiation of pre-chondroblasts into chondroblasts. A slower rate of differentiation results in a longer life-span of the cell. Unfortunately, no study

has been conducted to demonstrate whether increased cartilage matrix is produced along with a prolonged life-span of the pre-chondroblasts.

1.6. Re-evaluation of the Experimental Evidence for Growth Modification in the Subchondral Bone

Again, following from the previous section, the proliferation and differentiation of bone cells are discussed in this section.

1.6.1. Hypothesis No.3: functional appliance treatment increases condylar growth through inducing more cells to differentiate into osteoblasts and produce more bone matrix

According to Petrovic and co-workers (1990), a functional appliance primarily induces an amplification in skeletoblast mitotic activity and, an acceleration in differentiation of skeletoblasts resulting in acceleration of endochondral bone growth. According to current views, the limitation for the concepts given by Petrovic and co-workers is their lack of detail, namely acceleration of endochondral bone growth needs to be specified as increased bone matrix formation, or increased bone matrix mineralisation, or both.

Mechanical factors have been found to be important during bone growth. Although the implication of compressive force on endochondral ossification has not been studied in the mandibular condyle, the change of mechanical loading on cartilage has been widely recognised in long bone growth plates. This has been reviewed by Frost and Jee (1994 a;b). They found that after

resorption of the mineralised horizontal septa between the chondrocytes (which are former chondroblasts), honeycomblike vertical tunnels are created extending upward into the mineralised cartilage. At this time, mineralised cartilage still carries the growth plate's mechanical loads, which must commensurately increase the loads on the remaining septa. They also pointed out that this observation is uncontroversial. Therefore, one can believe that increased compressive force on mineralised cartilage is compulsory for bone matrix formation during endochondral ossification.

The influence of compressive force on bone cells during the process of endochondral ossification has not been reported, but compressive force has long been recognised to influence osteoblast and osteoclast behaviour during orthodontic treatment. A dramatic decline in the bone surface covered by osteoblasts and an increase in the bone surface covered by osteoclasts have been reported in alveolar bone and periodontal ligament during tooth movement *in vivo* in rats (King & Keeling, 1995), and preliminary results obtained from *in vitro* study on metatarsal bones from young rats also suggested compressive force stimulates osteogenesis by stimulating osteoblast proliferation (Lozupone et al., 1992).

To date, most of these studies have concentrated on the influences of compressive force on bone matrix formation (measured by the separation of double fluorescent labels) and matrix mineralisation (measured using osteoid seam thickness). Limited studies have reported the changes in the subchondral bone subsequent to the changes in the cartilage in the

mandibular condyle. Although the distribution and magnitude of the strain applied to the condyle is currently unable to be measured, it is assumed that jaw movement has a rhythmical nature. Therefore, the occlusal is comparable to cyclic dynamic loads (Gazit et al., 1987). Two studies are outstanding amongst the others. Sugiyama et al. (1999) fed rats with a soft diet and they found the condyle showed a thinner overall cartilage thickness and less subchondral trabecular bone. Gazit et al. (1987) treated rabbits with a uni-lateral bite-raising appliance and they found the cartilage thickness and bone formation rate increased on the treated side of the rabbits' condyle.

These results agreed with those obtained from *in vitro* studies of foetal mouse calvariae or long bones, which demonstrated the link between increased compressive force and increased bone matrix formation (Klein-Nulend et al, 1987) and decreased bone matrix resorption (Klein-Nulend et al, 1987, 1990).

Although the mode of bone formation in the TMJ is not known, from the investigations listed above, one can conclude that changed mechanical environments have an impact on bone matrix formation, mineralisation and resorption. Experimental data show that functional appliance treatment increases bone mineralisation in the mandible (Petrovic et al., 1990). Nevertheless, insufficient evidence supports the view that functional appliance treatment increases bone matrix formation. Moreover, the influence of functional appliance treatment on bone resorption has not been studied.

1.6.2. Hypothesis No. 4. Functional appliance treatment increases condylar growth by increasing both the life-span of bone cells and deposition of bone matrix during endochondral ossification

Bone cell differentiation rate can be measured by means of the life-span of cells. The normal life-span of human osteoblasts has been suggested to be 12 or 13 days (Parfitt, 1984; Jilka et al, 1998) and the life-span of osteoclasts, 9 or 10 days (Tsay et al, 1999).

The differentiation of osteoblasts and osteoclasts from partially differentiated marrow stromal cells of different lineage has been found to have interaction with each other. For example, the development of osteoclasts from hematopoietic support cells in hematopoietic lineage has been suggested to be dependent on osteogenic cells of mesenchymal lineage. Cytokines such as interleukin-6 and interleukin-11 produced by osteogenic stromal cells are critically responsible in osteoclastogenesis. However, consistent evidence for a specific "activating factor" is lacking, and the argument is presented that the isolated osteoclast resorption assays have not been shown to convincingly be the assays of osteoclast activation (Martin et al., 1994; Manolagas et al., 1995). Therefore, the factor or factors which may influence the differentiation of bone cells and which lead to change in the life-span have not been identified.

Recently, terminal deoxynucleotide transferase end-labels procedures and DNA fragmentation have been used to determine osteoblast life-span. Unfortunately, this method has not yet been used to study whether or not

increased compressive force would change the life span of osteoblasts or osteoclasts during functional appliance treatment. To date, no study address these questions. It is not clear if functional appliance treatment influences bone cell proliferation or differentiation by acting on any single marrow stromal cell lineage.

1.7. Re-evaluation of the Experimental Evidence for the Mechanical Environment and Changed Direction of Growth

An alternative to the hypothesis that functional appliance treatment increases condylar growth, other hypotheses have dealt with changes in the shape of the mandible during growth. This change may be induced either during endochondral ossification or during intramembranous ossification. In this section, two different hypotheses are evaluated regarding endochondral ossification and intramembranous ossification.

1.7.1. Hypothesis No. 5. Changed compressive force changes the pattern of angiogenesis resulting in the changed shape of the condyle

In Section 1.4 of this Chapter, the changed shape of mandibular condyle and the changed trabecular pattern within the condyle were introduced both in rodents and non-human primates. In this section we discuss angiogenesis as the factor associated with these changes.

Trabeculae are the products of endochondral ossification. The build up of trabeculae has been described by Frost and Jee (1994 a;b) as follows. In the growth plates, woven bone begins to form on the remaining vertical chondral septa to form the primary spongiosa. The trabeculae then become thicker, fewer and further apart than the vertical septa in the un-resorbed but mineralised cartilage above. The trabeculae must also be stiffer. Newly formed bone needs time to mineralise and fully stiffen. Then, an activation-resorption-formation-type of basic multicellular unit-based remodelling begins to replace the primary spongiosa with lamellar bone. The above authors also stated that the primary spongiosa progressively converts into secondary spongiosa made of lamellar bone. This remodelling continues as long as a secondary spongiosa exists, but at varying rates.

Bone matrix in both cortical bone and trabecular bone is the tissue component that carries mechanical loads. Trabecular pattern and its relationship with stress and strain have been extensively studied and reviewed. Recently, Teng and Herring (1995, 1996) have analysed the trabecular pattern in pig mandibular condyle reporting that the relationship between mechanical environment and trabecular pattern is strongly correlated. Whilst the influence of mechanical factors on the formation of the trabecular pattern is well accepted, another fact is that bone trabeculae are always built up parallel to already ordered blood vessels. In both normal and abnormal materials, the constancy of metaphyseal vascular and trabecular pattern in long bone growth plate is remarkable, and is not dependent on the form of the matrix bars in the growth cartilage (Thorp, 1988; Thorp & Duff, 1988).

In recent experimental functional appliance treatment using rats, the expression of vascular endothelial growth factor in the mandibular condyle has been reported to increase significantly. This suggests that angiogenesis is enhanced in the mandibular condyle during forward mandibular positioning (Leung et al., 2001).

In summary, one could explain changed trabecular pattern as being a result of compressive force influencing bone matrix deposition in the mandibular condyle. This process is modified by angiogenesis. During functional appliance treatment, the altered condylar-fossa relationship may increase compressive force in the posterior region of the condyle with a decrease in the anterior region. This changed compressive force presumably leads to decreased angiogenesis and less bone matrix deposition in the anterior region whereas increased angiogenesis and more deposition in the posterior region. Unfortunately, no study has been performed to validate this hypothesis.

1.7.2. Hypothesis No. 6. Changed mechanical environment of the TMJ following functional appliance treatment increases bone formation under the periosteum in some regions resulting in condylar change

A change in shape of the mandibular condyle may be induced during intramembranous ossification as well as by the formally mentioned endochondral route. The perichondrium covers the condylar cartilage and it blends uninterruptedly with periosteum. There is a very close relationship

between the periosteum and the perichondrium during condylar growth. In particular, partially differentiated progenitor cells, belonging to mesenchymal lineage are found under both periosteum and perichondrium (Ten Cate, 1989). Furthermore, it is well known that the periosteal cell population consists widely of potentially chondrogenic cells (Thorogood, 1979). In deed, Meikle (1973 (a) (b)) has reported from histologic studies that the mitotic cell compartment of the condyle is located in its sub-articular layer and this structure has been suggested to represent the periosteum, in contrast to the explanation of condylar growth known as "endochondral ossification".

Considering the cellular components of both periosteum and perichondrium, Koski and Ronning (1985) have suggested the growth of mandible should be studied as a whole and not to separate endochondral ossification from intramenbranous ossification. Their argument was based on the fact that the basic process of the growth leading to continuous production of secondary cartilage in the mandibular condyle, is the mitotic activity of the relatively undifferentiated cells of the periosteum that cover the mandible and its condylar process. From this viewpoint, it is possible that functional appliance treatment influences condylar growth by influencing the periosteum.

Based on electron-microscopic morphological observations of the periosteum in epiphyseal growth plates of long bones, it has been suggested that mechanical force does influence the bone morphology by affecting the periosteum (Tonna, 1974; Dysart et al., 1989) although the reactions of bone that vary depending on the bone structure.

In long bones, mechanical forces resulting from, say, ambulatory activity are thought to be transmitted to bones and articular cartilages by the periosteum acting as an intermediary. Periosteal tension has been widely studied as the controller (Frankenhuis-van der Heuvel et al., 1991). Changes in long bone growth rate were reported following changes in periosteal tension. For example, increased long bone growth rate after osteotomy and periostomy suggested that periosteum exerts a restraining force on the epiphyses (Crilly, 1972). Inhibited long bone growth rate following periostomy suggests that the functional demands of individual long bones may also modify the periosteum and might play a more dominant role in the growth regulation of the epiphyses than commonly acknowledged (McLain & Vig, 1983). These results suggests that periosteal tension might directly influence the cartilage cells in the growth plate of long bones and the periosteum has even been assumed to be in direct control of the proliferation of cells of the epiphyseal cartilage plate (Crilly, 1972; McLain & Vig, 1983).

The same should hold true for the mandible. It has been reported that condylar growth is also reduced after periostomy (Koski & Ronning, 1982; 1985) indicating the importance of functional demands in the growth and development of mandible. Furthermore, mechanical force seemed to influence the mandiblar morphology by affecting periosteum following periosteal elevation (Sa'do & Tashiro, 1989). The moderate manipulation of periosteal tension while the muscle and frenum were attached and presumably functioning, was also followed by a decrease in the mitotic activity of the condylar cartilage (Koski & Ronning, 1985) as noticed also after the removal

of the muscle and the frenum (Petrovic, 1982). These results suggest that periosteum could have an even more significant role in the growth of the mandibular condylar cartilage than the epiphyseal cartilage. In addition, the structure and orientation of the periosteum in the condylar area suggests that the periosteum might have a regulatory effect on condylar cartilage (Chong et al., 1982; Frankenhuis-van der Heuvel et al., 1991).

Based on these studies, Kuijpers-Jagtman et al. (1988) have recommended that the fibrous periosteum acts as a mediator for other local regulatory factors, and McLain and Vig (1983) recommended that the functional demands of individual long bones may modify the periosteal influence in long bones (Frankenhuis-van der Heuval et al., 1991). Koski and Ronning (1982; 1985) as well as Petrovic and co-workers (1981) have recommended that the periosteum, in one or more ways, is the closest local controller of the proliferation of pre-chondrolblasts in the condylar cell population.

Unfortunately, there is no conclusive evidence so far to support the hypothesis that functional appliance treatment controls condylar growth by controlling cell proliferation through the periosteum. Two questions make it difficult to use the concept of periosteal tension to explain the results of functional appliance treatment: The first direct question is “how do functional appliances change the periosteal tension of the mandibular condyle during treatment?” There is no methodology so far available to directly or indirectly measure periosteal tension during normal TMJ function nor is there methodology to measure the compression of the mandibular condylar

cartilage. A second fundamental question is “how does changed periosteal tension influence bone cell proliferation and differentiation in the mandibular condyle?” The studies cited above were all simple morphological observations at light or electron microscope level. No study has attempted to identify cell proliferation or differentiation during this process using labelling or staining techniques.

Based on the studies conducted by Stutzmann and Petrovic (1990), the retro-discal pad (including the temporomandibular frenum and meniscal portion) has been suggested to be the essential control mechanism for the periosteum being the mediator during the functional appliance treatment. However, the detailed mechanism was not specified.

1.8. Functional Appliances Mode of Action: An Ongoing Investigation

The purpose of this section is to evaluate the scientific rationale for functional appliance treatment in the correction of Class II malocclusion.

1.8.1. Theories about functional appliance treatment based on animal experiments

As reviewed in previous sections, functional appliances are thought to increase the length and/or change the direction of mandibular growth. Functional appliances, which change the occlusion in animal experiments, have been found to induce many changes in masticatory muscles, particularly the LPM. As reviewed by Stutzmann and Petrovic (1990), four lines of

evidence have suggested that the LPM plays an important role in the physiologic control of the condylar cartilage growth rate.

i. After surgical resection of the LPM in the growing rat, untreated or treated with a functional appliance, a relative decrease in the growth of the condylar cartilage was observed.
ii. Electromyographic (EMG) records of the LPM in monkeys treated with functional appliance showed increased electrical activity.
iii. Microelectronic stimulation of the LPM in the young rat produces an increased rate of condylar cartilage growth. The LPM was directly stimulated by means of intermittent electric shocks.
iv. After treatment with the functional appliance, there was a significant increase in the proportion of fast non-fatiguable fibres in the young rats LPM and a significant decrease in the number of serial sarcomeres in the same muscle. Thus, even without an increase in contractile activity, the mandible could be maintained in a more forward position.

Based on these studies, Stutzmann and Petrovic (1990) assumed that both increased cartilage thickness and increased bone deposition were induced by tensile force, generated by the LPM, applied to periosteum. These studies also led to the hypothesis that the LPM controls growth of the mandibular condyle through the covering tissues, including periosteum and perichondrium. The histological changes observed from animal experimentations were also attributable to increased compressive force on the mandibular condyle. This increased compressive force slowed the

differentiation of pre-chondroblasts leading to the thickening of cartilage in the posterior region of the mandibular condyle. Furthermore, this increased compressive force induced bone matrix deposition in the same region of the mandibular condyle resulting in a posteriorly rotated trabecular orientation.

1.8.2. Limitations of the lateral pterygoid muscle hypothesis

The change of compressive force seems to be unlikely to account for the observed remodelling response in humans since the retro-condylar region is generally assumed to be a non-functioning region in the human TMJ (Sicher & DuBrul, 1970).

In human subjects, it is still unclear which factor or factors induce TMJ tissue responses during functional appliance treatment. The lack of understanding is partly due to the difficulty involved in ethically studying the responses in human subjects. For example, using endosseous implant markers, serial cephalograms and histological examination are inappropriate. Moreover, although proper control of the environmental factors is of great importance, complicated individual variations arise from patient compliance, growth response and factors related to treatment planning (Woodside, 1998). Consequently, human studies usually have limitations of weak research design, small sample size, or incomplete collection of important information (Tulloch et al., 1990).

Clinical observations, implicate neural and muscular intercommunication that facilitates the functional role of the oral anatomic structures (Hictt & Gartner,

1987). Occlusion is the result of neuromuscular control of the masticatory system (Kawamura, 1967).

Unfortunately, for the experimental evidence showing the importance of the LPM in functional appliance treatment, other experimental evidence has indicated inconsistent changes in activity of the masticatory muscles following functional appliance treatment. Increased, decreased or unchanged muscle activities have all been reported. In non-human primates, an increased activity of the LPM and masseter muscles has been noted using EMG measured with bipolar implanted hook electrodes (McNamara, 1980). Conversely, a decreased activity of those muscles has also been reported following the use of a vary similar method (Sessel et al., 1990; Yamin-Lacouture et al., 1997). In human subjects, there are reports of an increased activity of the anterior temporalis and masseter muscles (Aggarwal et al., 1999) using EMG measured with bipolar surface disk electrodes, whereas no significant change was identified using maximum muscle protrusive force and fatigue time (Chintakanon et al., 2000 (a)).

The inconsistencies may be due to the difficulties in measuring the muscle activity. Previous studies have been focussed on the jaw protrusive muscles such as the LPM. The LPM is difficult to access beneath the overlaying structures and it is not possible to directly palpate clinically (Wanman & Agerbery, 1986). Any attempt to do so leads to the possibility of confusion with the sensitivity of the medial pterygoid or temporalis muscles (Johnstone & Templeton, 1980; White, 1985). Using EMG is generally invasive with minor to

severe consequences (Koole et al., 1990), further limiting its widespread use. When an alternative approach is used to assess a combination of all the protrusive muscles using maximum protrusive force and fatigue time, the possible change of muscle fibre type with treatment makes the maximum protrusive force and fatigue time less representative of the muscle activity (Chintakanon et al., 2000 (a)).

Another issue that leads to the inconsistency is related to the optimal time to measure the muscle activity. Changes in activity of masticatory muscles diminishes shortly after appliance insertion and before correction of the jaw relationship is achieved (Auf der Maur, 1980; Sessle et al., 1990; Hiyama 1996; Yamin-Lacouture et al., 1997). The difference in time when the measurements were performed could also contribute to the variation in the reported results.

1.8.3. To develop new theories to support our understanding of functional appliance treatment and related issues

Functional appliances have been reported to achieve correction of Class II discrepancy in human subjects by increasing the mandibular length (Pancherz, 1979; Vargervik & Harvold, 1985; Illing et al., 1998) and by rotating the mandible (Williams & Melson,1982; Birkebaek et al., 1984). It has been suggested that these changes result from stimulation of mandibular condylar growth beyond that which would occur in normal developmental growth as well as redirection of condylar growth from an upward and forward vector to a more posterior orientation (Woodside, 1998).

With mounting evidence disproving the LPM hypothesis (Chintakanon et al., 2000 (a)), other hypothesis have been formulated to explain the factors that induce the TMJ tissue responses during functional appliance treatment. One example has been given by Voudouris and Kuftinec (2000). They claim that growth pattern modification during functional appliance treatment involves condyle displacement and active relocation of the fossa. All the non-calcified tissues were thought to be involved in determining the TMJ remodelling. These tissues included synovial fluid, retro-discal tissues, LPM perimysium and the fibrous capsule, as well as TMJ tendons, ligaments and bodily fluids.

So far, no theory is perfect but should be based on two clinical questions. The first being; is this patient suitable for dentofacial orthopaedic treatment using functional appliances? The second being, if functional appliance treatment is selected, how it will work? In answering the first question, problems are encountered when the growth potential of the facial skeleton is the decisive factor for using either dentofacial orthopaedic treatment, a surgical approach or dento-alveolar masking of the underlying skeletal discrepancy. Growth potential, which determines the skeletal growth adaptability, has long been thought to be important in dentofacial orthopaedic treatment. Knowing the growth potential of a patient is very helpful in determining when to start the treatment, how long the treatment will take and if the outcome will be beneficial.

The second question deals with the quality of the clinical management and was introduced in section 1.2.4. *Efficacy* of the treatment deals with its

capacity for growth modification. Knowing this will help clinicians to define the appropriate time to start the intervention. The growth modification is more likely to succeed during a fast growing period. The theory should be able to provide clinical guidance, e.g., biochemical markers, to estimate the bone developmental stage in the patients.

Effectiveness of the treatment deals with the magnitude of the achievable growth modification. Knowing this will help clinicians determine the treatment objectives. Since the growth modification effects consist of dentoalveolar changes and skeletal changes. Theory should be able to quantify the achievable skeletal changes based on the estimate of bone developmental stage of the patient.

Efficiency of the treatment deals with the time frame for the growth modification. Knowing this will help clinicians predict over what period the treatment objectives can best be achieved. Individual variation in bone development requires individualised treatment planning designed for each specific patient. A theory should be able to aid prediction of the length of time required for the treatment correlated to the bone developmental stage for each individual patient.

The development of such theory requires knowledge of the adaptations in bone and all the soft tissues along with their interactions in the TMJ. With knowledge of the TMJ hard tissue gradually accumulating, there are still a few important questions left.

First, during functional appliance treatment, does the altered condylar-fossa relationship increase the compressive force in one part of the condyle and decrease it in another? Does this changed compressive force lead to accelerated pre-chondroblast differentiation in one region and slow cell differentiation in another? Does this change lead to increased cartilage thickness? The main question is whether or not functional appliance treatment increases the total number of pre-chondroblasts?

Second, is the rate of differentiation changed in pre-chondroblasts, whether or not cartilage matrix synthesis is increased, and is this associated with prolonged life-span of the pre-chondroblasts?

Third, increased new bone formation in the TMJ has been found in human subjects during functional appliance treatment observed with MRI. In addition, increased bone matrix mineralisation has been reported in animal experiments using histological techniques. The question is whether the treatment increases the quantity of bone matrix formation and what is the impact on bone resorption?

Fourth, do functional appliances have any impact on the life-span of osteoblasts and osteoclasts within the TMJ? Will this change the total quantity of bone matrix formation?

Fifth, is the changed trabecular pattern a result of changed blood vessel invasion during endochondral ossification?

Sixth, what is the mechanism controlling the intramembranous ossification during functional appliance treatment and what is the role of tensile forces.

To link these fundamental questions to clinical practice, the questions can be summarised according to whether functional appliance treatment increases the quantity of bone formed during treatment, or whether treatment changes the distribution of bone. The former suggests that functional appliance treatment creates additional growth, whereas the later suggests that the treatment only changes the shape of a structure. Perhaps both occur? The answer to these questions on the responses of TMJ tissue to functional appliance treatment can best be derived from animal experiments.

1.8.4. Objectives and hypotheses to be tested

General Objectives

The general objectives of this project are listed below.

First, to test the effectiveness of a functional appliance specially designed for sheep and to clarify whether or not forward mandibular displacement in sheep is associated with accelerated and/or redirected condylar growth.

Second, to evaluate the sheep as a model for dentofacial orthopaedic research by comparing the mandibular condylar growth in sheep and humans.

Third, to investigate in detail the position of the mandible during forward mandibular posturing and the effects of mandibular forward displacement on modelling and remodelling of the mandibular condyle.

Specific Objectives

Six questions were listed in the previous section regarding the development of a theory to guide the clinical practice of dentofacial orthopaedics. Due to time limitations, this project focuses on the 3rd and the 4th questions involving investigation of the bone responses to the functional appliance treatment.

The prime objective of this project is to answer the question: “Does functional appliance treatment increase the quantity of bone formed during the treatment, or does the treatment change the distribution of bone? Or both?” Based on the answer to this question, it can be determined which step of bone formation (bone matrix formation vs. bone matrix mineralisation) can be used to detect the skeletal changes induced by the functional appliance treatment.

This project will test the following null hypotheses:

(i): functional appliance treatment has no effect on bone matrix formation, bone matrix mineralisation or bone resorption in the TMJ.

(ii): functional appliance treatment has no effect on the mineralisation lag time. Mineralisation lag time is quantitatively equal to the life span of the osteoid which is the same as the life span of active osteoblasts, and is the time period

from which the osteoblasts start to produce osteoid untill the osteoblasts become trapped within the mineralised bone matrix and some osteoblasts undergo apoptosis. Therefore, the change in the mineralisation lag time implies the changed life span of active osteoblasts (detail is provided in *Chapter 2*).

Chapter 2
Materials and Methods

Part I: Rationale of the Methodology

2.1. Analysis of the Mandibular Position

Functional appliances have been found to change the position of the mandible (Lund & Sandler, 1998; Tulloch et al., 1997). The purpose of this section is to introduce the methods used to measure the mandibular position.

2.1.1. The limitations of the interpretation of conventional two-dimensional cephalometry

In orthodontics, the facial skeleton is usually observed radiographically using cephalograms. As reviewed by Quintero et al. (1999), there are several sources of error in conventional two-dimensional (2-D) cephalometry. They include *internal orientation error*, *external orientation error*, *geometric error*, *association error* and *landmark identification error*.

Internal orientation error refers to the three-dimensional (3-D) relationship of the patient relative to the central X-ray beam. The error becomes minimal when the head position is specific and consistent. *External orientation error* refers to the 3-D spatial relationship or alignment of the imaging device, patient stabilising device and image recording device. It is assumed that minimal error can be achieved if the X-ray source is 60 inches (152.4 cm) from mid-cephalostat, with the central beam passing through the ear rods and the beam parallel to the horizon and perpendicular to the film plane. *Geometric error* refers to the differential magnification created by projection

distance between the imaging device, recording device and the patient's head as a 3-D object. *Association error* refers to the difficulty in identifying the same point in two or more cephalograms acquired from different points of view. The same landmark may be viewed in a different location due to change in its magnitude when the X-ray is projected from a different angle.

Landmark identification error refers to difficulty in identifying the anatomical landmarks on the facial skeleton resulting from the lack of well-defined outlines, hard edges and/or shadows. Trpkova et al. (1997) have summarised the errors associated with the landmark identification using a meta analysis. They found point B (B), point A (A), pterygomaxillary fissure inferior (Ptm), sella (S), and gonion (Go) have high reproducibility and are considered to be reliable landmarks which can be identified from lateral cephalograms. However, based on a meta-analysis of 6 studies trying to identified the error of 15 landmarks, they suggested the accepted total error computed from the meta-analysis for the *x* and *y* coordinates were 0.59 mm and 0.56 mm respectively. They also recommended that in clinical practice, a separate analysis to estimate the errors of the landmark identification is a prerequisite.

Although errors cannot be eliminated from conventional cephalometry, efforts have been made to increase the resolution of the cephalograms, especially in the TMJ region, in order to reduce the landmark identification error. Hickman et al. (1996) have introduced one method using a dual sensitivity screen-cassette system. They claim that by using this system, the reproducibility of the measurement is significantly increased for 7 selected landmarks including

S, N, orbitale (Or), Pog, gnathion (Gn), menton (Me) and Go. When the error was measured as “range-of-variability” (ROV), which is the 95% confidence interval of the standard deviation, the error for the *x* and *y* coordinates were reduced to 0.34 mm and 0.40 mm respectively.

During the landmark identification of the present project, the author assigned errors of 0.56 mm to 0.59 mm to be too large and, in addition errors of 0.34 mm to 0.40 mm were also inadequate. Therefore, anatomical landmarks were not selected for analysis in this study. Instead, endosseous implants were to be used as artificial landmarks. The detail will be introduced in the next section.

2.1.2. Innovative approaches to three-dimensional craniofacial imaging methods using conventional two-dimensional cephalometry

In order to deal with the errors inherent in 2-D cephalometry and maintain the use of the equipment designed for such cephalometry in a current clinical setting, innovative approaches have been made to institute 3-D measurement. Spolyar et al. (1993) introduced an early method called “image corrected cephalometric analysis (ICCA)” where by it is possible to achieve an accurate analysis of displacement and growth. Comments were made by Rune (1993) on the stability of implant markers. Spolyar’s response to this comment was there was no technique which could ensure that the implants were stable, and re-affirmed that bone implants would be the most beneficial method over a short study period (Spolyar, 1993).

Recently, by combining digital technology with modern stereo photogrammetry, new software has been developed by Hatcher and co-workers (Acuscape^{TM}). This software can assist in identifying the same feature on more than one cephalogram and allows for 3-D measurement of the distance between those features. (Quintero et al., 1999). Being new computer software, no work using this technique has been published so far.

2.2. Analysis of Bone Architecture in the Mandibular Condyle

The purpose of this section is to introduce the methods used to measure the bone structures assessed in this project, with particular emphasis on the reliability and statistical methods.

2.2.1. The measurement error in bone histomorphometry

In general terms, histomorphometry, implies measuring dimensions of organic structures and counting and measuring their elements by observing tissue sections at the microscope level. There are two methods in bone histomorphometry: the traditional manual analysis measurement technique and the computerised image analysis system. In manual analysis measurement, test grids and test lines are used to measure the distance or length of trabeculae and to count intercepts of test lines with bone trabeculae. Trabecular bone surface and area can be calculated based on these counts and measurements (Fazzalari et al., 1983; Baddeley et al., 1986). In the computerised image analysis system, images are first constructed on a computer screen by pixels which are the smallest resolvable areas detected

by the video camera or microscope. The desired parameters can then be calculated directly by computer (Fazzalari, 1980).

As with other quantitative analysis, in this system error will occur from incorrect discrimination between two different tissues. Such error consists of systematic error, from equipment, etc, and random error, which may be caused by incorrect judgement made by observers, etc. Within these two kinds of errors, random error can be observed by the variability of observations. This variability is concerning since it directly influences the reliability of the results obtained from these analyses.

In order to study the variance within one single observer using the same methods, Compston et al. (1986) double checked 6 biopsies with intervals ranging from 1 to 18 months. The following parameters were compared; total trabecular bone volume (TBV), relative osteoid volume (OV), relative osteoid surface (OS), mean cortical thickness (MCT), mineral appositional rate in trabecular bone (MAR), fractional labelled surfaces (S fract(lab)) , fractional double labelled surfaces (S fract(d lab)), fractional single labelled surfaces (S fract(s lab)), total resorption surface (TRS) and mean osteoid seam width (WOS). The percentage variation (Variance%) and coefficient variation (CV%) were used together with the mean and standard deviation (SD). They found parameters related to bone tissue, such as TBV, MCT and MAR, had smaller variance compared with other osteoid related parameters. The CV% of TBV, MCT and MAR was 3.8% to 9.4%, 3.8% to 4.0% and 3.1% respectively. The largest CV% was found in TRS as 15.6% to 21.9%.

The variance between observers is also not negligible. In order to study the variance between different observers using the same method, Compston et al. (1986) double checked 20 biopsies with two independent observers. TBV, OV, OS, MCT, MAR, S fract (lab), S fract (d lab), S fract (s lab) TRS and WOS were compared. Variance and CV% were used together with mean and SD. They found the variance was larger compared with the variance within one single observer. Parameters related to bone tissue, such as TBV, MCT and MAR, also had smaller variance compared with osteoid when related to other parameters. The CV% of TBV, MCT and MAR was 10.2%, 14.2% and 8.5% respectively. The largest CV% was found in OV as 68.7%.

Inter-observer variability has been further studied by White et al. (1992) using both a manual image analysis system and a semi-automatic computer image analysis. Bone area (B.Ar), osteoid width (O.Wi) and osteoid perimeter (O.Pm) were analysed and it was found that in both of the comparisons the variance of B.Ar was smaller than the other two parameters with CV% at 17.01%. The variance of O.Pm was the largest with CV% at 39.47%.

In order to study the variance between manual and computerised methods, Chavassieux et al. (1985) analysed 100 biopsies for the parameters of TBV, trabecular resorption surface, trabecular osteoid surface, trabecular osteoid volume thickness index of osteoid seams and calcification rate. As with the previous studies, TBV was found to have the smallest variance amongst others (CV% = 6.2%) whereas osteoid volume was found to have the largest variance (CV% = 25.2%). In order to study the difference between two

different staining processes, Wright et al. (1992) studied B.Ar, O.Wi and O.Pm in original sections and re-stained sections using a semi-automatic computerised image analysis system. They also found that the variance of B.Ar was smaller than the other two parameters with CV% at 24.1%. The variance of O.Pm was the largest with CV% at 73.4%.

Based on the above studies, it is concluded that bone volume (BV/TV) has a relatively small variance both between methods and observers when systematic error and random error are taken into account. As stated by Chavassieux et al. (1985) and Wright et al. (1992), bone parameters, which can be easily discriminated by observers, have relatively low variation compared with osteoid parameters which are hard to discriminate. Poor differentiation of osteoid structures possibly leads to the inaccuracy and creates a larger variability.

2.2.2. Image magnitude and contrast in bone histomorphometry

Together with the variance caused by system error and random error, another source of variance was found when sections were analysed with different magnification. Parkinson and Fazzalari (1994) observed two sections at 8 different magnifications, which included 5 microscope settings (×32, ×64, ×160, ×320, and ×640) and 3 macro-viewer settings (×4, ×9, ×13, or ×4, ×8, ×12). They found that mineral bone surface (BS/TV) increased as the magnification increased but trabecular thickness (Tb.Th) decreased as the magnification increased. The measured mineral bone volume (BV/TV) had no

change. A single specific magnification was recommended for all comparative studies for trabecular bone.

Without a high contrast image, error may occur by inaccurately discriminating bone from marrow. Unfortunately, for sections stained with other kinds of techniques with poor contrast, such as H&E, and when they are not able to be re-stained, manual editing of the image becomes compulsory. Measurement errors will definitely exist with manually edited, non-binary images especially for beginners.

In summary, in histomorphometric image analysis, both manual and computerised, binary images are necessary. High contrast between bone and osteoid makes it easier to discriminate these two structures. Binary imaging seems more important for computerised image analysis systems, since high contrast of the image helps the computer detect bone correctly and to construct the structure of bone by pixels. Van Gieson staining for decalcified sections and von Kossa staining for undecalcified sections are currently used to produce binary images. Manual editing of images is almost unnecessary with the discrimination of these two stains.

2.2.3. Statistical procedures to compare the data obtained from control and experimental groups

All the parameters measured using histomorphometric methods have been compared between control and experimental groups, according to the principles given by Zar (1996).

The parametric test used in this project was Student's *t*-test. As reviewed by Zar (1996), the *t*-test for two independent samples assumes that both samples come at random from a normal population with equal variances. While there is no evidence, so far, to validate the data in our project following this assumption, Zar pointed out that numerous studies have shown that the *t*-test is, nevertheless, robust enough to stand considerable departure from this assumption, especially if the sample size is equal or nearly equal, and especially when two-tailed hypotheses are considered. The two variances were compared before conducting the *t*-test in this project. However, considering the variance-comparison test performs so poorly when the distribution is not normal, the *t*-test was used in both the circumstances of an equal or un-equal variance was detected.

Without knowing the distribution of the data, non-parametric tests were also used in this project, and the results were compared with those obtained from *t*-tests. The reason to do this was to compare the outcomes from these two different statistical methods and to explore the nature of our data. If the results were the same or similar, the assumptions that both samples were selected at random from a normal population with equal variances was accepted.

Part II: Animal Experiment: The Protocol

In order to clarify the procedure of the animal experiment, detailed protocol is provided in this section.

2.3. The Detail of the Protocol

2.3.1. *Outline*

Twelve, 4-month old, castrated male Merino sheep were purchased from the Veterinary Services Division of The Institute of Medical and Veterinary Science (IMVS) and randomly assigned to 2 experimental groups or control group with 4 in each group: protrusive appliance, in-activated appliance and control. During the experimental period, the animals were kept at the animal house of the IMVS. Each animal was kept in an individual pen for about one week after surgical placement of the endosseous implant markers. The detail of the surgery will be described in section 2.3.2. Subsequently, animals were kept as a group in a large pen. All animals were fed with lucerne chaff during the experimental period.

Dental casts were manufactured for the animals both in the experimental groups and in the control group at the beginning and at the end of the experiment. See section 2.3.3. The animals in the experimental groups received functional appliance treatment. See section 2.3.4. The experimental period was 15 weeks. Cephalograms and weight of each animal were taken every five weeks. See sections 2.3.5. and 2.3.6 respectively. Three

fluorescent bone labels were administrated to all the animals. See section 2.3.7. This experiment was approved by the Animal Ethical Committees of Adelaide University and the IMVS (approval number: 69/99).

2.3.2. *Surgical placement of the implant markers*

Four stainless steel implants were surgically placed in all animals as radio-opaque markers on each side of the head; one near the chin, one at the gonial angle, one near the mandibular condyle and one in the zygomatic process of the temporal bone (Figure 2.1.). In order to minimise the impact of the surgery and the impact of the implants on bone growth, the periosteum was sutured to cover the implants. In some cases the incision in the periosteum could not be closed directly, requiring the periosteum near the implant to be minimally elevated using a periosteal elevator to release the tension.

In order to reduce the variability of the surgical procedure caused by the differences of the operator's skill, most of the animals were operated by the author with the assistance from two senior research fellows with the similar training background. In 2 animals, the operations were performed by only the author. The duration of the operations were recorded for each animal. The median and range as well as the mean and standard deviation of the operation time were calculated. The operation time was then compared between the control and the experimental group.

Figure 2.1. Surgical placement of the implants. During the surgery, an implant was first placed in the zygomatic process (Zy), then in the condyle (Co), followed by the gonion (Go), finally in the chin (Ch). Implants were placed bilaterally.

2.3.3. *Dental casts*

In order to monitor changes in the dentition, full arch impressions were taken for all sheep under general anaesthesia using silicon impression material (Kerr®, U.S.A.). The animals were postured to make their occlusal plane parallel to the horizontal plane when the impressions were taken. A pair of universal trays were made, based on a dry skull, and designed to fit all the dental arches. An individual tray was only made if obvious impairment between the universal tray and the dental arches was observed. In fact, individual trays were made for 4 animals. Vacuum suction was used while the impressions were taken to avoid suffocation. Plaster models for each animal were obtained at the beginning and again at the end of the experimental period. The models were then trimmed to standard shape (Figure 2.2).

Figure 2.2. Standard dental casts were used to study the change in occlusion during growth and functional appliance treatment.

2.3.4. *Fabrication and placement of the appliance*

One pair of refractory models was duplicated for each experimental animal. Because the upper arch in the sheep is wider than the lower arch and the teeth cannot occlude on both sides simultaneously, both the upper and lower refractory models were separated along the midline. The occlusion of the left or right sides was established according to the pattern of tooth attrition. Completed refractory models were mounted in a semi-adjustable articulator. In the protrusive appliance group, protrusive occlusion was achieved by setting the upper member of the articulator horizontally backward for approximately 4 millimeters. The wax pattern of the appliance was then constructed at the protrusive position with minimal occlusal interference. In the in-activated appliance group, the wax pattern of the in-activated appliance was constructed at the centric occlusion with minimal occlusal interference. Finally, the cast appliance was fabricated using chrome cobalt dental alloy (Dentaurum®, Germany) (Figure 2.3.). The appliance was cemented using glass ionomer cement (GC Fuji IX®, Japan) about one week after the impressions were taken. The total treatment period was 15 weeks.

Although 3 groups were initially designed, unfortunately, two of the four animals in the inactivated-appliance group died during the experiment. One died from aspiration on the first day after the implant markers were inserted. The other died five days after the insertion of the appliances from not intake fluid voluntarily. With the mortality abnormally high, in this group we thought to include the rest two animals in the analysis was inappropriate, and we decided to analysis the data only from the control and the experimental groups. In the following sections of this thesis, only the protrusive appliance group and the control group are discussed and they are named as the experimental and the control groups respectively. Nevertheless, the animals in the in-activated appliance group provided great opportunity for accumulating experience in manufacturing the appliances.

2.3.5. *Cephalograms*

Cephalograms were taken under general anaesthesia with the sheep head fixed in a specially designed cephalostat (Figure 2.4.), which included a head frame for assisting calibration (A) and a holder to stabilise the sheep head and the head frame (B). The anaesthetic tube was set to deliberately pass through the edentulous space to ensure occlusal contact.

Figure 2.4. Cephalostat designed for sheep. A: Head frame with metal markers assisting calibration. B: Holder to stabilise the sheep head and the head frame.

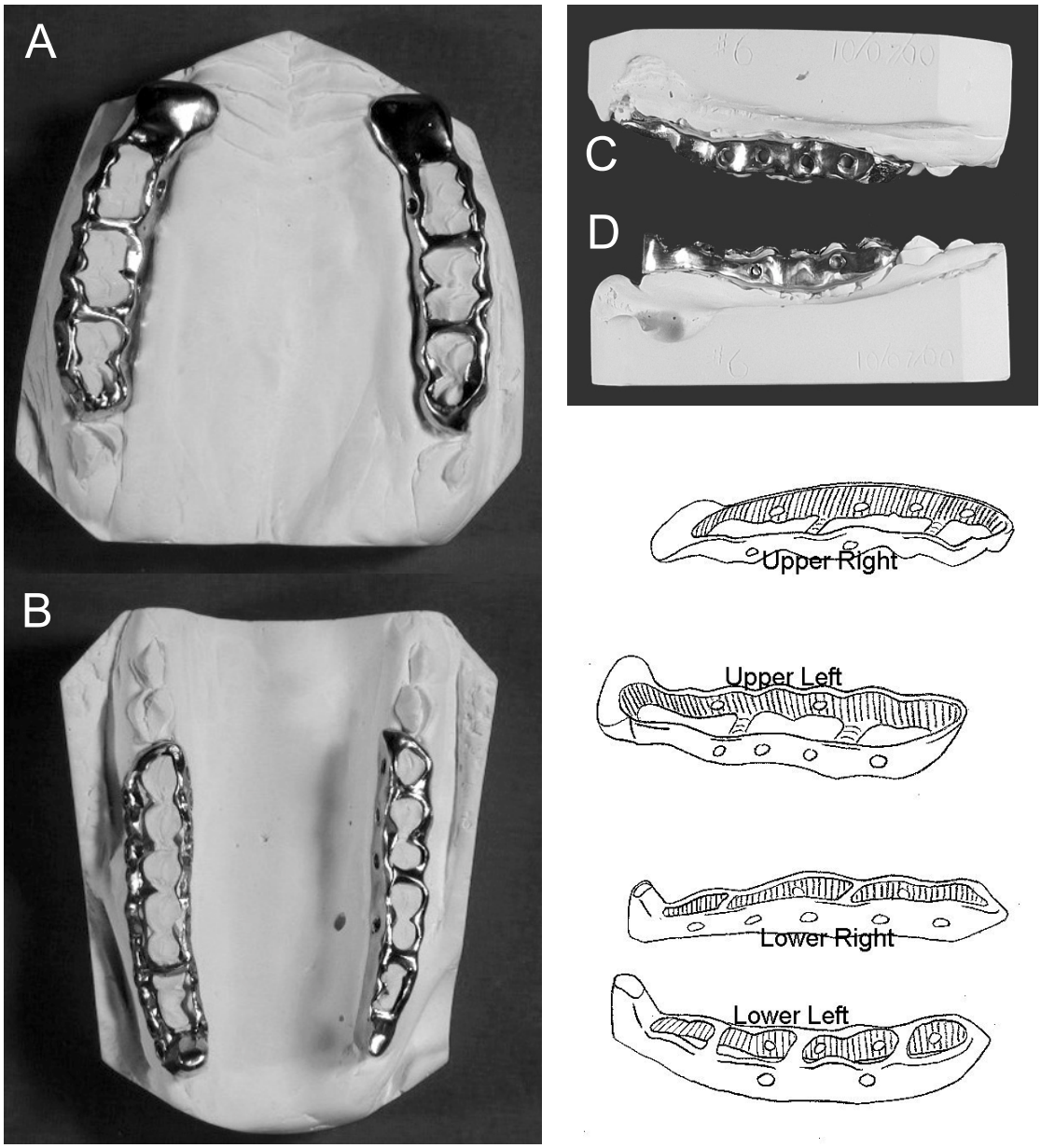


Figure 2.3. The appliance designed for forward mandibular displacement in sheep. A: Occlusal view of upper components. B: Occlusal view of lower components. C: Lateral view of upper components. D: Lateral view of lower components. Lower Right: Schematic presentation showing the relative position of the quadrants.
The in-activated appliance was constructed following the same principle, but no ramps were constructed.

In this study, an innovative method of 3-D craniofacial imaging using conventional 2-D cephalometry was used. The basic concept of this method is to view the same object cephalographically from 3 different angles. The shape of this object must be different among the 3 cephalograms due to the angle of projection and the magnification of the object. Of more importance, the magnification of different parts of the object differs according to their distances to the film. The actual size of the object and the magnified size of the object that we observed from the cephalograms follows strictly the principles of photogrammetry, and the actual size of the object can be calculated based on a reference body with its actual size and shape is known.

The reference body used in this study is the head frame. The key issue of this method is to maintain the sheep's head position unchanged within the frame while taking the 3 cephalograms. In this study, during taking the cephalograms, the anaesthetised sheep was stabilised onto a portable platform ensuring the head position was absolutely unchanged during the taking of the 3 cephalograms. The sheep was rotated or elevated in order to adjust the head position to meet the requirement for each cephalogram.

The procedures for taking the 3 cephalograms is illustrated in Figure 2.5 including two lateral view cephalograms and one vertex view cephalogram. Viewed from above, a sheep has a V-shaped mandible with two relatively straight halves jointed at the symphysis. The 2 lateral view cephalograms were designed to arrange one half of the mandible parallel to the film. This enables to reduce the distortion of the image for each side at each time. In

order to facilitate viewing of the left and right TMJs separately, the central beam ten-degree oblique to the mid-sagittal plane was introduced. Based on a previous study, ten-degree oblique was chosen (Chintakanon, 1999). Radiographically, objects close to the film are less magnified and have clearer boundary than those further apart from the film. Ten-degree oblique of the central beam also facilitates differentiation of the right TMJ from the left by comparing their sizes and boundary.

One set of cephalograms for each animal in the experimental group was taken before and after the appliances were placed. Serial sets of cephalograms were taken monthly for each animal in both groups.

All the cephalograms were obtained under supervision of the author using the same radiographic machine and cephalostat. The focal spot to film distance was 150 cm and the object (mid-sagittal plane) film distance was 10.5 cm. The focal spot to object (mid-sagittal plane) distance was, therefore, 139.5 cm. The voltage used for the lateral view cephalograms was 60 kV (10 mA). The voltage used for the vertex view cephalograms was 66 kV (15 mA).

The anaesthetised sheep was stabilised onto a portable platform ensuring the head position of sheep was absolutely unchanged during the taking of the three cephalograms. The sheep was rotated or elevated in order to adjust the head position to meet the requirement for each cephalogram (Figure 2.5).

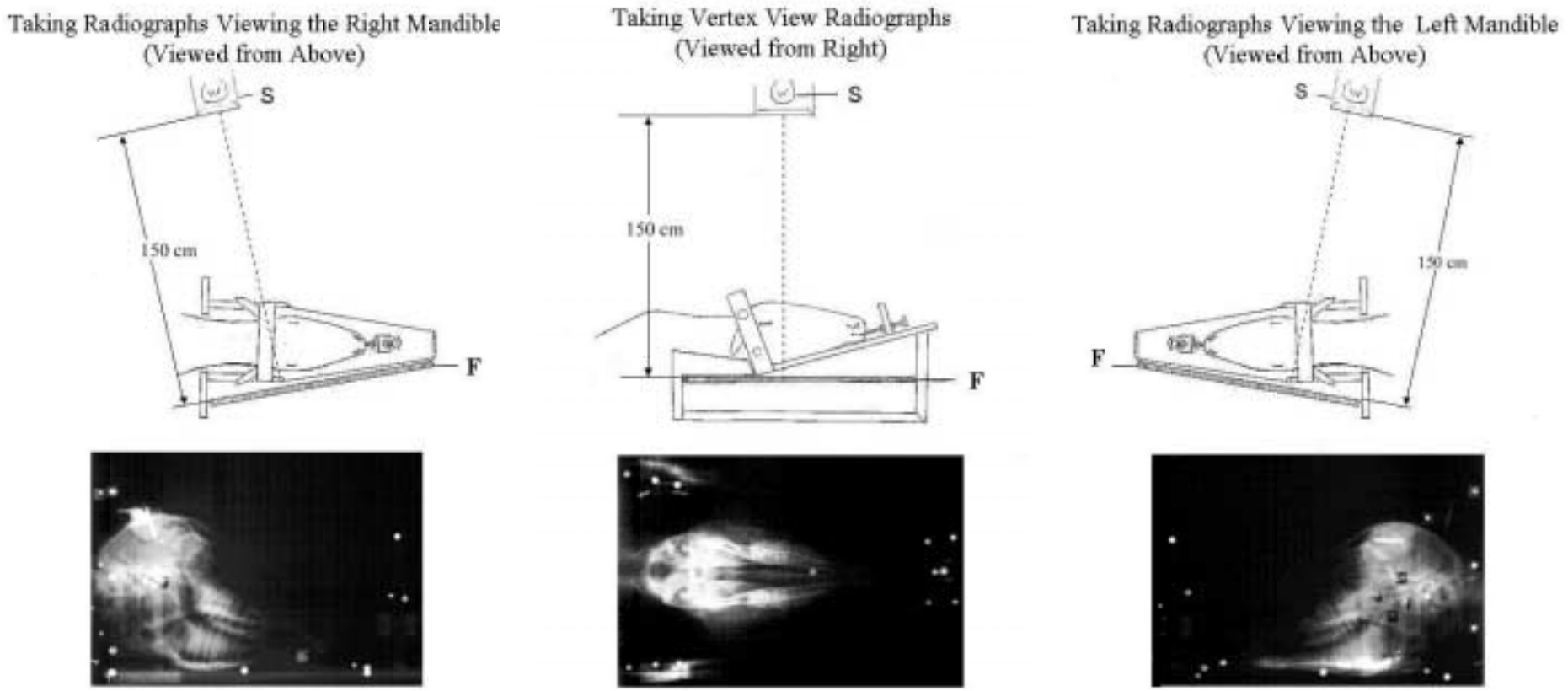


Figure 2.5. Taking three view cephalograms with the assistance of the cephalostat
S: X-ray source; F: Film holder

Based on observations from the dental casts, the control animals had a tooth wear pattern with sharp cusps in one arch interlocked with the ridges in the opposite arch. Wear patterns were seen in the experimental animals on the casting appliances soon after the appliances were placed. The occlusion of the sheep was determined according to wear pattern, i.e., tooth wear patterns in the control animals and appliance wear pattern in the experimental animals. For the newly placed appliance, the occlusion was determined according to the bite registration used in the appliance construction. Once the occlusion was achieved, the jaw relationship of the sheep was stabilised with a bandage around the jaws while the cephalograms were taken. In the experimental group, the appliances were not removed when the cephalograms were taken.

All of the eight implant markers were used as landmarks. They included the implants in the zygomatic process, mandibular condyle, gonial angle and chin, for both sides. The quality of the cephalograms was assessed by reference to the visibility of all the markers. The animals were not released from the platform until the films were processed and seen to be acceptable. On very few occasions the implants on the cephalogram were obscured by other metal objects, e.g., ear-tags, the end of endotracheal tube used for general anaesthesia. These cephalograms were retaken to ensure the visibility of the implants.

2.3.6. *Monitoring the weight gain of the animals*

Weight gain was used as an indicator of the animals' growth and development of in this study. The live weight (Butterfield, 1988) of each animal was

recorded every 5 weeks during the experimental period. All measurements were made at 9am (±30 minutes). Since the fleece contributes to the sheep's total weight by a few kilo-grams and it varies among animals, the sheep were maintained un-showered and un-shorn.

Because it is generally believed that a surgical operation is a significant factor influencing the growth in sheep, correlation of the weight gain and operation time was investigated.

2.3.7. *Fluorochrome administration*

At the beginning of the study, the fluorochrome bone marker calcein label, was given intra-muscularly to all the animals (5 mg/kg; Sigma Aldrich®, Germany). Tetracycline label (25mg/kg; Intervet Pty Ltd ®, Australia) and alizarin red S label (30mg/kg; Ajax Chemicals ®, Australia) were given intra-muscularly to all animals at 14-and 3-days, respectively, prior to sacrifice. The protocol for the animal experiment is summarised in Chart 2.1. Contingency procedures were instituted to deal with any infection after surgical placement of the implants as well as damage or loss of any appliances during the experimental period.

2.4. Tissue Sample Collection and Processing

2.4.1. *TMJ tissue sampling*

Animals were killed by intra-venous injection of Lethabarb® 0.5 ml/kg body weight. Subsequently, the gross dissections of the TMJ were performed

parallel or perpendicular to the occlusal plane to include the complete glenoid fossa, condylar process and articular eminence.

Chart 2.1: Flow-chart of the Animal Experiment

	Experimental Group	**Control Group**
Initial Procedure	Weight Impression Surgery ↓ (Construction of the appliances) ↓	Weight Impression Surgery Cephalogram (#1) ↓ ↓ ↓
Day 1 of Treatment	Cephalogram (#1) Cement the Appliance Cephalogram (#2) Calcein Injection ↓	↓ ↓ ↓ Calcein Injection ↓
5 Week in Treatment	Weight Cephalogram (#3) ↓	Weight Cephalogram (#2) ↓
10 Week in Treatment	Weight Cephalogram (#4) ↓	Weight Cephalogram (#3) ↓
13 Week in Treatment	Tetracycline Injection	Tetracycline Injection
15 Week in Treatment	Alizarin Red Injection Weight Impression Cephalogram (#5) Sacrifice	Alizarin Red Injection Weight Impression Cephalogram (#4) Sacrifice

Fixation in 70% ethanol:formal-saline for three days occurred immediately after dissection. Each TMJ block was then sagittally separated into lateral, central and medial parts using a low speed diamond saw (Buehler®, U.S.A.) at approximately 120 rpm. The 1st sagittal separation was made along the midline of the coronoid process. The 2nd separation was made 7-8 mm medially from the 1st section. A pilot study indicated that TMJ tissue less than 8 mm thick can be satisfactorily embedded with methylmethacrylate (MMA). Therefore, excess soft and hard tissues on the lateral side of the lateral block

and the medial side of the medial block were removed to achieve approximately 7-8 mm in thickness for each of the three parts (Figure 2.6.). Efforts were made to ensure the boundaries of the specimens were parallel to those previously made by the band saw. This ensured the maintenance of the boundaries of the specimens parallel or perpendicular to the occlusal plane.

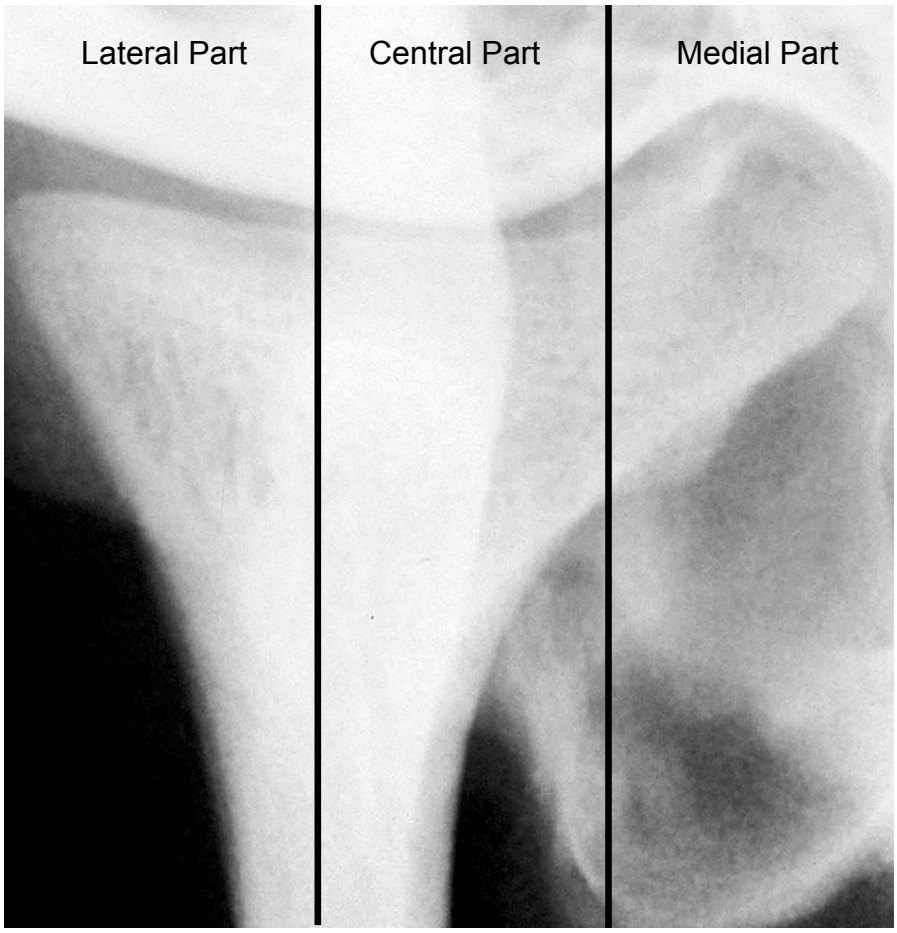


Figure 2.6. Standard separation of the temporomandibular joint.

Tissue samples were then dehydrated in graded ethanols (70%, 95% and 100%) for at least one day each and defatted in acetone. In this study, the purchased MMA (BDH Laboratory Supplies®, U.K.) was stabilised with 0.01% quinol. This, quinol was washed out with 5% sodium hydrochloride. After removing the water in the washed MMA with sufficient amount of dried

calcium chloride (granule size: ϕ1.5-2.5 mm; Ajax Chemicals ®, Australia), the tissue samples were embedded in MMA with the polymerisation induced by Perkadox 16 (Dobbs Ferry ®, USA). The detailed procedure for MMA embedding of the TMJ tissue is given in Table 2.1.

Table 2.1. The procedure for MMA embedding of the TMJ tissue.

Medium	Condition	Time
70% Ethanol	1st change	1 day
95% Ethanol	1st change	1 day
95% Ethanol	2nd change	1 day
95% Acetone	1st change	1 day
95% Acetone	2nd change	1 day
100%Acetone	1st change	1 day
100%Acetone	2nd change, under vacuum	1 day
100%Acetone	3rd change, under vacuum	1 day
MMA+100%Acetone(1:1)	under vacuum	3 days
MMA+Polyethylenglycol400(10:1)	under vacuum	7 days
MMA+Polyethylenglycol400(10:1)	+ initiator at 37 °C	1 day

Undecalcified sections, 5-7μm thick, were cut from the lateral surface of the central block using a microtome (Polycut®, Germany). The sections were then stained with von Kossa or von Kossa/haematoxylin-eosin (H&E). Undecalcified sections, 10 μm thick, were also cut from the same block for analysis and left unstained for fluorescent microscopy.

The von Kossa staining used in this study was performed as follows:

i. Bring MMA embedded sections to distilled water and wash with three changes of water.
ii. Place in 5% aqueous solution of silver nitrate and expose to ultra-violet light for an hour.
iii. Wash with three changes of distilled water.
iv. Rinse in 5% sodium thiosulphate to remove excess silver nitrate.

v. Wash with three changes of distilled water.
vi. Spread sections on glass slide, press firmly with a pair of clips and dry in an incubator at 37 °C for 24 hours.
vii. Dissolve MMA with 100% acetone for 10 minutes.
viii. Clear in xylol.
ix. Mount in Histomount ®.

The von Kossa/H&E staining used in this study was performed by counterstaining the sections with 10% haematoxylin for 10 minutes at the stage “v” and then thoroughly washing in distilled water for three changes. Finally the eosin staining was completed at stage “vii”.

2.4.2. *Metacarpus tissue sample*

In young sheep, histomorphometric analysis of the metacarpus has been recognised to be reliable in assessing overall skeletal growth (Pastoureau et al, 1991). Therefore, the left metacarpus was also collected to monitor bone growth. The appearance of a fresh specimen is presented in Figure 2.7. Unstained 10-micrometre thickness mid-sagittal sections from each TMJ and metacarpus in the standard region were cut on a microtome (Polycut®, Germany) for examination with fluorescent and light microscopy.

2.5. Linear Measurements from Histological Sections

2.5.1. *Condylar cartilage thickness*

Three mid-sagittal sections of each condyle were selected and the total

thickness of the condylar cartilage was measured for the anterior, intermediate and posterior regions according to the method described by McNamara and Carlson (1979). The measurements were performed perpendicular to the articular surface from the articular surface to cartilage-bone interface defined by the presence of angiogenesis using bright field light microscopy with a ×4 microscope objective and with the aid of an ocular graticule (accuracy 20 μm). The average cartilage thickness in the anterior, intermediate and posterior regions for each condyle was then recorded (Figure 2.8).

Figure 2.7. Sagittal section of the left metacarpus along the midline (fresh specimen). The growth plate of the metacarpus (GP) can be clearly seen.

2.5.2. *The growth within the metacarpus*

The growth within the growth plate of metacarpus was determined by measuring the distance along the subchondral trabeculae in the direction of the shaft bone between the calcein and alizarin red fluorescent bands (Pastoureau et al, 1991) with a vernier calliper attached to a fluorescent

microscope (Zeiss®, Germany) with an accuracy to 0.1 mm following the methods given by Hansson (1967). A $\times$ 6.3 microscope objective was selected to ensure fluorescent bands were clearly seen. The measurements were performed on three sections and the averages were calculated. The results of the condylar cartilage thickness and the growth of metacarpus are presented in *Chapter 3*.

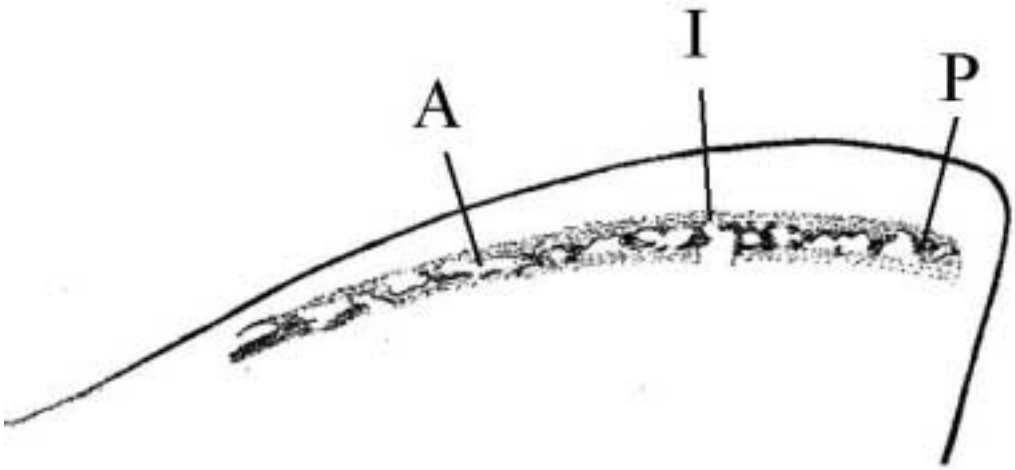


Figure 2.8. Thickness of the condylar cartilage. The thickness was measured perpendicular to the articular surface at anterior (A), intermediate (I) and posterior (P) regions from each section. Three mid-sagittal sections were selected for each condyle and the average thickness of the condylar cartilage in anterior, intermediate and posterior regions were calculated.

Chapter 3 also describes an evaluation of a number of other features in the experimental and control animals. They are: (i) chewing, (ii) growth, i.e., weight gain and the growth of metacarpus, (iii) anatomy of the dental arches compared from dental casts (iv) displacement of the mandible in the experimental group which was evaluated by superimposition of the cephalogram tracings taken before and after the placement of the appliance using cranial-base structures as the reference and (v) the histological features

of the TMJ, including the mandibular and the temporal components, observed in von Kossa/H&E stained sections.

Means and standard deviations were calculated for body weights, metacarpus growth and condylar cartilage thickness. The differences between the groups were tested with the *t* test for two independent samples. The correlation between the operation time and the weight gain of the animals was also performed. A *p*-value <0.05 was adopted for statistical significance. The SPSS software package (version 9.0; Chicago, U.S.A.) was used for all analysis.

2.5.3. *Mandibular condylar growth*

The distance between the calcein and alizarin red fluorescent bands was measured along four directions which included height perpendicular to, and length parallel to the occlusal plane (Fig. 2.9). This procedure was developed from a protocol designed for quantifying mandibular condylar dimension in rats (Kantomaa & Pirttiniemi, 1996). Fluorescent bands were clearly defined with a × 6.3 microscope objective. The measurements were performed on three unstained sections per TMJ. The minimal separation between each section was 270 μm. All the measurements were performed by the author with a vernier calliper attached to a fluorescent microscope (Zeiss®, Germany) to an accuracy of 0.1 mm.

Means and standard deviations were calculated for mandibular condylar growth in four different directions (both left and right side) as well as

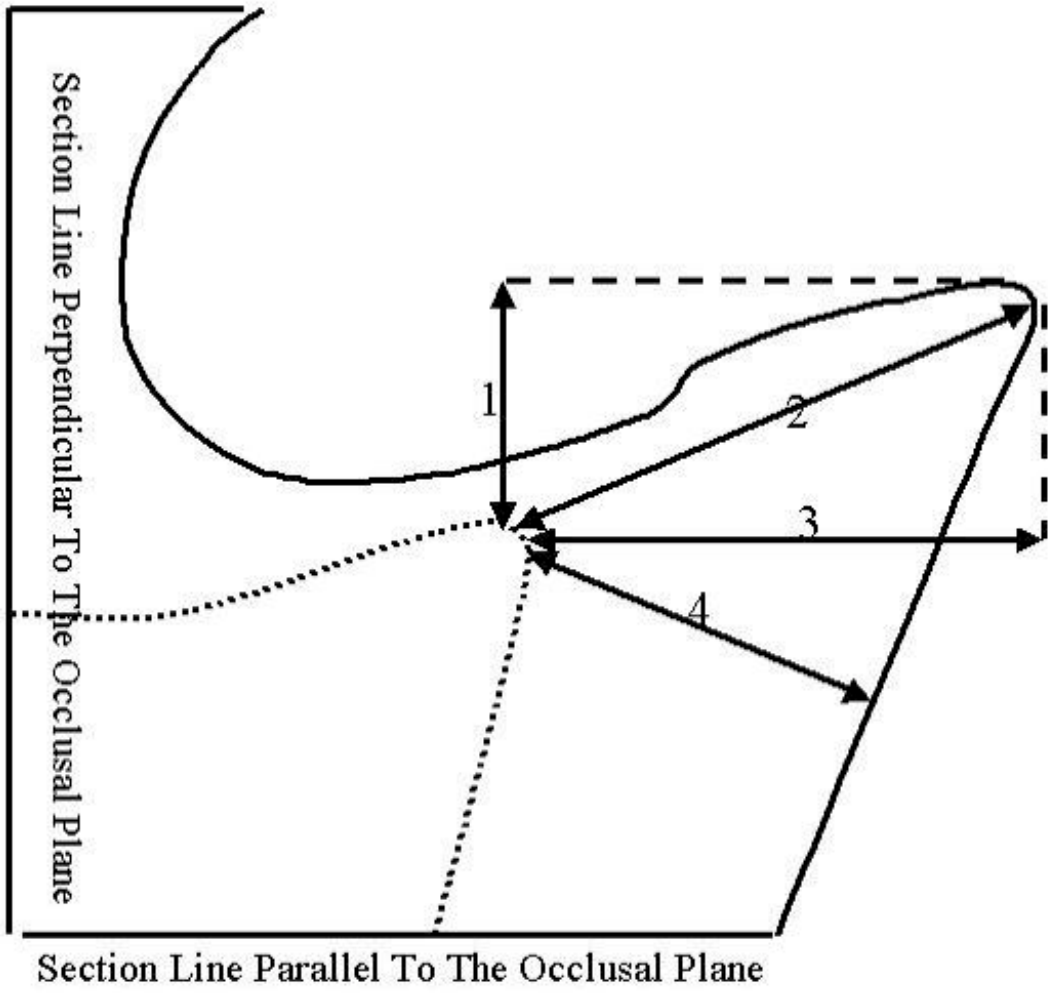


Figure 2.9. Measurements made from the mandibular condyles of sheep injected with fluorochrome bone labels.

1. Growth in height perpendicular to the occlusal plane.
2. Growth in a posterosuperior direction measured parallel to the axis of the condylar process. This is the longest distance between the two fluorescent bands.
3. Growth in length parallel to the occlusal plane.
4. Growth in a posteroinferior direction measured as the shortest distance between the two fluorescent bands.

metacarpus longitudinal growth and weight gain among the animals in both the control and experimental groups. They are described in *Chapter 4*. Growth data for the condyle were analysed using multivariate analysis of variance (MANOVA) with “control or treatment”, “left or right side” as fixed factors, “metacarpus growth” and “weight gain” as covariates. Insignificant factors were removed from the analysis in a stepwise manner. Condylar growth in four directions was then adjusted by multivariate analysis of covariance (MANCOVA). When appropriate, analysis of covariance (ANCOVA) was performed, marginal means and mean differences were estimated. The SPSS software package (version 9.0; Chicago, U.S.A.) was used for all analysis. A *p*-value < 0.05 indicated statistical significance.

2.6. Error Study of the Linear Measurements

For evaluation of the measurement error of the three linear measurements, the measurements was repeated after three months by the same observer (B.M.) and the error was also calculated according to Dahlberg (1940):

$$S = \sqrt{\Sigma d^2 / 2n}$$

where *S* is the measurement error, *d* is the difference between the repeated measurements and *n* is the number of the repeated measurement. The results appear both in *Chapter 3 and* in *Chapter 4*.

2.7. Bone Histomorphometry

2.7.1. *Structural, static and dynamic indices of the trabecular bone in the mandibular condyle*

All the variables measured in this study are summarised in Table 2.3. Histologic sections were subjected to the measurement of different variables according to their thickness. Unstained 10 μm thickness sections were used for fluorescent microscopy and for the measurement of dynamic variables of bone formation. A computerised image analysis system (Quantiment 500®, Cambridge, UK) was used to analyse von Kossa stained 5 μm thickness sections for structural variables of trabecular bone. Static variables of bone-forming and -resorbing activities were measured from von Kossa/haematoxylin-eosin (H&E) stained 5 μm thickness sections. Dynamic variables included: mineral apposition rate (MAR, [μm/day]), bone formation rate with respect to bone mineral surface (BFR/BS, [mm^3/mm^2/day]) and bone formation rate with respect to bone mineral volume (BFR/BV, [%/year]). Structural variables included: bone volume fraction (BV/TV, [%]), bone surface (BS/TV, [mm^2/mm^3]), specific bone surface (BS/BV, [mm^2/mm^3]), trabecular thickness (Tb.Th, [mm]), trabecular separation (Tb.Sp, [mm]) and trabecular number (Tb.N, [#/mm]). Static variables of bone-forming and resorbing activity included: osteoid surface (OS/BS, [%]), eroded surface (ES/BS, [%]) and quiescent surface (QS/BS, [%]) (Parfitt et al., 1987). In order to clarify the relationship between the rates of matrix synthesis and mineralisation, and the thickness of osteoid seams, the mineralisation lag time (MLT, [Day]) was also calculated according to the formula given by Baylink (1970).

MLT=OSW/MAR

where OSW is osteoid seam width.

Under fluorescence microscopy, a dotted reference line was drawn on each un-stained glass slide from the most postero-superior point of the alizarin red label (A) to the most postero-superior point of the calcein label (B). Line AB represented the condylar axis, and was extended for 5 mm to C. The dimension of the trabecular bone varies in size among histologic sections. The distance of 5 mm was determined from a basis of the smallest dimension of the trabecular bone. Two lines were drawn perpendicular to line ABC from point B and point C (Figure 2.10). Therefore, in the subchondral region of the condyle, the region between A and B comprised of the bone that had developed during the experimental period. The central region of the condyle (between B and C) comprised of the bone which existed before the experiment. The dotted line was erased when all the dynamic parameters were measured to avoid observer or computer artifact. Within the regions of interest, only trabecular bone was taken into account (Figure 2.10). The adjacent sections stained with von Kossa or von Kossa/H&E were superimposed upon unstained sections and the regions of interest were outlined from the unstained sections (Figure 2.11). Measurements were performed on three sections of each mandibular condyle. The minimal separation between each section was 270 μm. The results are presented in *Chapter 5*.

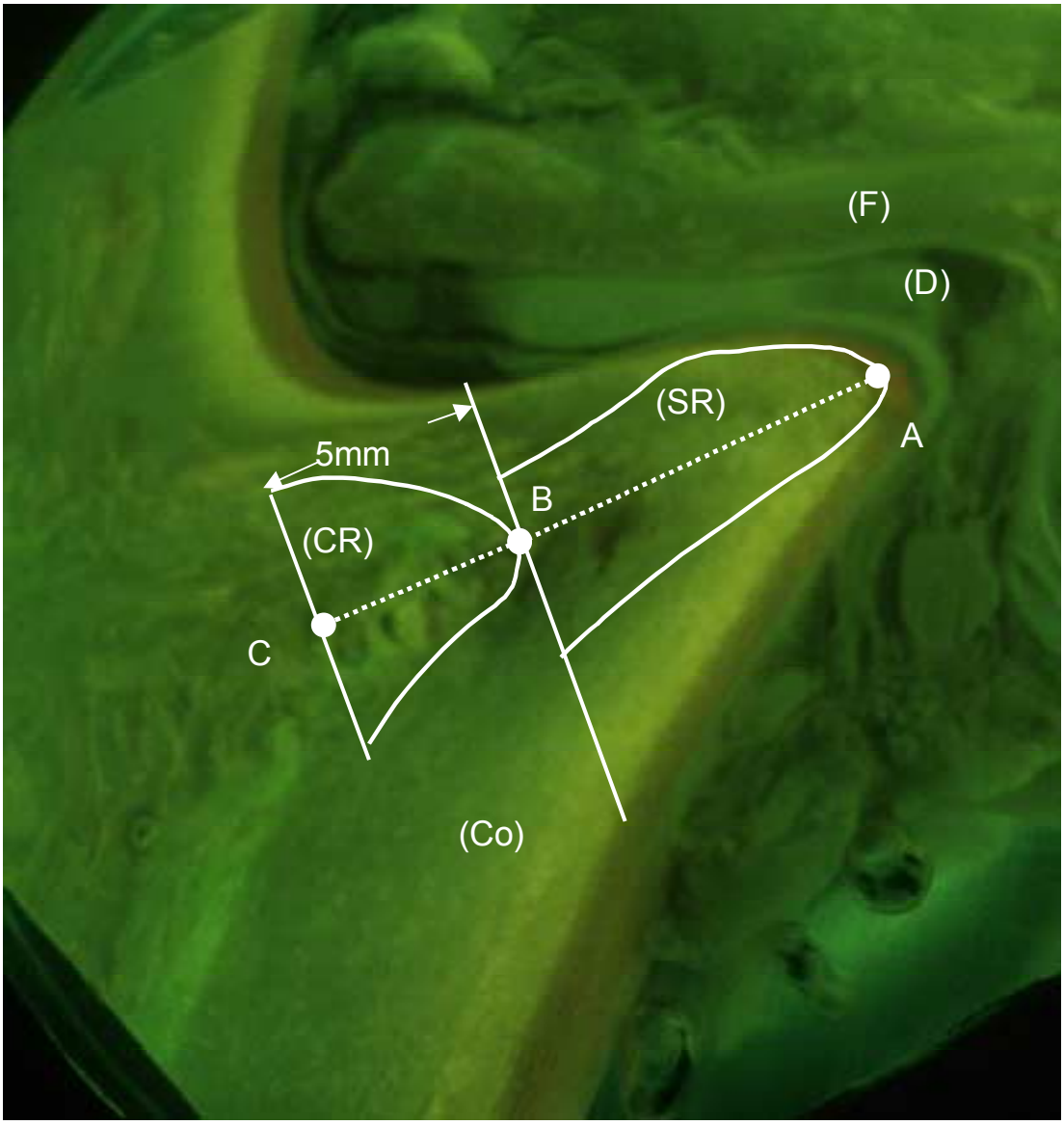


Figure 2.10. Mid-sagittal section of sheep TMJ viewed under ultra-violet light showing fossa (F), disc (D) and condyle (Co).
A. The most postero-superior point of the alizarin red label.
B. The most postero-superior point of the calcein label.
C. 5 mm extension of the line AB.
Two lines were drawn perpendicular to line ABC from point B and point C, and outlined areas in the subchondral region (SR) and the central region (CR) are regions of interest determined by the characteristics of trabecular bone.

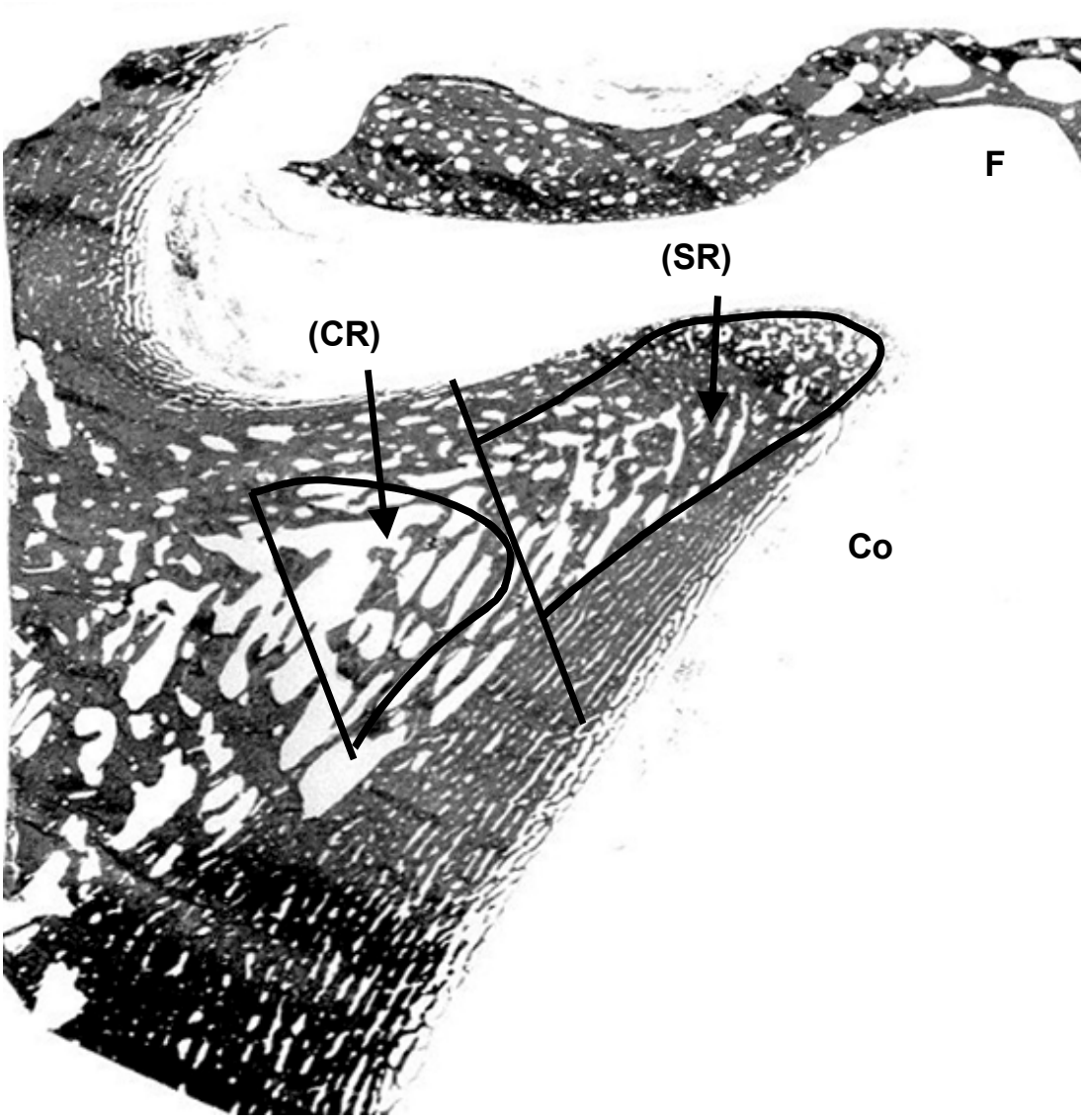


Figure 2.11. Mid-sagittal section of sheep TMJ (von Kossa stained) showing fossa (F) and condyle (Co) as well as the defined regions of interests (see Fig. 2.10).
von Kossa or von Kossa/H&E stained sections were superimposed upon adjacent unstained sections and the regions of interest were outlined from the unstained sections. The regions of interest for a von Kossa stained section are illustrated in the above figure.
(SR): the subchondral region.
(CR): the central region.

Wilcoxon signed ranks test was used to compare the difference for each variable between the subchondral region and the central region within the condyle. The Mann-Whitney test was used to compare differences for each variable between control and experimental groups. Because the individual variation exists for the variable ES/BS and in order to increase the precision of analysis when the comparison is made between the control and the experimental groups, analysis of covariance (ANCOVA) was applied to deal with the variable ES/BS. Static variables of bone forming and resorbing indices were subjected to ANCOVA analysis with “control or treatment”, “left or right side” as fixed factors, and ES/BS as covariates. Insignificant factors were removed from the analysis in a stepwise manner. OS/BS was then compared as the dependent variable after the assumption of using ANCOVA had been evaluated. The SPSS software package (version 9.0; Chicago, U.S.A.) was used for all analyses. A p value < 0.05 indicated statistical significance.

2.7.2. *Structural indices of the cortical bone in mandibular condyle*

In the cortical bone, an index for cortical bone thickness (Ct.Th, [μm]) was measured in the anterior and posterior regions of the mandibular condyle (Figure 2.12). The measurements were performed from the anterior border of the condylar cartilage (A) and the posterior border of the condylar cartilage (P). Ct.Th was measured using Quantiment logic modules in conjunction with Microsoft® Excel 2000. When performing the measure in the anterior region, the section was rotated counter-clock-wise to ensure the border of the cortical bone was perpendicular to the horizontal test grid (Figure 2.13). Ct.Th was

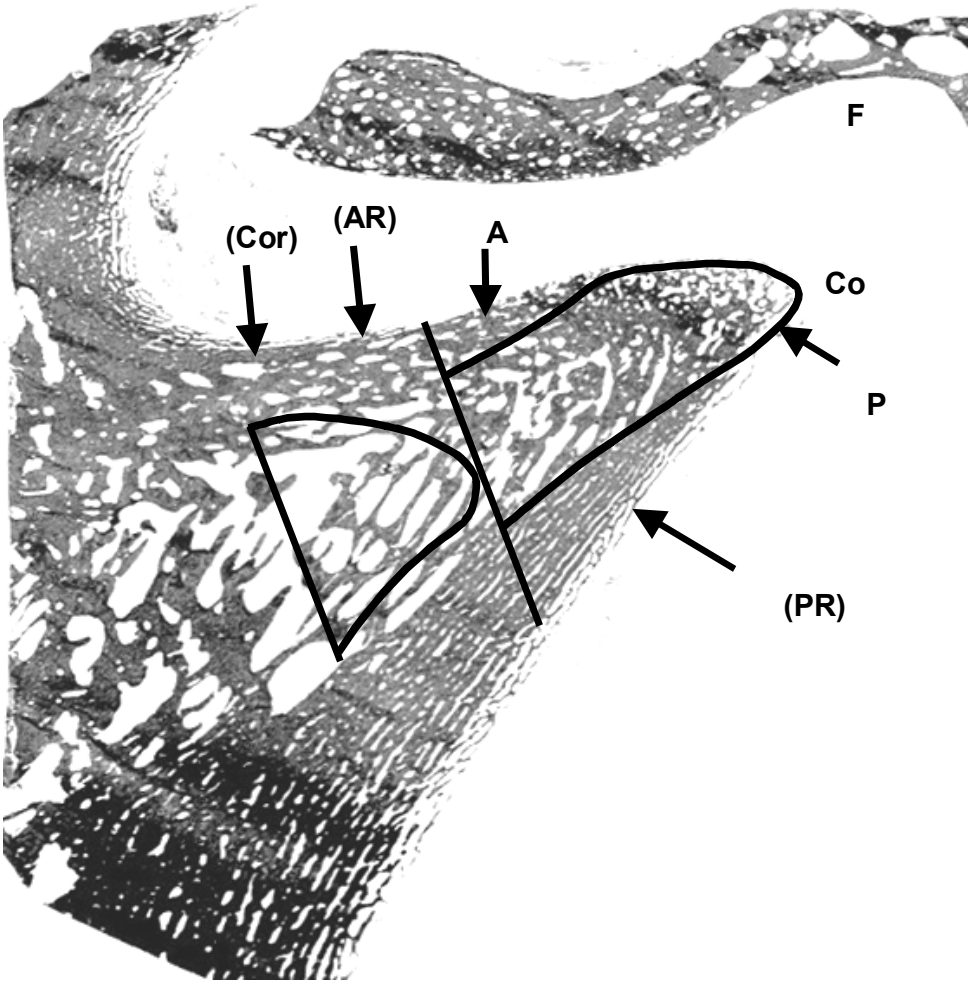


Figure 2.12. Mid-sagittal section of sheep TMJ (von Kossa stained) showing fossa (F) and condyle (Co). This von Kossa stained section was superimposed upon adjacent unstained sections and the regions of interest were outlined from the unstained section.
A: the anterior border of condylar cartilage.
P: the posterior border of condylar cartilage.
(AR): the subchondral region.
(PR): the central region.
(Cor): The transverse part of the coronoid process.

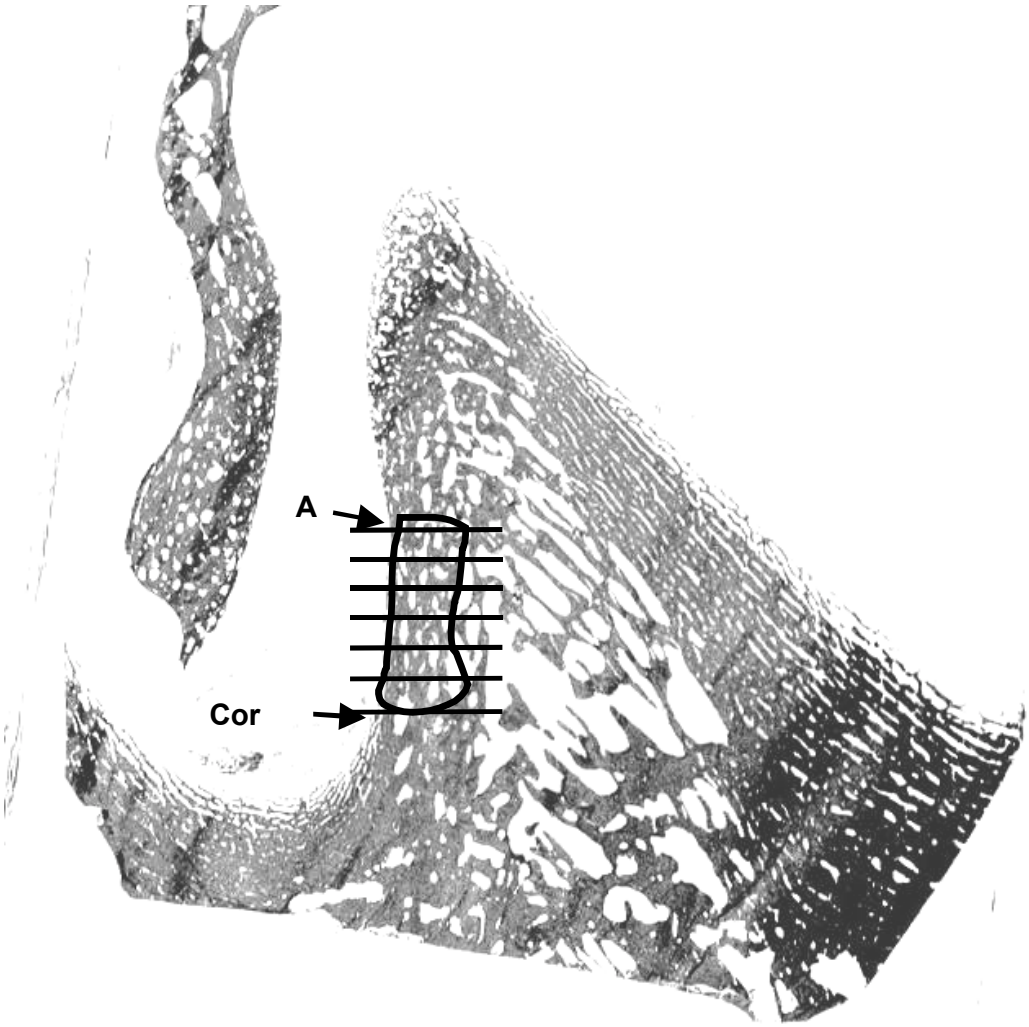


Figure 2.13. The orientation of the specimen at which the cortical bone thickness was measured in the anterior region. Paralleled horizontal lines represent the test lines of the computer programme.

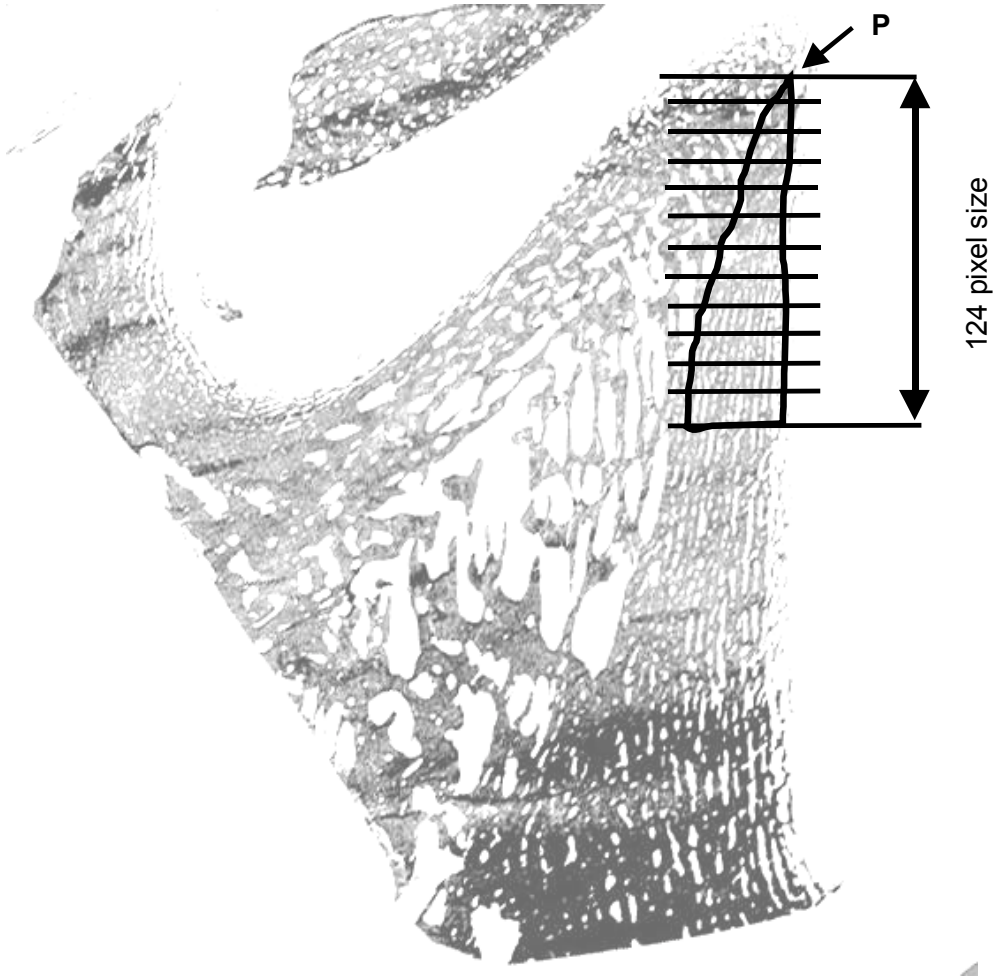


Figure 2.14. The orientation of the specimen at which the cortical bone thickness was measured in the posterior region. Paralleled horizontal lines represent the test lines of the computer programme.

then measured superiorly to the transverse part of the coronoid process (Cor). Likewise, the section was also rotated when the measure was performed in the posterior region (Figure 2.14). A total length of 124-pixel size was defined to determine the region of interest. The dimension of the cortical bone varies in size among histologic sections. The length of 124-pixel size was determined on the basis of the smallest dimension of the cortical bone.

In each section, chord length was first measured in the anterior region, then the posterior region. The average chord length was calculated for the anterior region and the chord length at the position 124 pixel size (approximately 3.8 mm) inferior to the posterior border of the condylar cartilage was recorded. The bone volume fraction (CtV/TV, [%]) of the cortex, cortical bone porosity (1-CtV/TV, [%]) and effective cortical thickness (Ct.Th×(CtV/TV/100), [μm]) were also calculated. Because of the different morphology of cortical bone in the anterior and the posterior region, only BV/TV and 1-CtV/TV were subjected to the intra-condylar comparison.

2.7.3. *Trabecular anisotropy in the mandibular condyle*

An index for trabecular anisotropy (Tb.An) was calculated as the ratio of horizontal to vertical intercepts of trabeculae. This was assessed by measuring Tb.An with the specimen of mandibular condyle orientated at angles ranging from 0° to 180° to test for maxima and minima in Tb.An (Figure 2.15).

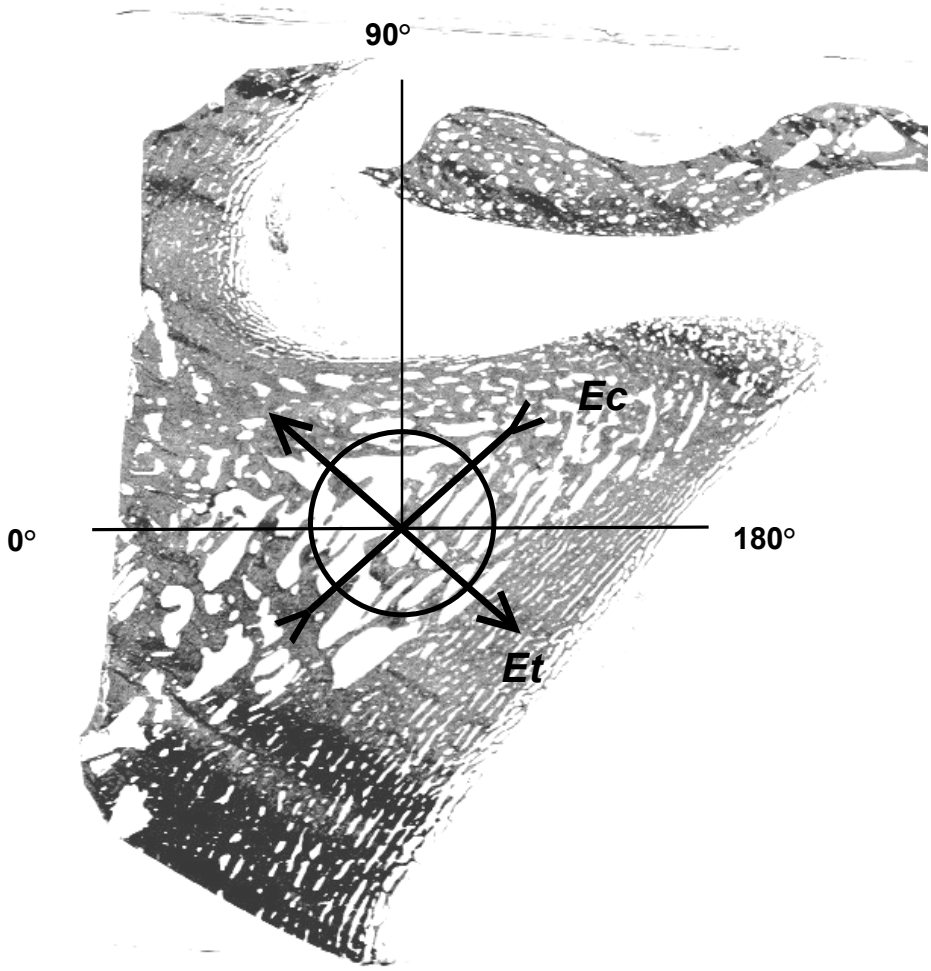


Figure 2.15. Mid-sagittal section of sheep TMJ (von Kossa stained) showing the orientation where Tb.An was measured. The definition of principle compressive (*Ec*) and tensile (*Et*) strain angles (bold arrows) were used for comparison with trabecular alignment.

This index differs from that used by Goulet et al. (1994); Teng & Herring (1995), which was derived from measurements of mean intercept length of test lines projected at differing orientations onto the trabecular structure. The index used in the current study for Tb.An is comparable with that of Biewener et al. (1996) who used the resulting "best-fit" line to describe trabecular orientation. As Biewener et al. (1996) stated that increased Tb.An indicates increasing alignment of trabeculae with the compressive strain axis.

In each section, Tb.An was first measured in the central region. The Tb.An was measured in the subchondral region by sliding the section into the fixed focused field with the angulation of the section unchanged. Tb.An was calculated and plotted along the angles where the Tb.An was measured. The data derived from the subchondral region and the central region were plotted on the same chart. An example is presented in Figure 2.16.

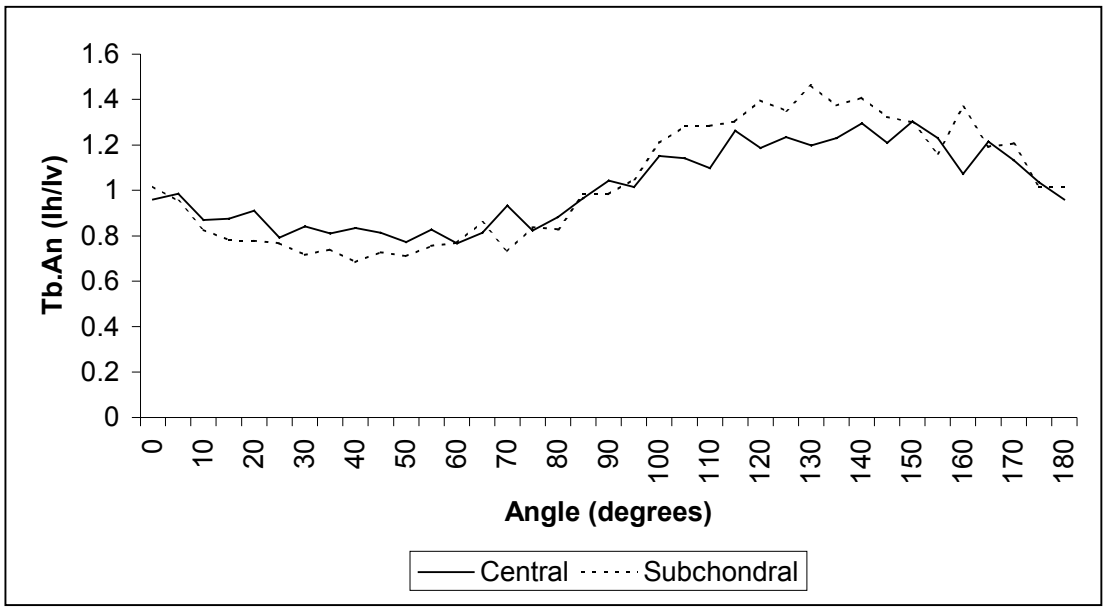


Figure 2.16. Trabecular anisotropy (Tb.An) measured as the ratio of horizontal line intercepts to vertical line intercepts (I_h/I_v) from 0°to 180°.

The distribution of Tb.An presents a sinusoidal shape. It is generally believed that the order for the trend line to be three creates a best-fit to the line in sinusoidal shape. The accuracy of using the cubic function to fit the sinusoidal shaped distribution of Tb.An is evaluated in section 2.7.4 of this chapter.

The best-fit line for the Tb.An in each region was generated on the chart using Microsoft Excel ®, Microsoft Office 2000^{TM}. By using the programme, a polynomial trend line with the order as three was produced to fit to the data obtained from both the subchondral and the central region. An equation, to four decimal points, was also produced to describe the regression line. The equations in the example are presented in Figure 2.17.

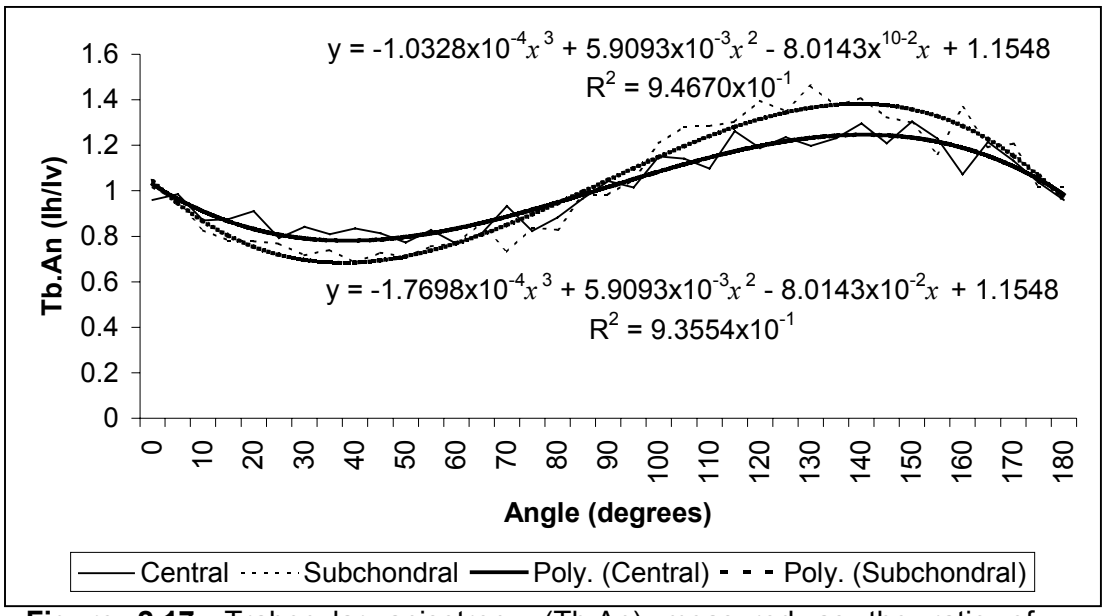


Figure 2.17. Trabecular anisotropy (Tb.An) measured as the ratio of horizontal line intercepts to vertical line intercepts (I_h/I_v) from 0°to 180° with the best-fit lines and the equations of the lines.

The maximum and the minium for each line were derived by calculating the local extremes using the first order derivative of the equation derived from the best-fit line. In the above example, the following equation was derived by the computer programme:

$$y = -1.0328\times10^{-4}\, x^3 + 5.9093\times10^{-3}\, x^2 - 8.0143\times10^{-2}\, x + 1.1080 \quad (1)$$

The first order derivative was than calculated as

$$dy/dx = -3.0984\times10^{-4}\, x^2 + 1.1819\times10^{-2}\, x - 8.0143\times10^{-2} \quad (2)$$

and the local extreme can be calculated by setting the dy/dx as 0. By solving the equation:

$$-3.0984\times10^{-4}\, x^2 + 1.1819\times10^{-2}\, x - 8.0143\times10^{-2} = 0 \quad (3)$$

it was determined that $x_1 = 8.8210$ and $x_2 = 29.3232$, where they are the sequential numbers of the specimen orientation starting at 0° and rotated every 5° to 180°. Based on equation (1) and the sequential numbers, y_1 and y_2 were then calculated respectively, i.e. the minium of the Tb.An was 0.7900 and the maximum of the Tb.An was 1.2350.

The angle at which the maximum Tb.An occurred indicated the orientation along which most trabeculae were aligned. From the mechanical view point, it was this orientation that was deemed to represent a nominal compressive force. Therefore, this angle was called the compressive principle strain angle (*Ec)*. The angle at which the minimum Tb.An occurred indicated the orientation along which least trabeculae were aligned. Also, from the mechanical viewpoint, it was this orientation that was deemed to represent a nominal tensile force. Therefore, this angle was called the tensile principle strain angle (*Et*). *Et* and *Ec* were calculated following the formulae:

$$Et = x_1 \times 5 - 5 \quad (4)$$

$$Ec = x_2 \times 5 - 5 \quad (5)$$

Therefore *Et* and *Ec* in this example were 39.1° and 141.6° respectively. The minimum and the maximum in Tb.An, the angle between tensile and compressive principle strain angles (*Ec-Et*), as well as the specimen angle where the minimum (*Tb.An-min*) and the maximum (*Tb.An-max*) values occurred were recorded. A schematic representation is presented in Figure 2.18. Measurements were performed on 3 sections of each mandibular condyle. The minimal separation between each section was 270 μm. The average from the three sections was calculated for the comparison between the two groups. These results are presented in *Chapter 7*.

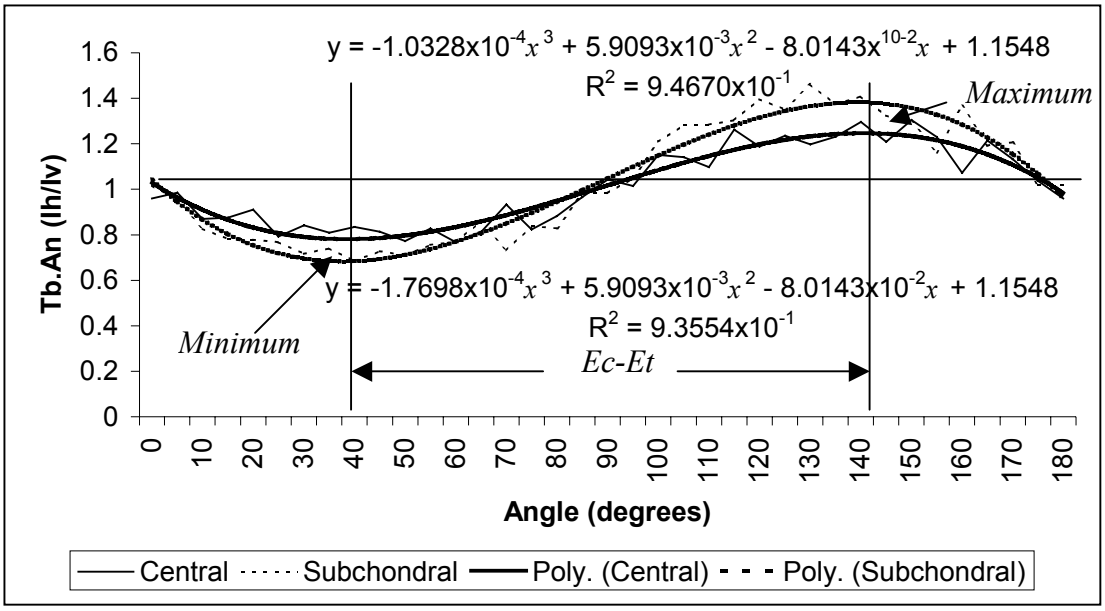


Figure 2.18. Trabecular anisotropy (Tb.An) measured as the ratio of horizontal line intercepts to vertical line intercepts (I_h/I_v) from 0°to 180° with the best-fit lines and the equations of the lines. A value of 1 (horizontal line) indicates elements (i.e. isotropy). In the example, maximum in Tb.An was observed at approximately 140°. Similarly, minimum in Tb.An was observed near 40°. Angles of 0° and 180° correspond to the occlusal plane.

2.7.4. *The accuracy of using cubic function to fit the distribution of Tb.An.*

2.7.4.A. Method and results

The method described in the previous section advances the technique of Biewener et al., (1996). In order to evaluate the accuracy of using cubic function to fit the distribution of Tb.An, a data set with the distribution of sinusoidal shape was created with the function as:

$$y = \cos(2x+90°) + 1.5$$

where x starts from 0° and increases for every 5° to 180°. The minimal and maximal values (y) and the corresponding angles (x) of the data set are known, i.e. at $x_1=45°$, the minimal value $y_1=0.5$; at $x_2=135°$, the maximal value $y_2=2.5$ (Figure 2.19).

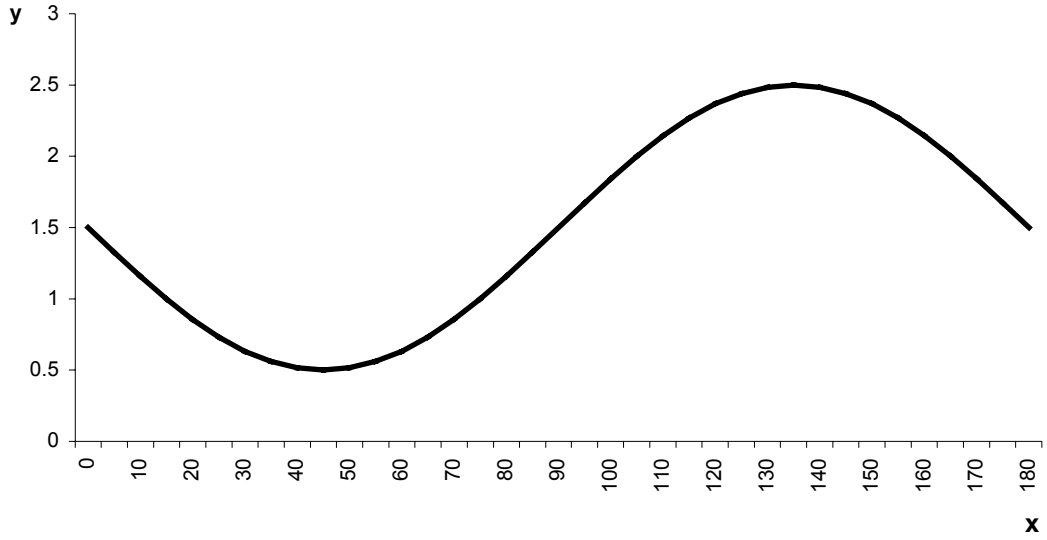


Figure 2.19. The data set showing a similar distribution to Tb.An. The data set is created using the function $y= \cos(2x+90°) + 1.5$.

The same method as described in section 2.7.3 was used to create the best-fit line and to estimate the local minimum and maximum of the line. The

regression line and its equation are provided in Figure 2.20. The correlation co-efficient is found to be high.

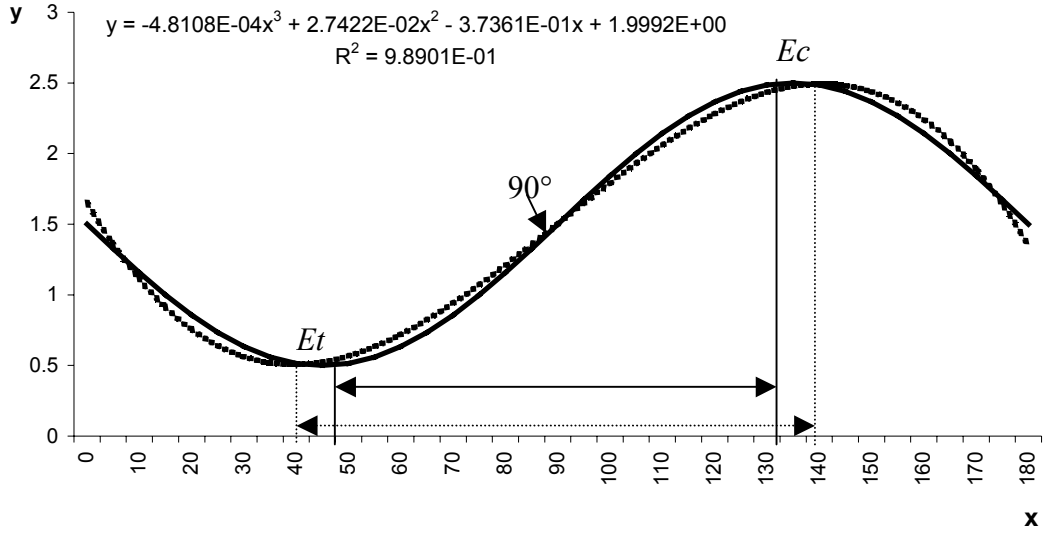


Figure 2.20. The data set showing a similar distribution to Tb.An (solid line) and its "best-fit" line (dotted line) generated on the chart using Microsoft Excel ®, Microsoft Office 2000™. The intercept of data set and the best-fit line locates at x = 90°. Vertical solid lines indicate the location of the local minimum and local maximum of the data set. Vertical dotted lines indicate the local minimum and local maximum of the best-fit line. Solid double arrow indicates the angle *Et-Ec* of the data set. Dotted double arrow indicates the estimated angle *Et-Ec* of the best-fit line.

The calculated minimal and maximal values of the function were compared to those true values. Although the calculated "y" is very close to the true value, the deviation from the true values was found as 5.53° for the calculated "x" values (Table 2.2). Graphically, the intercept of the data set and its best-fit line locates at x = 90°. Based on Figure 2.20, it was concluded that, for a given y, estimated x is always less than the true x when x < 90°; whereas estimated x is always more than the true x when x > 90°.

2.7.4.B. Discussion

The distribution of Tb.An presents a sinusoidal shape. Due to the unavailability of the computer programme which generates the sinusoidal function to fit the data, a cubic function available from Microsoft Excel ®, Microsoft Office 2000™ is used to create the best-fit line. For the given data set with the distribution of sinusoidal shape, cubic function generated a biased result with the estimated x value 5.53° less than the true value for minimal y and the estimated x value 5.53° more than the true value for maximal y. As presented in Figure 2.18, *Et* is always less than 90° and *Ec* is always more than 90°. These results indicate the estimated *Et* values are always smaller than the true value, where as the estimated *Ec* values are always larger than the true value.

Table 2.2. The accuracy of using cubic function to fit the distribution of Tb.An

	Minimum		Maximum	
	x	y	x	y
True value	45.00 °	0.50	135.00 °	2.50
Calculated value	39.47	0.51	140.53	2.49

Acknowledging the bias in estimating the minimal and the maximal Tb.An value, caution must be taken when the results are interpreted. When the descriptions of *Et* or *Ec* are made, a value of 5.53° must be added and subtracted respectively to compensate the bias to provide an estimate of *Et* or *Ec*. When the description of *Et-Ec* is made, a value of 11.06° must be subtracted to compensate the bias to provide an estimate of *Et-Ec*.

When the comparisons of *Et* or *Ec* are made between the control and the experimental groups, or the comparison are made between different regions

within the mandibular condyle (details are provided in *Chapter 7*), the measurement bias cancelled, therefore the results of angles of local minimum and maximum of Tb.An (*Et* and *Ec* respectively) are reliable. In this project, the Tb.An, *Et*, *Ec* and *Et-Ec* are measured merely for the comparison between the control and the experimental groups or between different regions within the mandibular condyle. Therefore, the estimated *Et*, *Ec* and *Et-Ec* are not corrected for the bias and presented as original in this thesis.

2.7.5. *Statistical analysis of Tb.An*

The *t*-test for paired samples was used to compare the means for 1-CtV/TV between the anterior region and the posterior region within the condyle, and for Tb.An between the central region and the subchondral region. Similarly, the *t*-test for two independent samples was used to compare means for each variable between control and experimental groups in conjunction with Levene's test for equality of variance.

In order to characterise the anisotropic nature of the trabeculae within the mandibular condyle, ratios of Tb.An-max and Tb.An-min were further calculated. As illustrated in Figure 2.18, Tb.An has the value of 1 which indicates isotropy. A larger value of Tb.An indicates more trabeculae are aligned with that orientation whereas a smaller value of Tb.An indicates less trabeculae are aligned with that orientation. Because Tb.An-max and Tb.An-min only describe the anisotropic nature of the trabeculae at 2 prescribed angles (Ec and Et respectively), the ratio of Tb.An-max and Tb.An-min deems to describe the anisotropic nature of the trabeculae two-dimensionally.

The ratios calculated in this study are illustrated in Figure 2.21. In the central region, the ratio of Tb.An-min/Tb.An-max (II/I) describes the least trabeculae alignment in this region whereas the ratio of Tb.An-max/Tb.An-min (I/II) describes the most trabeculae alignment in this region. Because the central region is also thought to be the region comprising of pre-existing bone and the subchondral region is thought to comprise of newly formed bone, the ratio of Tb.An-max in the subchondral region to the Tb.An-max in the central region (III/I) and the ratio of Tb.An-min in the subchondral region to the Tb.An-min in the central region (IV/II) describe the status of trabecular alignment during growth along the similar direction of nominal mechanical force. The ratio of Tb.An-max in the subchondral region to Tb.An-min in the central region (III/II) and the ratio of Tb.An-min in the subchondral region to Tb.An-max in the central region (IV/I) describe the trabecular alignment during growth along direction of different nominal mechanical force. The means of the ratios were compared using the *t*-test for two independent samples.

The SPSS software package (version 9.0; Chicago, U.S.A.) was used for all the statistical analyses. A p-value < 0.05 indicated statistical significance.

2.8. Error in the Bone Histomorphometric Method Used in This Study

2.8.1. *Background*

The method for estimating the measurement error from the linear measurements has been described previously in this chapter. The method for

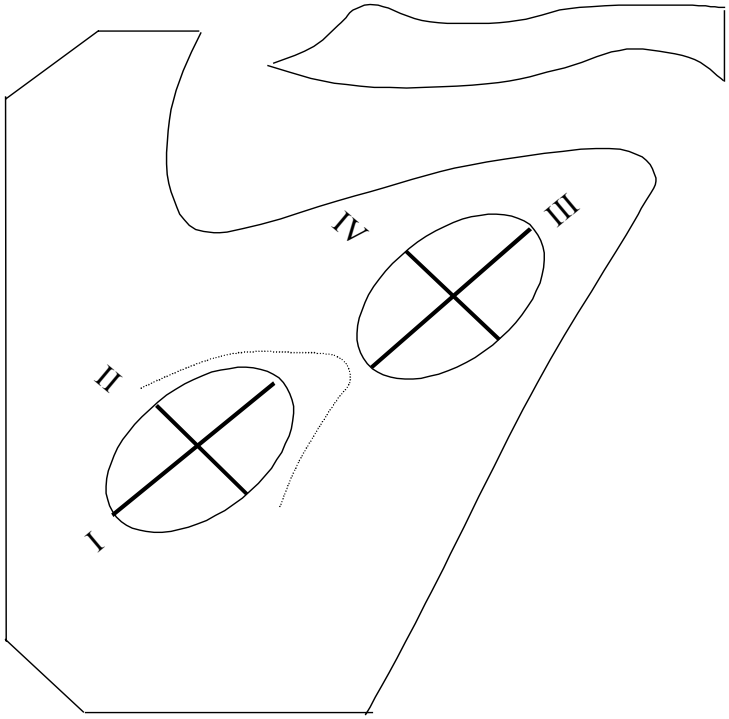


Figure 2.21. Schematic illustration of the mandibular condyle model represented by four functions (I, II, III and IV) that define the two ellipses where:
I: maximum of TbAn in the central region
II: minimum of TbAn in the central region
III: maximum of TbAn in the subchondral region
IV: minimum of TbAn in the subchondral region

II/I: the least trabeculae alignment in the central region.
I/II: the most trabeculae alignment in the central region.
III/I: the trabecular alignment during growth along the nominal compressive force.
IV/II: the trabecular alignment during growth along the nominal tensile force.
III/II: the trabecular alignment during growth along the nominal compressive force based on the nominal tensile force.
IV/I: the trabecular alignment during growth along the nominal tensile force based on the nominal compressive force.

estimating the error from histomorphometric measurements was dealt with in this section.

As a well developed technique, the error of bone histomorphometry has been extensively studied (de Vernejoul et al, 1981; Compston et al, 1986; Wright et al, 1992) and the details have been presented in section 2.2. The standard procedures were used in the present study and the measurement error appears to be comparable with previous studies. Indeed, to justify the testing of error of bone histomorphometry by means of determining regions of interest is an alternative method. The methods described by Dahlberg (1940) were considered time consuming and complex.

Table 2.3. All the histomorphometric variables measured in this study.

Structural, static and dynamic indices of the trabecular bone			
Type of the Section	Variable	Abbreviation	Unit
Unstained,	Mineral apposition rate	MAR	μm/day
10 μm	Bone formation rate with respect to bone mineral surface	BFR/BS	mm^3/mm^2/day
	Bone formation rate with respect to bone mineral volume	BFR/BV	%/year
	Mineralisation lag time	MLT	Day
von Kossa stained,	Bone volume fraction	BV/TV	%
5-7μm	Bone surface	BS/TV	mm^2/mm^3
	Specific bone surface	BS/BV	mm^2/mm^3
	Trabecular thickness	Tb.Th	mm
	Trabecular separation	Tb.Sp	mm
	Trabecular number	Tb.N	#/mm
von Kossa/H&E stained	Osteoid surface	OS/BS	%
5-7μm	Eroded surface	ES/BS	%
	Quiescent surface	QS/BS	%
Structural indices of the cortical bone in mandibular condyle			
Type of the Section	Variable	Abbreviation	Unit
von Kossa stained,	Cortical thickness	Ct.Th	μm
5-7μm	Bone volume fraction	CtV/TV	%
	Cortical bone porosity	1-CtV/TV	%
	Effective cortical thickness	Ct.Th×(CtV/TV/100)	μm
Trabecular anisotropy in the mandibular condyle			
Type of the Section	Variable	Abbreviation	Unit
von Kossa stained,	Trabecular anisotropy	Tb.An	Ratio, no unit
5-7μm	Principle tensile strain angle	Et	º
	Principle compressive strain angle	Ec	º

As stated in *Chapter 1*, characteristics of all bones can be identified in sagittal sections of the mandibular condyle. Ten Cate (1989) described the bony structure of the condyle, beneath the periosteum, which includes a dense outer sheet of cortical bone and an inner marrow cavity. The marrow cavity is interrupted throughout the length of mandible by a network of trabecular bone particularly at the end of the condyle.

It can be clearly seen from any graphic figure of the TMJ presented in this chapter that in young growing sheep, no clear boundary of the cortical bone and trabecular bone can be identified in the mandibular condyle. However, in order to facilitate the comparison of the TMJ morphology between the control group and the experimental group, a boundary needed to be arbitrarily but consistently defined for the cortical bone and the trabecular bone to deal with the variations of size and shape in the condyle among the animals. In each comparison, the regions thought to be comparable among the sections from different TMJs were determined to be the "region of interest" for that particular comparison. In this study, the following regions defined as the regions of interests are

i. The cortical bone in the anterior region,
ii. The cortical bone in the posterior region,
iii. The trabecular bone in the subchondral region and
iv. The Trabecular bone in the central region.

The anterior and posterior regions can be easily defined according to the anatomy. The subchondral and central regions can be easily defined

according to the fluorochromes. The error of determining the cortical bone from the trabecular bone was defined to be the primary source of error.

2.8.2. *Procedures*

The cortical bone thickness (Ct.Th, [μm]) was selected to test the error in determining the boundary between the cortical bone and the trabecular bone. Sixteen von-Kossa stained sections were randomly selected from both the control group and the experimental group. Ct.Th was measured in the anterior and posterior regions of the mandibular condyle. Measurements were repeated after three months and was performed by the same observer (B.M.). The error of the measurement was tested using *t*-test for paired samples.

2.8.3. *Outcomes and conclusions*

In the anterior region, the Ct.Th distribution of repeated measures on one section was presented on Figure 2.22. Repeated measurements produced very similar results. No significant difference was detected by paired *t*-test (t= 0.812, $p>0.05$). The standard deviation of the difference between the repeated measurements (random error) is 21.6%.

In the posterior region, the Ct.Th distribution of repeated measures on one section is presented in Figure 2.23. Repeated measurements also produced very similar results. No significant difference was detected by paired *t*-test (t= 2.070, $p>0.05$). The standard deviation of the difference between the repeated measurements (random error) is 13.9%.

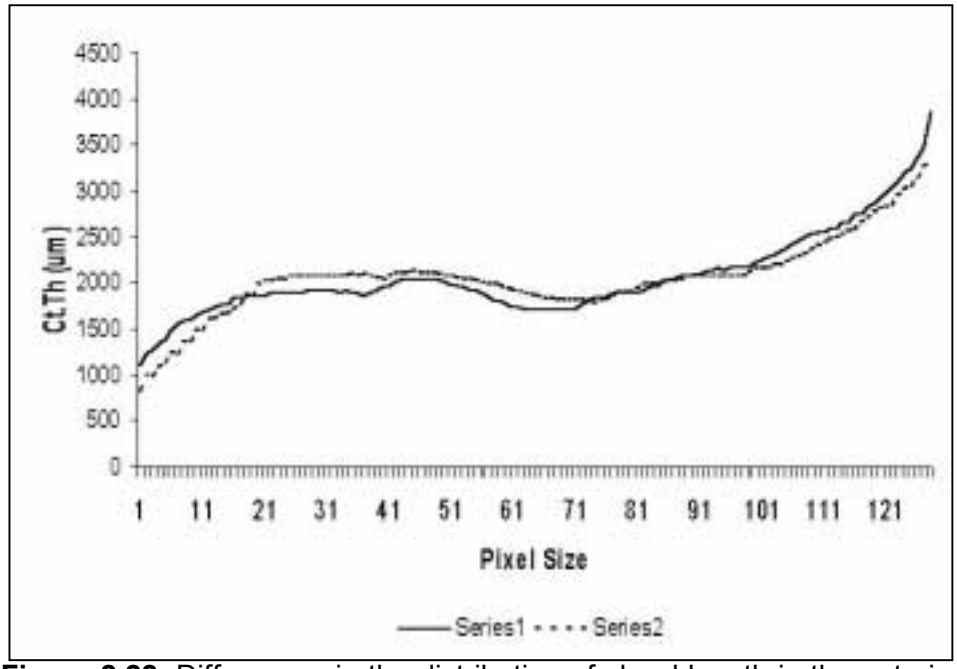


Figure 2.22. Differences in the distribution of chord length in the anterior region of the mandibular condyle between the initial and repeated measurement.

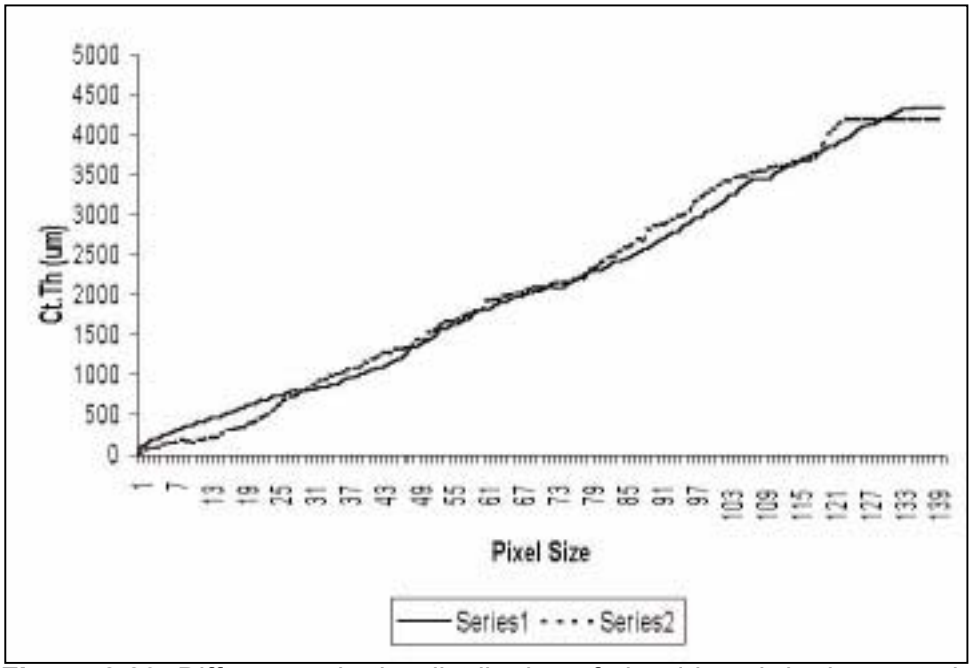


Figure 2.23. Differences in the distribution of chord length in the posterior region of the mandibular condyle between the initial and repeated measurement.

2.9. *Three-dimensional measurement of the distance between implants*

Three-dimensional (3-D) distances between every two markers were measured from the three radiographic views and using recently developed software Acuscape Sculptor™ 1.0 (Acuscape International Inc, USA). This software has a self-calibration feature based on images of the head frame seen on the three radiographs and using the principles of photogrammetry. This self-calibration feature can compensate for the magnification and rotation effects.

Cephalograms were scanned with a resolution of 150 dpi using Jasc Paint Shop Pro © 3" (JASC Software Inc., USA) and stored as JPEG files. The three cephalograms from any one set, taken on the same day, were imported into Acuscape Sculptor™. The 3-D measurements comprise *calibration* and *landmark digitising*.

2.9.1 *Calibration*

Calibration was necessary to match the programme's digitized co-ordinates of the calibration frame with the 13 metal markers in the calibration frame viewed on the scanned cephalograms. The landmarks were re-digitised several times until all the digitised landmarks matched well with the calibration frame image. The process was repeated for the other two cephalograms in the same set. At the end of the calibration, the three cephalograms in the same set were

correlated in 3-D according to the calibration frame. An example is presented showing one set of cephalograms after calibration (Figure 2.24).

2.9.2 *Landmark digitising*

Landmark digitising involved identification of the 3-D location of each implant for one subject from the one set of three cephalograms. First, the three calibrated cephalograms were tiled vertically together on the screen for comparison. A landmark was then identified on one of the three cephalograms. Once the landmark was identified on that cephalogram, the positions of that landmark on the other two cephalograms were implied by the computer programme by an epipolar line. The landmarks were re-digitised several times until the digitised landmarks matched well with the landmark shown on the three cephalograms. An example is presented in Figure 2.25 showing the digitisation of the zygomatic implant on the right side.

2.10. The Accuracy of Three-Dimensional Measurements

2.10.1. *Background*

Compared with the well developed bone histomorphometry technique, 3-D measurements of the distances between the endosseous implants is a newly developed method and its accuracy needed to be evaluated. While performing the measurements of the animals in both control and experimental groups, this accuracy was also evaluated. The test of accuracy comprised of, (i): evaluation of the three-dimensional measurement system, and (ii): estimation

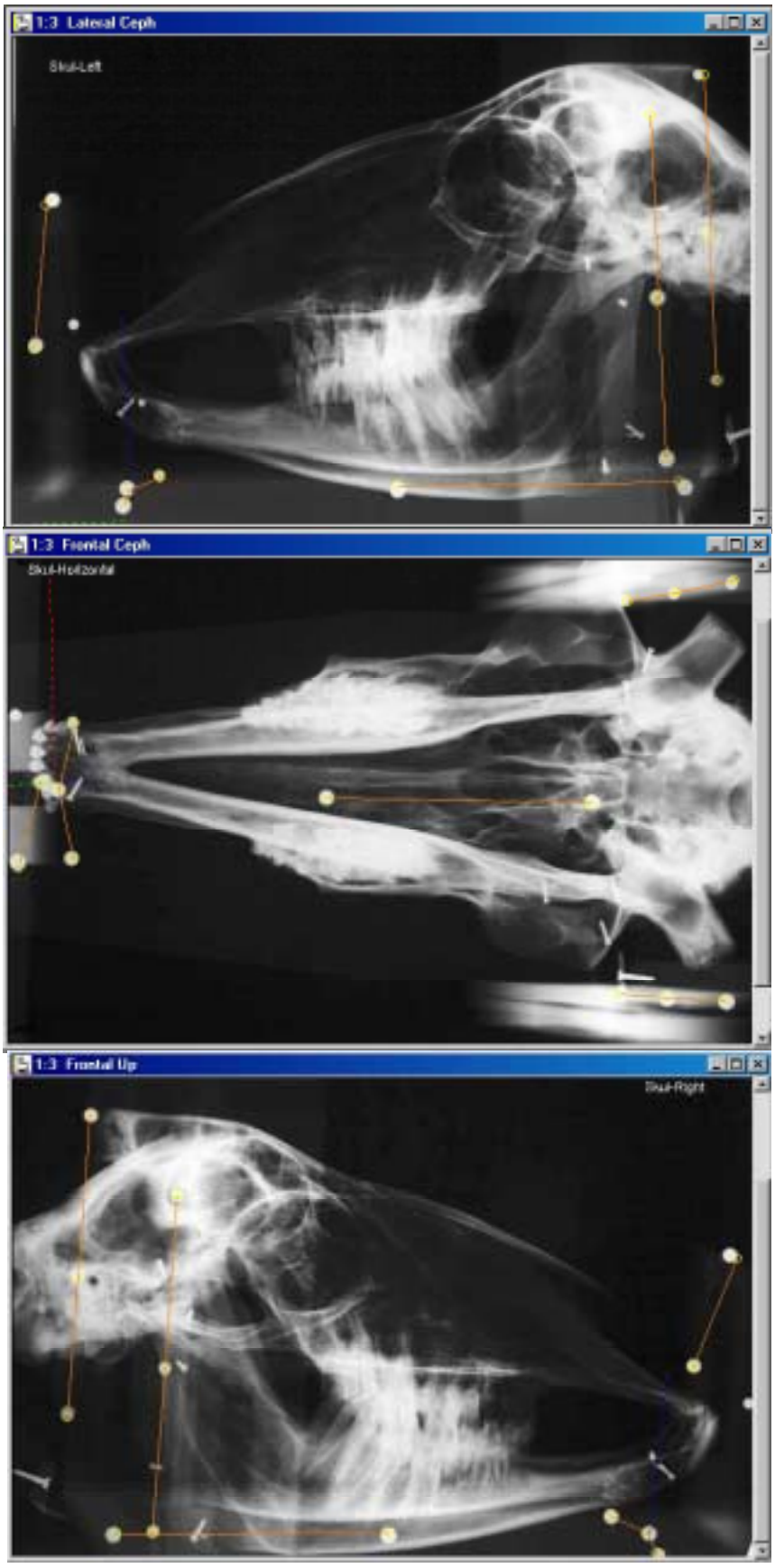


Figure 2.24. Images from three cephalograms and their corresponding calibrations.

Figure 2.25. Epipolar line for zygomatic landmark from one cephalogram to the others according to the calibrated co-ordinates.

of the measurement error with particular reference to the radio-opaque markers used in the present animal experiment.

2.10.2. *Procedures*

The evaluation of the 3-D measurement system was performed in a plastic rectangular cube with eight ball bearings attached to each of the eight corners (Figure 2.26a). Theoretically, the regular shape of the cube would enable the observer to achieve a higher measurement accuracy by using a vernier calliper (Mitutoyo™, Japan). The vernier calliper used in this study had an accuracy to 0.02mm.

The distances between the ball bearings were measured by the same observer (B.M.) six times. The average of the measurements was cumulatively calculated. For example, the average distances between ball bearings were calculated for the first two repeated measurements, then for the first three repeated measurements, and finally the average of all the six repeated measurements. By plotting these averages with the times of measurement, we can easily see the change of the averages and can identify the reliability of the results. The average of the six measurements would have been considered to be the "gold standard" if the reliability of the measurements were high.

The computer measurements for the distances between ball bearings were performed on one set of radiographs six times. The averages were also calculated in a cumulative manner.

The average values of all the computer measurements were compared with "gold standards" in a pair wise manner. The standard deviation of the difference between the two measurements represents the random error of the computer measurement. Paired t-test was used to identify the statistical significance. If a significant difference was identified between the computer measurement and the "gold standard", the average of the difference between the two measurements was used to represent the bias of the computer measurement.

The same procedure was then performed on a dry sheep skull (Figure 2.26b). The purpose of this measurement was to mimic the procedure of measuring the distances between the implants, which was thought to be more representative of the measurement error in animals.

In the dry sheep skull, the distances were measured between the implants with a vernier caliper (accuracy to 1mm; Siber Hegner Co.A.G.^{TM}, Swiss) for six times. The computer measurements were performed nine times according to the tradition. The data were analysed in the same manner as that of the data obtained measuring the plastic rectangular cube.

Based on the accuracy of the measure, the occasions of the repeated measures were identified. As a new method, raw data are presented in helping to validate the method.

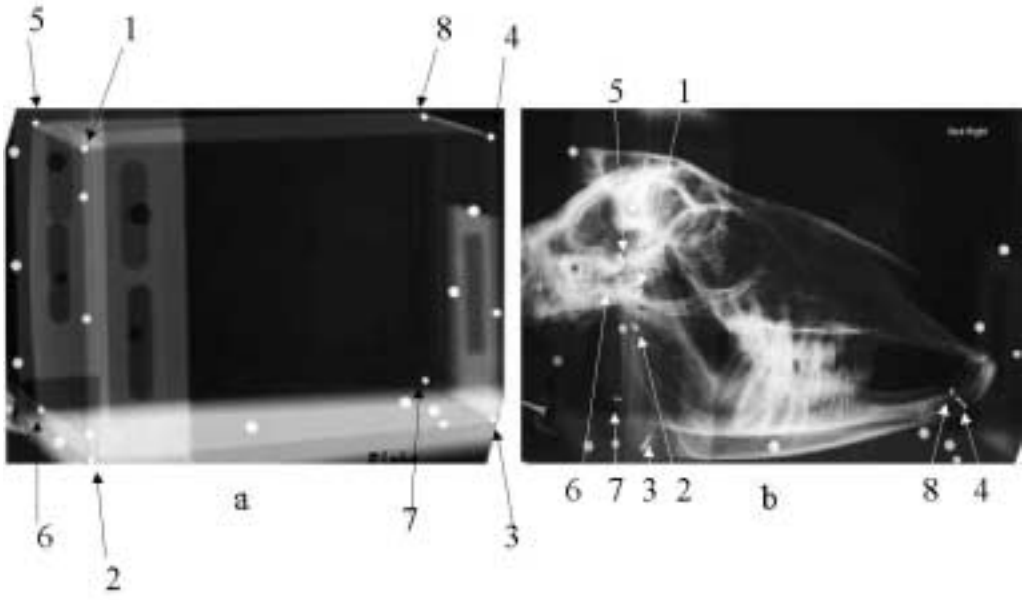


Figure 2.26. Systems used to evaluate the accuracy of the 3-D cephalometric computer programme. a: the position of a rectangular cube within the head frame; b: the position of a sheep skull within the head frame.

2.10.3. *Outcomes and conclusion*.

2.10.3.A. *Plastic cube*

There were 28 combinations of the distance between the 8 ball bearings attached to the plastic rectangular cube. In most of the 28 distances between each two ball bearings, the plotted lines fluctuated around an average value for the first one or two measurements. The line almost approached horizontal after three repeated measurements were performed. The average values of three repeated measurements were very close to the average of six manual measurements, with the distance being less than 0.02 mm. Therefore, it was concluded that the average was reliable as a “gold standard” (Figure 2.27).

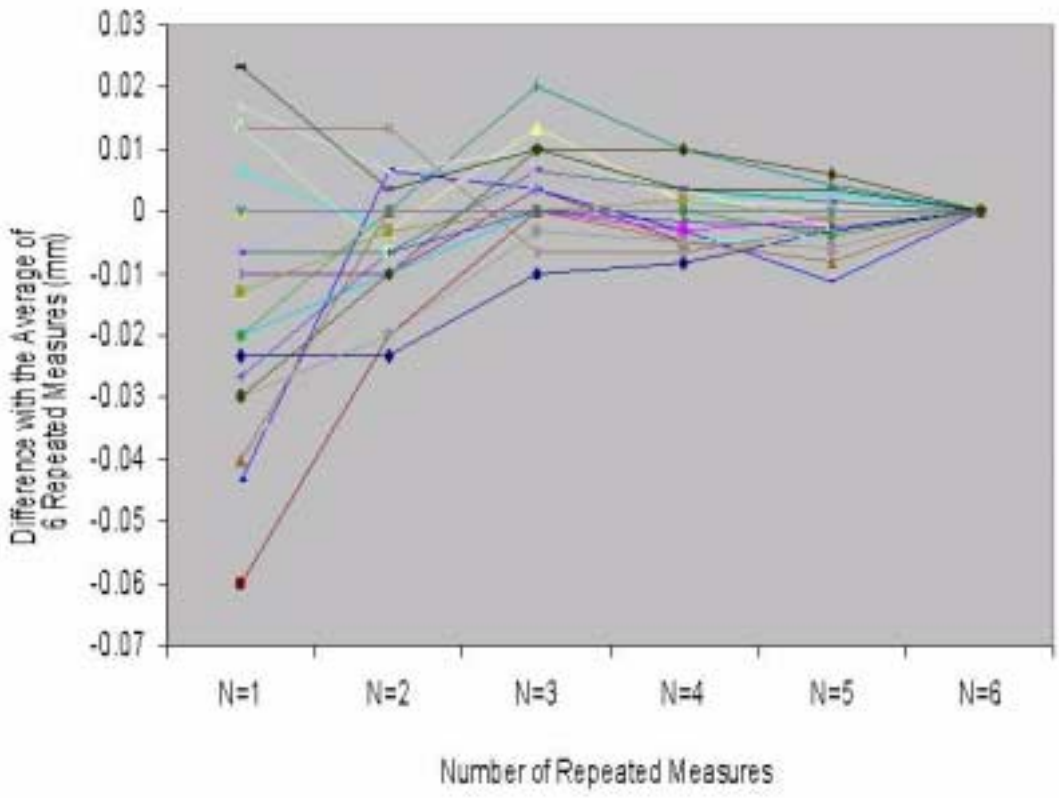


Figure 2.27. Differences in the distance between the ball bearings in repeated measurements. The average of 6 repeated measurements was set as the gold standard. Each measurement was compared with that in a cumulative manner.

The average of the six computer measurements was also calculated in a cumulative manner. The differences from the "gold standard" were then plotted against the number of repeated measurements. Since the lines were almost parallel to the x-axis, it was concluded that repeated computer measurements could not reduce the measurement error (Figure 2.28).

Based on the paired *t*-test, no significant difference was detected between the average of the nine computer measurements and the gold standard ($p=0.1752$). Based on the standard deviation and overall mean value, the random error of the computer measurement was estimated to be small at 0.46%.

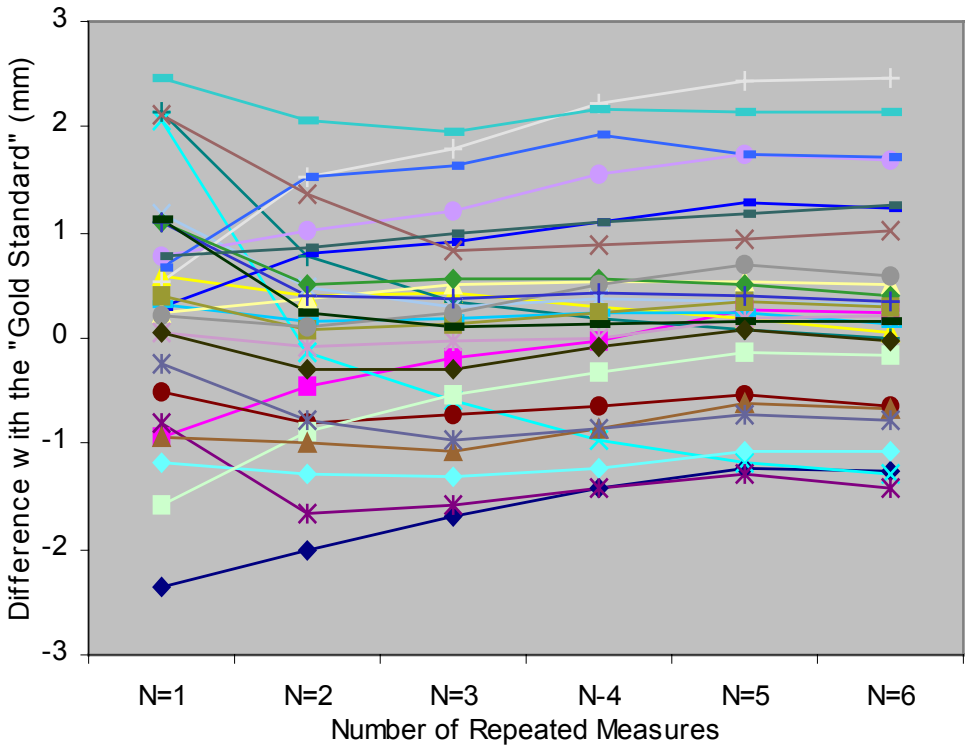


Figure 2. 28. Differences in the distance between the ball bearings measured by 3-D cephalometry compared with the direct measurement when 3-D cephalometry was performed 6 times.

The difference of each of the 28 measurements from the gold standard was also estimated (Figure 2.29) with the largest error being 2.12%.

2.10.3.B. *Sheep Skull*

There were 12 combinations of the distance between the implants. In most of the 12 distances, the plotted lines fluctuated around the average value for the first one or two measurements. The line almost approached horizontal after three repeated measurements were performed. The average values of three repeated measurements were very close to the average of six measurements, with the distance mostly less than 0.3 mm. Therefore, it was concluded that the average was reliable as a gold standard (Figure 2.30).

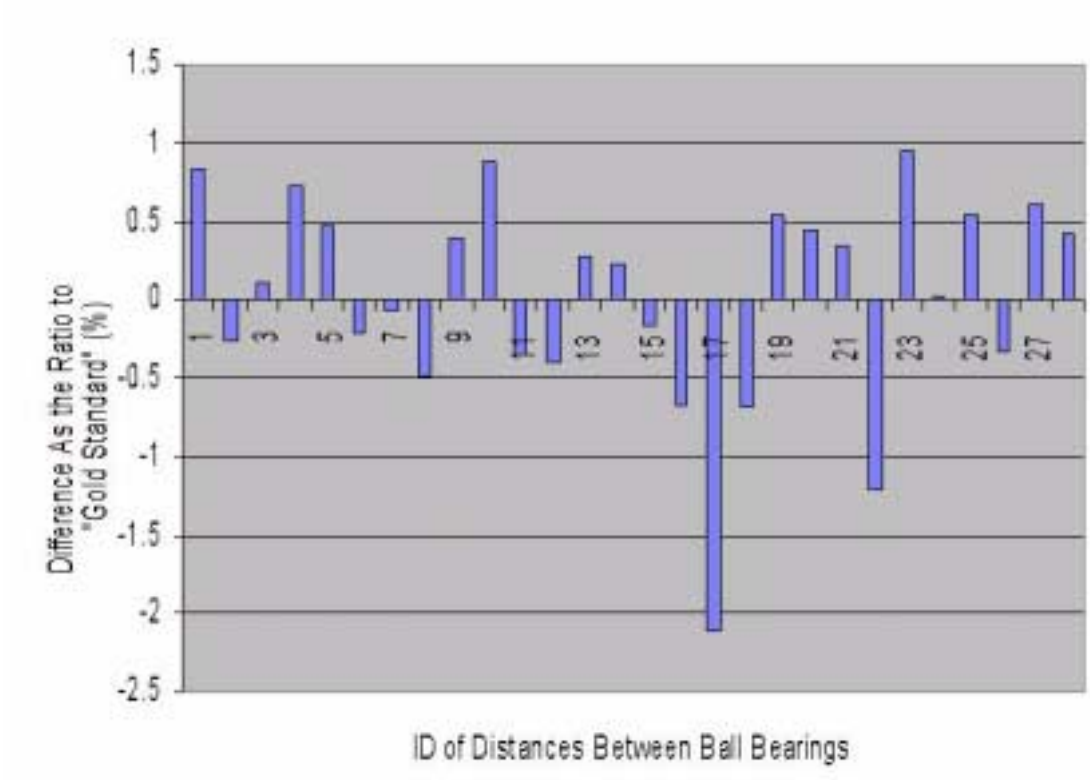


Figure 2.29. Differences in the distance between the ball bearings measured by 3-D cephalometry compared with the direct measurement. The ID of the 28 distances refers to **Figure 2.26 a**.

The average of the nine computer measurements was also calculated in a cumulative manner. The differences from the gold standard were then plotted against the number of repeated measurements. Since the lines were almost parallel to the x-axis, it was concluded that repeated measurements could not reduce the measurement error (Figure 2.31).

According to paired t-tests, the average of all the nine measures did not differ from the gold standard ($p=0.0914$). Based upon standard deviation and overall mean value, the random error of the computer measurement was estimated as 0.40%.

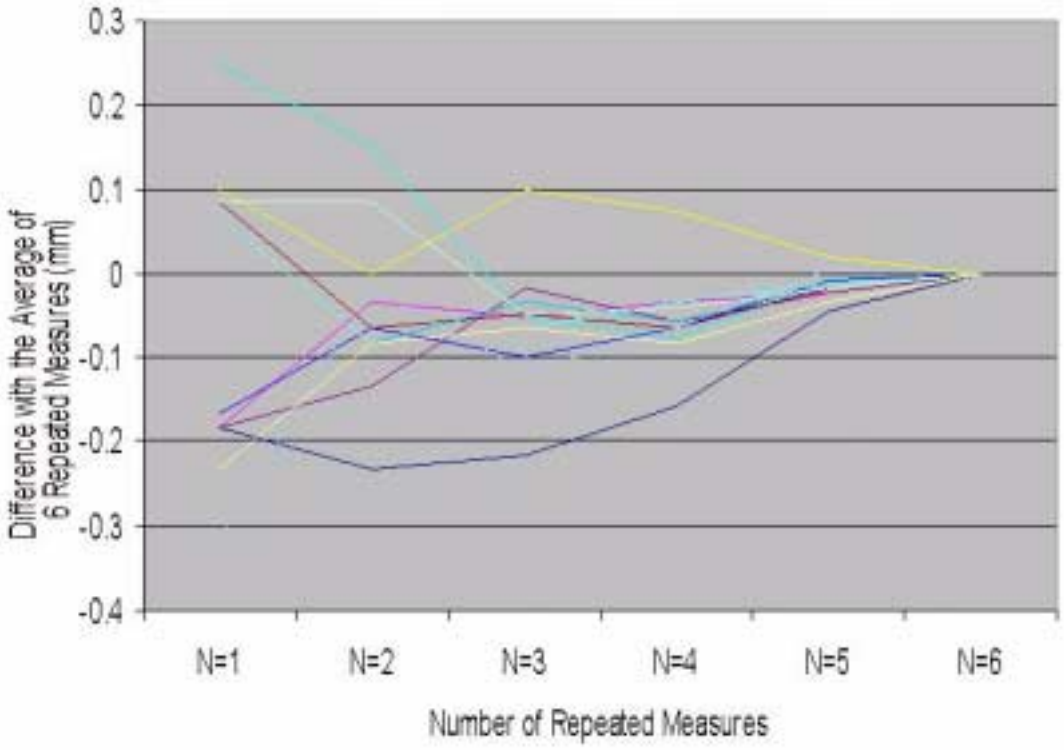


Figure 2.30. Differences in the distance between the implants in repeated measurements. The average of 6 repeated measurements was set as the gold standard. Each measurement was compared to that in a cumulative manner.

When comparing the nine single measurements to the gold standard, one significant difference was found (p=0.0385). The bias of this measurement was estimated to be 0.30%. The random error of this measurement was estimated to be 0.44%.

The difference of each measurement from the gold standard was also estimated. The largest error was found to be –1.79 % (Figure 2.32).

With regards to the error of the measurement, it was concluded that the average of three repeated measurements would provide a reliable measurement result.

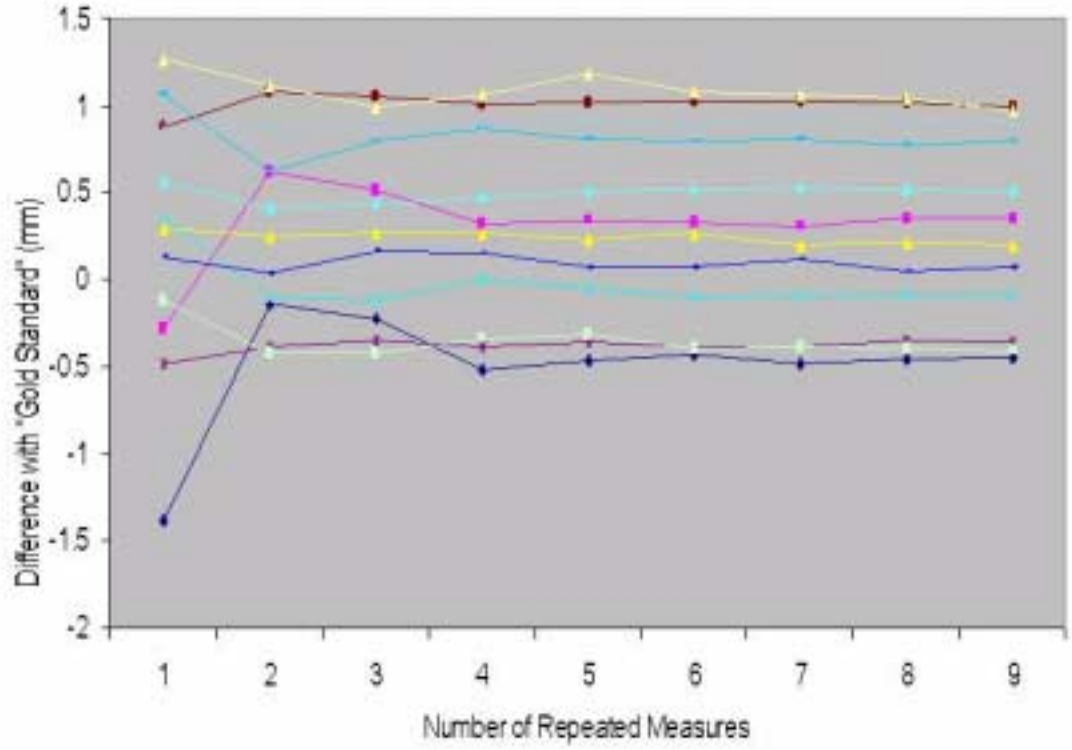


Figure 2.31. Differences in the distance between the implants measured by 3-D cephalometry compared with the direct measurements when repeated 9 times.

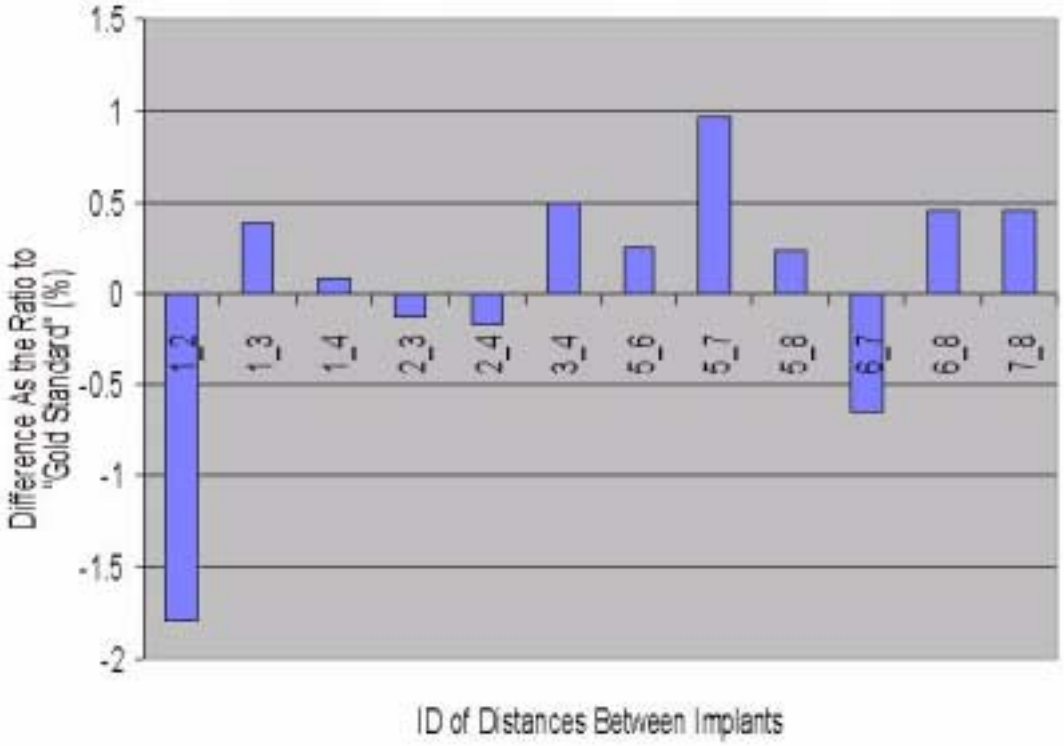


Figure 2.32. Differences in the distance between the implants measured by 3-D cephalometry compared with the direct measurement. The ID of the 12 distances refers to **Figure 2.26 b**.

Chapter 3
Experimental Forward Mandibular Displacement in Sheep

3.1. INTRODUCTION

In the treatment of class II malocclusion, growth modification in the temporomandibular joint (TMJ) by the use of functional appliances is of great interest to orthodontists (see *Chapter 1*). Currently, knowledge of the responses of TMJ components to functional appliance treatment is mainly derived from animal experiments. While radiography, computer tomography or magnetic resonance imaging can be performed on human subjects, specific histological evaluation is denied. Thus, in order to more extensively investigate the adaptability of the TMJ complex to procedures that mimic orthodontic treatment regimens, specific functional appliances have been fitted to various experimental animals, including non-human primates and rodents, to prompt the mandible into a protrusive position.

Non-human primates and humans have similar anatomy with respect to the cranial base, upper face and mandible, as well as the TMJ (Isaacson et al., 1990). The appliances used for experimental mandibular displacement in non-human primates are also similar to those used in humans, such as an inclined plane and the Herbst appliance. Reproducible morphological changes in non-human primate TMJs after treatment have been variously reported. They include thickening of the posterior mandibular condylar cartilage together with increased bone deposition both on the posterior border of the ramus and on the anterior surface of the postglenoid spine (Stockli & Willert, 1971, McNamara & Carlson, 1979; McNamara et al., 1982; Hinton & McNamara,

1984; Woodside et al., 1987). However, high cost and their relative unavailability make the widespread use of this animal model difficult.

These issues apply less to rodents and a number of investigations have reported the results of mandibular protrusion experiments in those animals. Regarding the main objective of this study, which concerns the questions relating to bone metabolism, using rodents as the experimental animal may be beneficial because many studies investigating bone metabolism use rodents and that knowledge may be helpful to this present investigation. However, aside from the obviously marked anatomical differences between the TMJs of rodents and humans, discrepancies in the adaptive response of the TMJ after treatment have been reported in rodents. For example, some studies have shown thickening of the mandibular condylar cartilage in the posterior region of the condyle (Charlier et al., 1969; Petrovic et al., 1975) while others report thinning of this cartilage (Ghafari & Degroote, 1986). Tsolakis and Spyropoulos (1997) have suggested that inconsistent outcomes in such observations are a result of the anatomical characteristics of the dental arch and mandible as well as the design and fit of the appliances. These inconsistent morphological changes in the TMJ may lead to confusion when bone metabolism in the TMJ is studied.

As an alternative to expensive non-human primates and rodents which provide inconsistent outcomes, sheep have been previously used as an animal model in craniofacial growth research (Prince et al., 1997) and in oral and maxillofacial surgery research (Karaharju-Suvanto et al., 1990; 1996).

Although some differences in function exist, sheep have TMJs of similar size and anatomy to humans (Bosanquet & Goss, 1987). Based on this similarity, the sheep TMJ has been used as a model to evaluate diagnostic techniques (Kuirata et al., 1994) and treatment approaches (Bosanquet & Goss, 1989) for several TMJ disorders, *e.g.* degenerative pathology of the TMJ, termed as osteoarthritis in humans (Ishimaru & Goss, 1992). Using the sheep as a model, the progress of induced pathological conditions has been studied, *e.g.* disc perforation (Bosanquet et al., 1991a) and occlusal loss (Ishimaru et al., 1994). The treatment of TMJ disorders have also been studied in sheep where modalities including silastic replacement following discectomy (Bosanquet et al., 1991b) and fascia repair (Bosanquet et al., 1991c) have been investigated.

The purpose of this present investigation was to evaluate the sheep as a model for dentofacial orthopaedic research. This chapter describes a novel appliance, which produces mandibular displacement in sheep and presents findings related to TMJ adaptation.

3.2. RESULTS

3.2.1. *Procedure validation*

A successful animal experimental procedure should cause less interference to growth and chewing pattern in the experimental animal. The growth of the animals was evaluated by weight gain and growth of the metacarpus of the animals.

The weight for each animal during the experiment is presented in Figure 3.1. The experimental animals maintained weight within the normal range for their age (Butterfield, 1988). The measurement error for growth of the metacarpus was 0.09 mm. No differences in the weight gain and metacarpus growth between the groups were detected (Table 3.1).

The operation period for inserting the implants varied from 1 hour to 4 hours 10 minutes with the median being 2 hours 30 minutes. The average operation time was 2 hours 32 minutes (SD: 1 hour 22 minutes). The number of occasions for repeated anaesthesia varied from 3 to 8 occasions with the median being 4 occasions.

Even though the operation time ranged from 1 to 4 hours 10 minutes, no correlation between the operation time and the weight gain was observed (Pearson correlation r=0.101, p=0.813). Furthermore, no correlation between the weight gain and the times of repeated anaesthesia was found (r=-0.263, p=0.529). Due to the lack of other information on sheep metabolism that has been monitored pre- and post-anaesthesia and the small sample size in this study, the present data was adopted to indicate that operation time and times of repeated anaesthesia had no relevant effect on the growth of the animals.

The chewing of the animals was evaluated by observation on a daily basis. Minor difficulties in chewing by the experimental animals were noticed within the first 2 or 3 days after placement of the appliance. These difficulties included irregular jaw motion and reduced chewing efficiency resulting in an

extended feeding time. Nevertheless, the animals grew and functioned normally.

Even though some components of the appliances did become displaced (11/16), they were promptly repaired and/or replaced (in 3 animals at < 1 week and 1 animal at < 2 weeks). However, it was observed that the mandible was still displaced forward during chewing when both of the lower components and at least one upper component were in place.

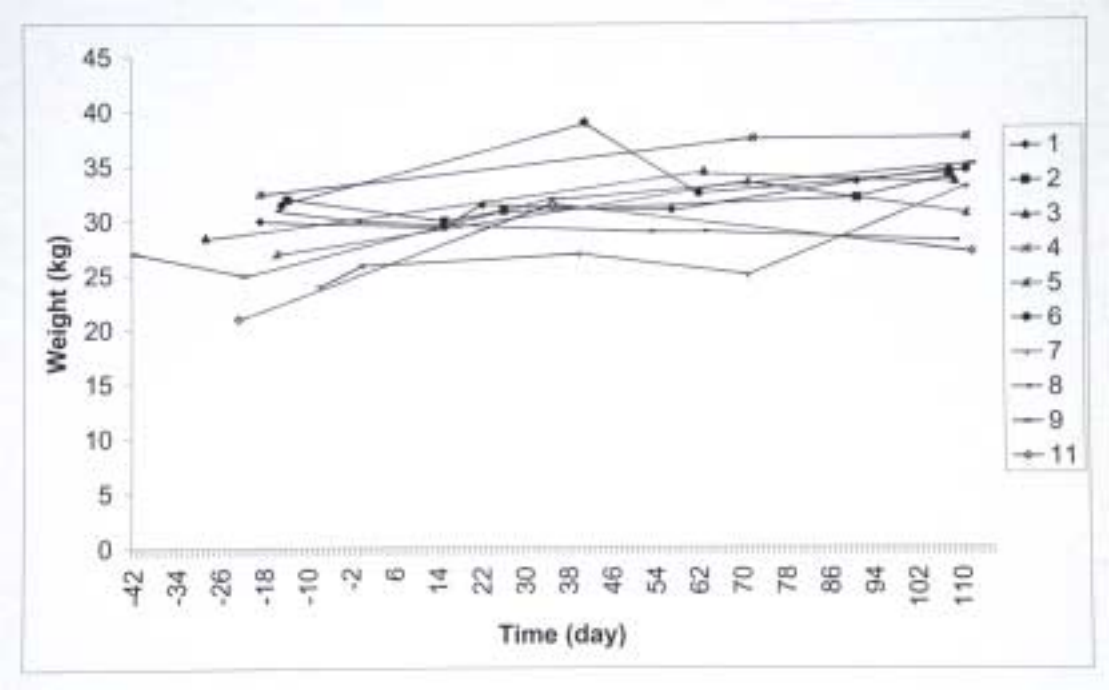


Figure 3.1. The weight of the animals during the experiment. The legend indicates the ID number of the animals in both the control and the experimental group. Negative values in time represent the pre-treatment observation period.
Protrusive appliance group: #1, #5, #6 and #8.
Inactivated appliance group: #4 and #7.
Control group: #2, #3, #9 and #11.

Table 3.1. Weight gain and metacarpus growth of the animals in the control group and the experimental group.

Group	Initial Weight (kg) (17 wk-old)		Final Weight (kg) (32 wk-old)		Weight Gain (kg)		Metacarpus Growth (mm)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control (N=4)	27.1	4.59	32.4	3.64	5.3	2.50	6.60	1.22
Experimental (N=4)	29.9	2.02	31.9	3.20	2.0	3.39	6.08	0.86

- No significance was found between the two groups by two-sample *t*-test.

3.2.2. *Observations from dental casts*

Based on observations from the dental casts, the control animals had a normal tooth wear pattern with sharp cusps in one arch interlocked with the ridges in the opposite arch. The experimental animals had a flat occlusal surface induced by the appliance (Figure 3.2) creating a cusp-against-cusp occlusion.

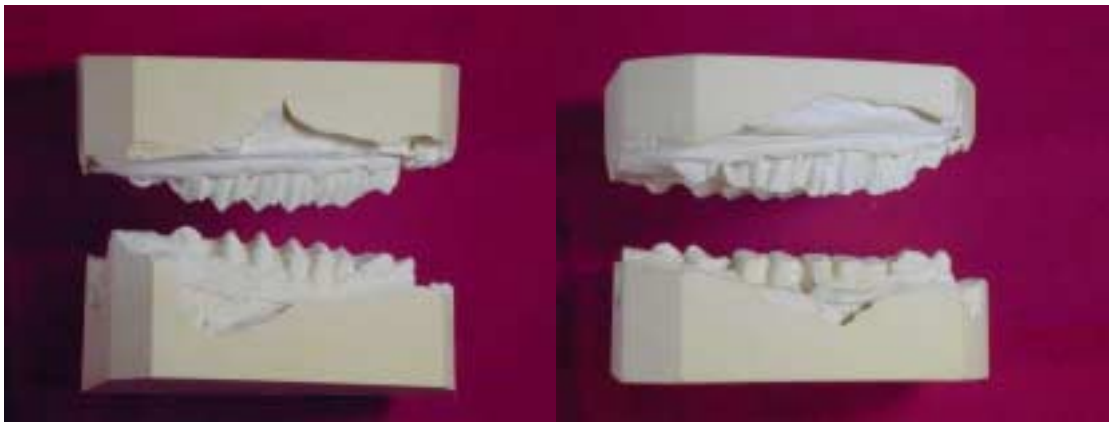


Figure 3.2. Tooth wear pattern shows differences between the control and experimental animals.
Left: Normal tooth wear pattern with sharp cusps (control).
Right: Flat occlusal surface (experimental).

3.2.3. *Observations from cephalograms*

By superimposing the tracings of cephalograms taken before and immediately after the placement of the appliance, downward and forward displacement of

the mandible was observed in the experimental animals (Figure 3.3). Detailed analysis of the effects of displacement is the subject of continuing investigation, the preliminary results of which are described in *Chapter 7*.

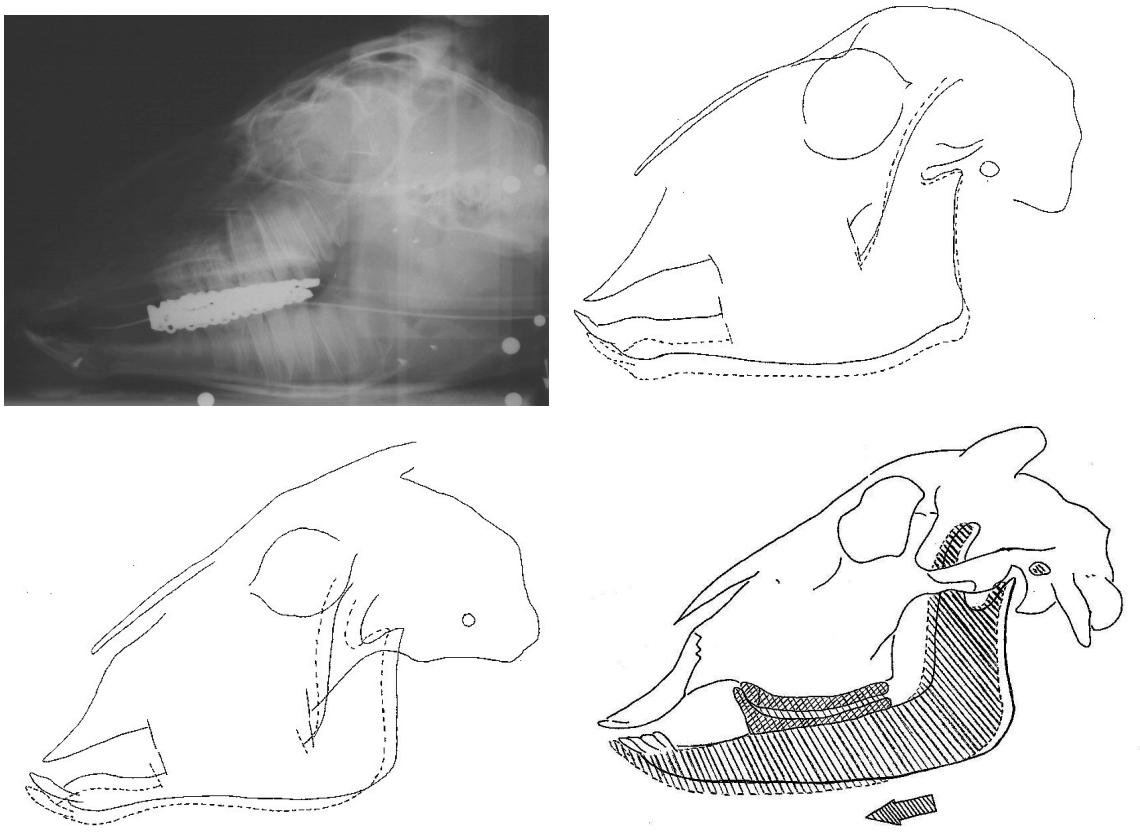


Figure 3.3. Functional appliance effects in sheep. Upper left: Lateral cephalometric radiograph of a sheep wearing the appliance; Upper right: Dot line shows the mandible at rest position; Lower left: Dot line shows the position of the mandible when occluding; Lower right: schematic presentation of the effectiveness of the appliance; arrow demonstrates the displacement of the mandible.

3.2.4. *Histological Investigation*

Microscopic observations of the histology of the TMJ revealed the following adaptive responses to the insertion of functional appliances.

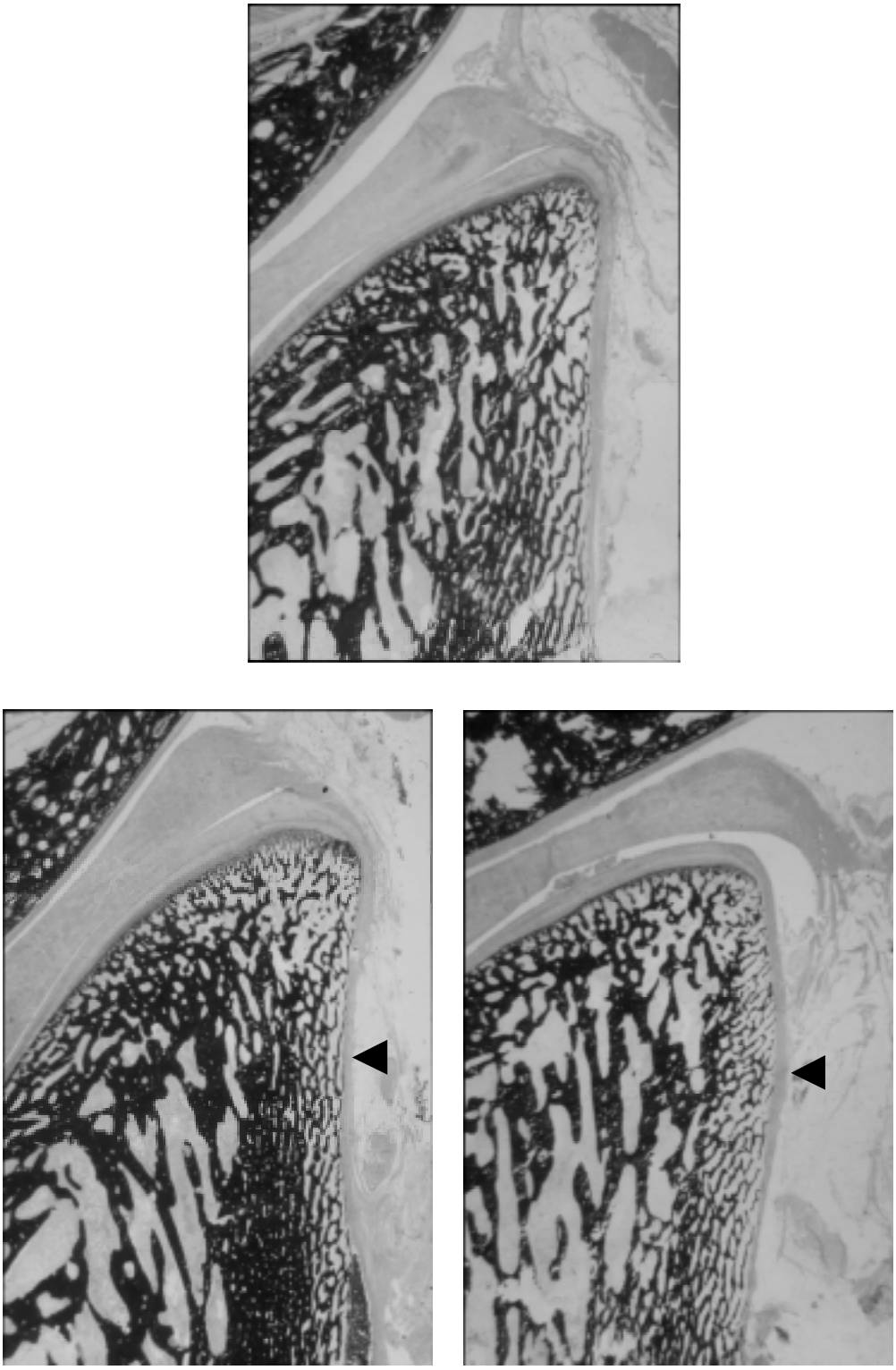


Figure 3.4. Ramal dimorphisms. Upper: Straight ramus-control. Lower Left: Concave flexure in the ramus-experimental (arrow). Lower right: Convex flexure in the ramus-experimental (arrow) (×1 objective, von Kossa/H&E stain).

Mandibular Condyle. The condylar process was less tapered and rounder in the experimental group than in the controls. A flexure (Loth & Henneberg, 1996) in the posterior border of the ramus was evident on at least one side of the mandible in each of the animals. Such flexure was either convex or concave in form (Figure 3.4).

The condylar cartilage covering the superior bony layer of the condyle comprised a hypertrophic cartilage layer (hypertrophic zone), a proliferative cell layer (proliferative zone) and a fibrous articular layer (articular zone). In the control animals, the condylar cartilage was thinnest in the anterior region and gradually thickened posteriorly. In three out of the four experimental animals, anterior thickening of the condylar cartilage was evident in at least one side of the condyle (Figure 3.5).

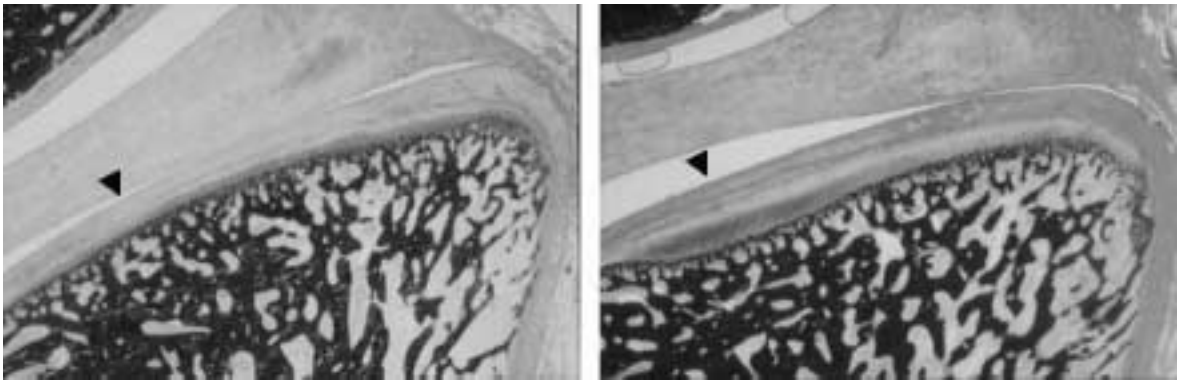


Figure 3.5. Mandibular condyle, disc and portion of fossa. Anteriorly thickened condylar cartilage in the experimental group. Left: Condylar cartilage-control (arrow). Right: Same region of the condylar cartilage-experimental (arrow) (×2 objective, von Kossa /H&E stain).

The proliferative zone and hypertrophic zone were distinguishable by the presence of dense spindle-shaped cells and intensely stained extracellular

matrix, respectively. In the experimental animals a thickening of both the proliferative and hypertrophic zones was found in the anterior region (Figure 3.6).

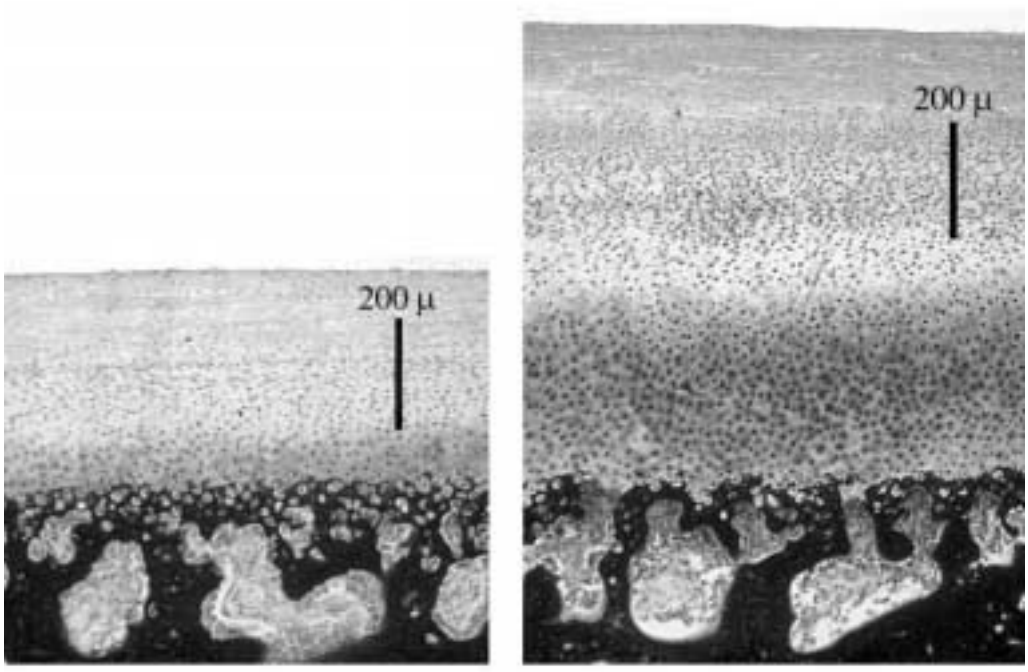


Figure 3.6. The anterior region of the mandibular condylar cartilage of the sheep. Left: Control. Right: Experimental showing thickened proliferative and hypertrophic zones (×10 objective, von Kossa /H&E).

Table 3.2. Microscopic measurements made of condylar cartilage thickness (μm) from all TMJs in the experimental and control groups.

	Control (N=8)		**Experimental (N=8)**	
	Mean	SD	Mean	SD
Anterior (μm)	401.3	58.8	538.3	170.9 *
Intermediate (μm)	543.3	74.2	531.7	138.7
Posterior (μm)	634.0	124.3	618.8	178.6

* $P<0.05$; *t* test for two independent samples.

The condylar cartilage thickness was found to be significantly increased in the anterior region in the experimental group when measurements for both left and right sides were pooled for analysis (Table 3.2). The measurement error for condylar cartilage thickness was estimated to be 7.8 μm.

Posterior Wall of the Glenoid Fossa. The posterior wall of the glenoid fossa consists of a superficial layer of articular tissue and compact bone enveloping the underlying bony trabecular meshwork. The normal structure in control animals comprises a thin compact bony layer in front of the marrow cavity with superficial islands of newly formed bone parallel to the articular surface. A difference was observed on the anterior aspect of the compact bone layer in the experimental animals where a thick compact bone layer was observed in the experimental animals with merging or merged islands of newly formed bone (Figure 3.7).

3.3. DISCUSSION

3.3.1. *Functional appliance and the mandibular displacement: static analysis vs. dynamic analysis*

In human subjects, functional appliances help to correct the Class II malocclusion either through relocating the mandible forward, increasing the growth of the mandible or inducing a re-direction in growth of the mandible (see *Chapter 1*). In sheep, a forward mandibular displacement was observed through superimposition of the cephalograms before and immediately after placement of the appliance. The quantity of the mandibular displacement achieved by the appliance associated with growth is described in *Chapter 8*. These measurements only represent the mandibular positions at fixed times while the cephalograms were taken, therefore they are referred to as static analysis of the mandibular position. The dynamic analysis of the mandibular

position modified by the functional appliances in this study referred to the mandibular position during chewing.

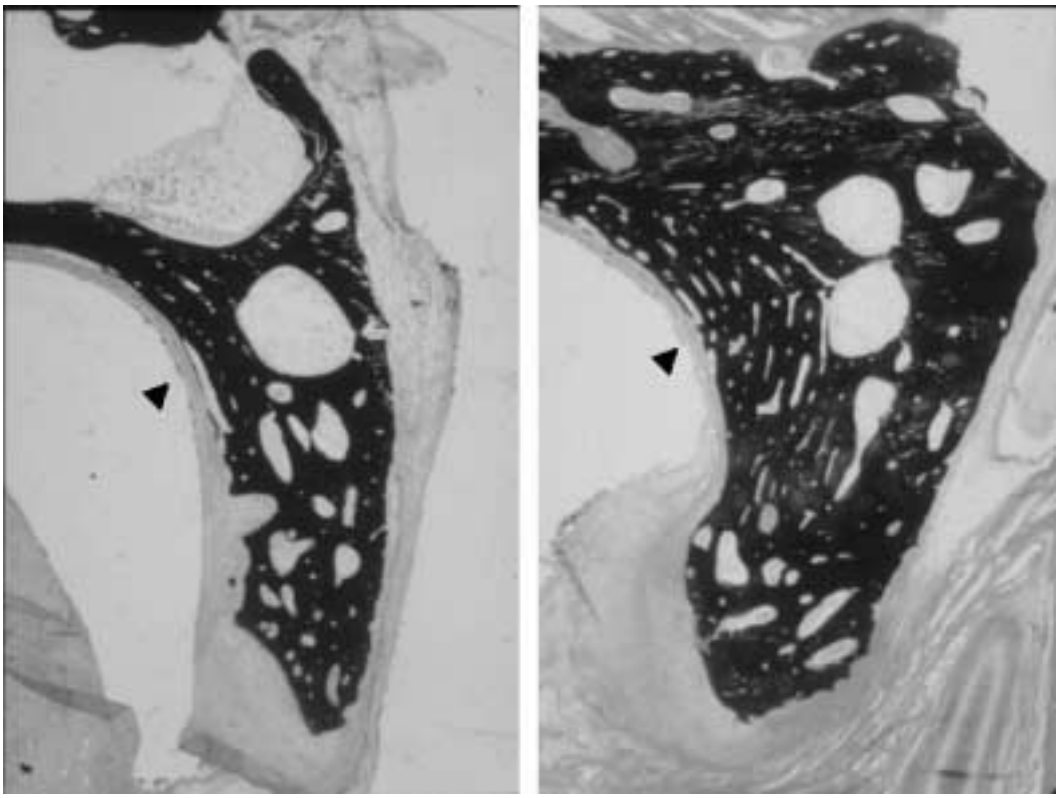


Figure 3.7. Adaptive response in the posterior wall of the glenoid fossa following insertion of the functional appliances. Left: The wall in the control animals (arrow). Right: Same region of the wall in the experimental animals showing thickened compact bone (arrow) (×2 objective, von Kossa /H&E stain).

Mandibular movement in sheep is primarily medio-lateral in character which enables the crushing and grinding of the food to break up cellulose fibres (Dovitch & Herzberg, 1968). Excursive movements and several anatomical characteristics, such as no maxillary incisors, do not allow appliances to be placed on the incisors. Anatomically, sheep have a wider upper arch than the lower arch which does not allow bilateral occlusion. In addition, sheep have an edentulous space of up to 9 cm between the mandibular incisor region and the molar teeth. Moreover, the symphysis of the mandible in young sheep allows free lateral and rotational movement of the lower jaw. Consequently,

an appliance placed on the incisors cannot create a forward displacement of the mandible during occlusion because there are no upper incisors and sheep can easily move the mandible laterally to avoid incisor guidance. Therefore, the forward displacement of the mandible in this study was created with the appliances placed on the molar segments. As a result, sheep had to protrude their mandible during mandibular excursion when the ramps of the upper and lower appliance components engaged.

The patterns of jaw motion can be observed by continuous video recording. However, the technical difficulty is the mobility of the animals. Effective video recording would need either restraint of the animal or the use of multiple video cameras. The limitations of restraining animals carry further biases in amassing; e.g. the animals may bite differently when they are restrained and cannot access the feed freely. Continuous restraint of the animal is both harmful from the viewpoint of animal husbandry and also involves ethical concerns.

Limitation of using multiple video cameras involves the determination of accuracy of the observation. In deed, the chewing pattern of sheep cannot be observed well from a distance especially when animals are not directly facing the video camera. Moreover, sheep generally eat with their heads down and the view of their jaw motion is obstructed. Thus, in this study the jaw motion of sheep was assessed only by visual assessment in this study.

3.3.2. *Appliance retention and mandibular displacement*

During the experimental period, 11 out of 16 components of the appliances became displaced at least once. Although it was observed that the mandible was still displaced forward during chewing when both of the lower components and at least one upper component were in place, the magnitude of mandibular displacement might have been changed. In addition, a partially displaced appliance may influence the pattern of jaw motion. The jaw motion pattern may also have an important role in the outcome of functional appliance treatment with the underlying mechanism of changed jaw muscle activity. Furthermore, after the appliances were repaired and put back, another change in the magnitude of mandibular displacement likely to have occurred.

The effect of change in the magnitude of mandibular displacement might be similar to reactivation of the appliance, as it is performed in human subjects to displace the mandible in a stepwise manner. However, any changes caused by the damage and/or the loss of the appliance in the present animal experiment could not be quantified. The changed magnitude of mandibular displacement could be measured using 3-D cephalometry, but this would require additional general anaesthesia.

3.3.3. *Adaptations in the TMJ*

Sheep have a flat mandibular condyle with the medio-lateral dimension being larger than the antero-posterior dimension. In this study, in order to standardise the location of the sagittal histological sections, the coronoid

process was used as a reference structure. With sectioning through the centre of the coronoid process, standardised sections of the condyle could be reliably obtained.

The posterior wall of the glenoid fossa is a feature which appears in the medial and central parts of the sheep TMJ. Viewed from the anterior aspect of the joint, the wall has a roughly triangular shape with its long side sloping laterally. As a result, the wall is usually not visible in parasagittal sections in the lateral part of the joint. By measuring the height of this structure, standardised sections for comparison of the fossa can be identified within consecutive parasagittal sections. Dramatic adaptive responses were induced in the TMJ. The changes in the glenoid fossa were very similar to those reported in non-human primates. Hinton and McNamara (1984) have described compact layers of newly-formed bone which was increased on the anterior surface of the postglenoid spine. In the present study, increased formation of new bone was observed on the posterior wall of the glenoid fossa which is analogous to that observed in the postglenoid spine in the primate.

The condylar cartilage in experimental sheep was found to thicken anteriorly. This observation concurs with the observations of Kantomaa (1984) on rabbits but differs from those of McNamara and Carlson (1979) on monkeys and of Charlier et al. (1969) on rats. However, in these studies two different approaches were used to induce similar inter-relational changes between the condyle and the fossa. One was to use intra-oral appliances to prompt the mandible to a protrusive position (Charlier et al., 1969; McNamara & Carlson,

1979). The other was surgical fixation of the interparietal, temporoparietal and both lambdoidal sutures to gradually displace the fossa to a backward position (Kantomaa, 1984). In this later study, the anteriorly thickened condylar cartilage was interpreted to be a consequence of the posterior migration of the glenoid fossa. As a result, the anterior condylar surface no longer participated in the articular function, thus, leading the mesenchymal cells in this region to differentiate into fibroblasts and they contributed to the thickening of the fibrous layer.

In contrast to the maxillofacial skeleton of the rabbit, no prominent articular eminence exists in the TMJ of the sheep. The condyle moves excursively within the fossa during function. It remains uncertain if protrusion of the mandible induced by the appliance would change the location of the articulating surface of the condyle. In the present study, thickening of the condylar cartilage anteriorly involved both the proliferative zone and the hypertrophic zone. This suggested that the progression of the mesenchymal cell lineage remained unchanged in the experimental sheep. While the observed increased thickness might be the result of a changed rate of cell differentiation, the reason for thickening of the condylar cartilage remains unanswered.

Convex or concave flexures of the posterior border of the mandibular ramus were observed in experimental animals. Ramal concave flexure at the level of the occlusal plane has been recorded in humans by Loth and Henneberg (1996). These authors have suggested “creation of the flexure is likely to

result from a change in the size, strength, or angulation of the muscles of mastication, especially the masseter and medial pterygoid muscles, which attach just below the level of flexure on the ramus". The concave flexure observed in the present study was well above the level of the occlusal plane. Convex flexure has not been reported in the human mandible. Further studies are required to assess the development of such dimorphism.

3.4. CONCLUSION

The results of the present study indicate that the sheep provides a valid model for studying growth modifications in the TMJ region after mandibular displacement. The appliance used in this study has been effective in inducing adaptive responses in the TMJ. Sheep were found to cope well with the experimental procedures. Relatively larger in size when compared with non-human primates and rodents, the sheep TMJ allows the application of stereological methods or other histomorphometrical methods for detailed quantitative analysis. A serious impediment to research in growth modification in the TMJ in humans is lack of the specific histological evaluation. The gross architectural and histological similarities of sheep and human TMJs afford us an opportunity for comparison that might allow for a better understanding of the changes likely to occur in humans where treatment involves appliances that induce mandibular displacement.

Chapter 4
Induced Mandibular Condylar Growth in a Sheep Model

4.1. INTRODUCTION

The value of the sheep for studying adaptive changes in the temporomandibular joint (TMJ) has been discussed In *Chapter 3*. This Chapter aims to further evaluate growth of the mandibular condyle in sheep following experimental functional appliance treatment.

4.1.1. *Functional appliance and the animal model*

Functional appliances have been reported to achieve correction of Class II discrepancy in human subjects through increasing the mandibular length (Pancherz, 1979; Vargervik & Harvold, 1985; Illing et al., 1998) and rotating the mandible (Williams & Melson, 1982; Birkebaek et al., 1984). These changes have been suggested to result from stimulation of mandibular condylar growth beyond that which would normally occur in growing children and a redirection of condylar growth from an upward and forward vector to a more posterior orientation (Woodside, 1998). While morphological changes in the mandibular condyle have been observed after functional appliance treatment (Paulsen et al., 1995; Pancherz, 2000), the mechanism remains unclear. The lack of understanding is partly due to the ethical difficulties involved with studying the responses in human subjects, for example, the use of endosseous implant markers, serial cephalograms and histological examination. Furthermore, the proper control of environmental factors can be a problem as well as the complicated individual variations that arise from patient compliance, growth, and factors related to treatment planning (Woodside, 1998). Consequently, human studies have inherent limitations of

weak research design, small sample size, or incomplete collection of important information (Tulloch et al., 1990).

Animal models have played an important role in investigating the mechanisms involved with functional appliance treatment. The limitations of using findings generated from animal studies to explain the human condition have also been acknowledged. For example, the sagittal discrepancy does not usually exist in animals and the experiments actually create a Class III malocclusion rather than effecting the correction of Class II malocclusion (Isaacson et al., 1991; Thilander et al., 2000). In addition, there are considerable differences between the anatomy of the cranial base, upper face and mandible of human and the anatomy of other animals. Even the use of non-human primates does not overcome this limitation because the human is unique in having a chin with lower incisors and the alveolar process grow more or less vertically (Isaacson et al., 1991). It is even more critical when quantitative analysis is proposed. The animal studies have shown significant growth modifications with various appliances, but the equivalent treatment and retention periods were too long to allow appropriate comparison with treatment in humans (Dermaut & Aelbers, 1996; Voudouris & Kuftinec, 2000). Furthermore, the growth rates in animals are usually greater than in humans, so interpretation of adaptive changes in the TMJ of animals is different. Consequently, caution about quantification of these changes for extrapolation to the human is necessary (Thilander et al., 2000).

Nevertheless, quantitative histological analysis of the biological responses to functional appliance intervention in animal models can provide valuable information regarding the mechanisms of the TMJ response to functional appliance treatment. It has been reported that sheep have similar anatomical features in the TMJ to those of humans (Bosanquet & Goss, 1987) and the adaptive responses in the TMJ to mandibular displacement have been found to be comparable with previous studies conducted on rodents and non-human primates (Charlier et al., 1969; McNamara & Carlson, 1979; Hinton & McNamara, 1984; Kantomaa, 1984; Ma et al., 2001). Hence, a sheep model was developed (see *Chapter 3*) to study simulated functional appliance treatment effects in the correction of Class II discrepancy with particular reference to the growth and development of the TMJ.

4.1.2. *Bone growth in the mandibular condyle*

The fundamental measure of bone growth in this chapter relies on fluorochrome bone labels. The distance between fluorochrome bone label bands is a well-accepted method of measuring long-bone growth and this method has also been applied to assess the growth of the mandibular condyle in rats (Suzuki, 1986). Here, fluorochrome bone markers have been used to record mandibular condylar growth in a sheep model over an active treatment and retention period. The timing of treatment was designed to represent a comparable period in humans. The related findings of normal condylar growth and induced condylar growth by functional appliance treatment in sheep are compared with other reported findings from relevant literature. The appropriateness of the sheep model for quantitative study is also discussed.

4.2. RESULTS

4.2.1. *Descriptive Analysis:*

In the bone of the embedded TMJ and the metacarpus samples, three fluorescent bands were clearly identified and their positions in the TMJ are illustrated in Figure 4.1. The metacarpus growth in the control sheep during the study period varied from 4.8 to 7.5 mm and in the experimental sheep, it varied from 5.0 to 7.1 mm.

The mandibular condylar growth in sheep was found to be greatest in the posterosuperior direction (variable 2), followed by growth in the posterior direction (variable 3) and posteroinferior direction (variable 4). The growth was least in the superior direction (variable 1). Condylar growth (variable 2) in the control sheep during the study period varied from 8.8 to 11.9 mm and in the experimental sheep, it varied from 8.5 to 13.3 mm.

Means and standard deviations among the animals of both the control and experimental groups for mandibular condylar growth and metacarpus longitudinal growth, as well as weight gain are shown in Table 4.1. The measurement error of inter-label distances in this study was estimated to be 0.35mm.

Data suggested that larger mandibular condylar growth was associated with a larger metacarpus growth and a larger weight gain. This trend was clearly

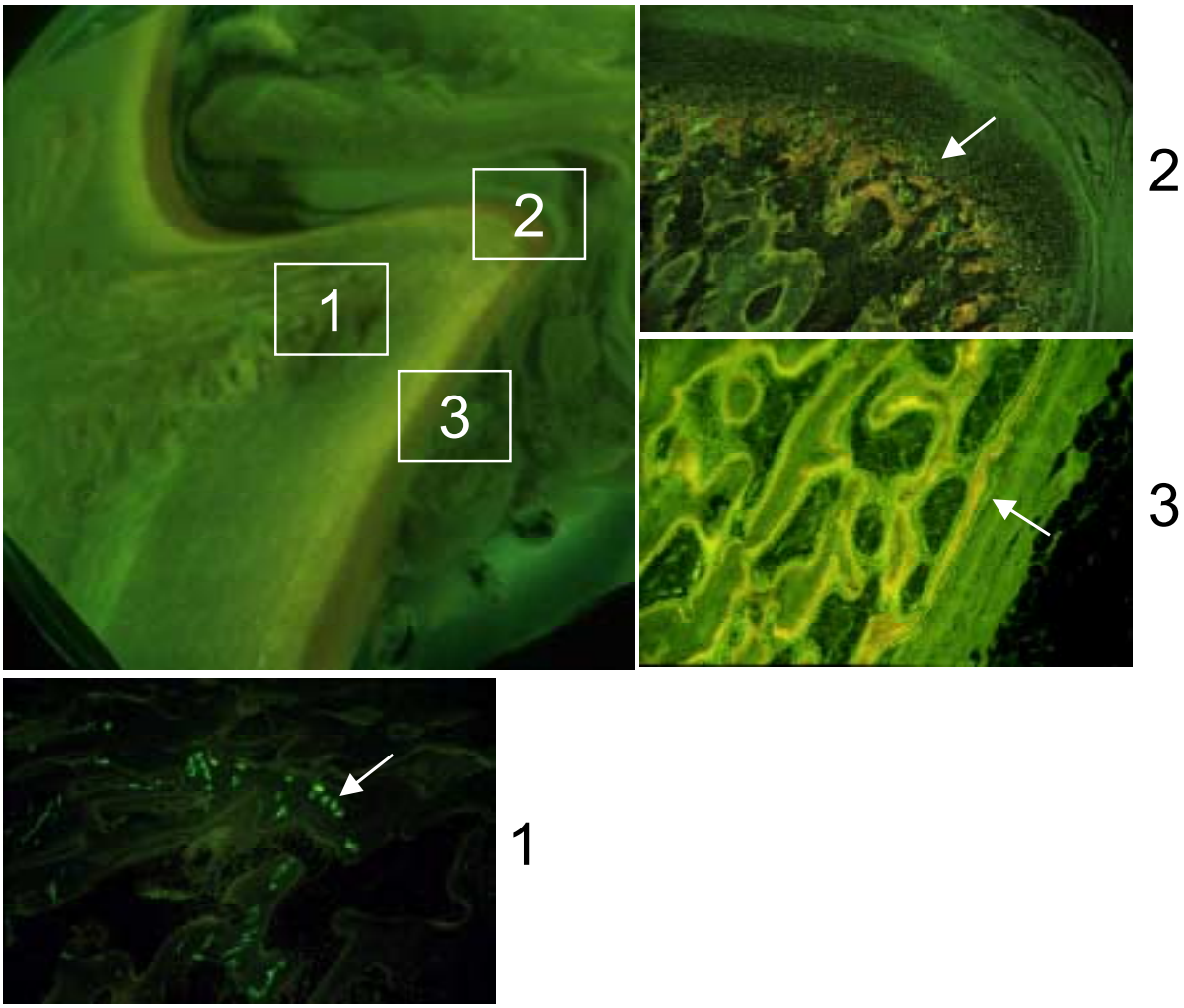


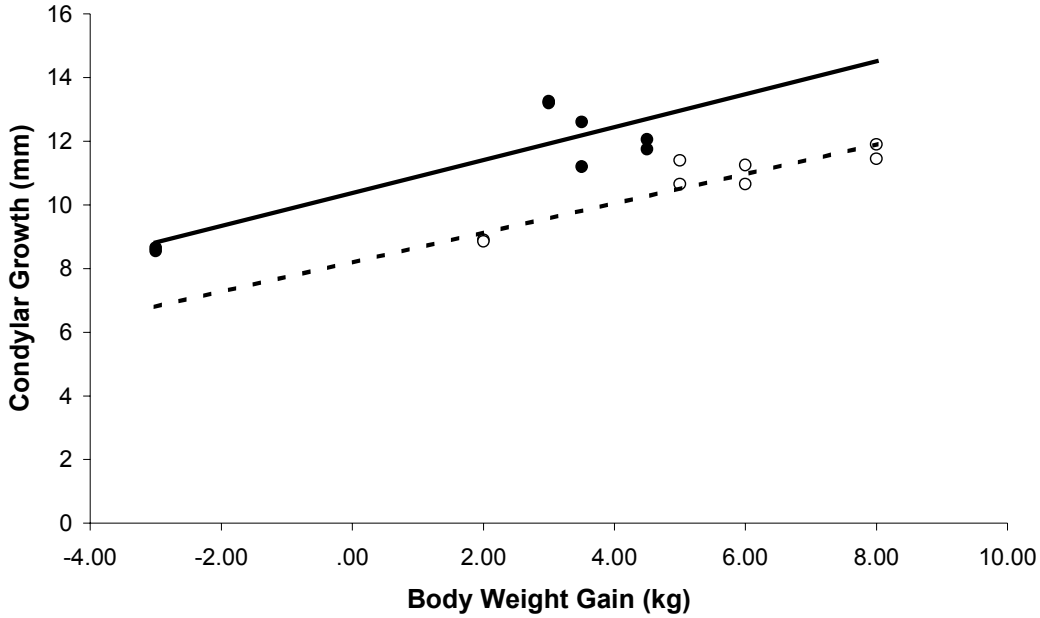
Figure 4.1. Fluorochrome bone labels calcein (green), tetracycline (yellow) and alizarin red (red) labels are clearly seen in embedded specimens from inner to outer parts of the mandibular condyle (upper left). 1: Calcein label (× 2.5); 2: Alizarin label in sub-chondral bone (× 6.3); 3: Alizarin label in periosteal bone (× 6.3).

presented on Figure 4.2 where the condylar growth was measured in its largest dimension (variable 2).

Table 4.1. Body weight gain, metacarpus growth and mandibular condylar growth in induced forward mandibular displacement (variables are illustrated in Figure 2.7 in *Chapter 2*).

Body Growth		**Metacarpus Growth (mm) (N=4)**		**Body Weight Gain (kg) (N=4)**	
		Mean	SD	Mean	SD
	Control	6.60	1.22	5.25	2.50
	Experimental	6.08	0.86	2.00	3.39
Condylar Growth Variable		**Left Side (mm) (N=4)**		**Right Side (mm) (N=4)**	
		Mean	**SD**	**Mean**	**SD**
1	Control	3.48	0.67	3.33	0.78
	Experimental	2.88	1.13	2.63	1.12
2	Control	10.63	1.20	10.65	1.34
	Experimental	11.65	2.07	11.20	2.01
3	Control	10.08	1.46	10.23	1.39
	Experimental	11.23	1.19	10.83	2.05
4	Control	7.10	1.25	6.38	1.30
	Experimental	7.90	1.49	6.43	1.02

4.2.2. *Multivariate Analysis:* From the charts presented in section 4.2.1, a larger condylar growth seems to be associated with larger metacarpus growth and larger weight gain. The purpose of multivariate analysis was to compare the difference in the condylar growth between the control group and the experimental group with the metacarpus growth and the weight gain held constant. In this investigation, multivariate analysis of covariance (MANCOVA) was selected following the procedure given by Tabachnick & Fidell (1989). Before proceeding with the MANCOVA, the variables were assessed with respect to practical limitations of the technique.



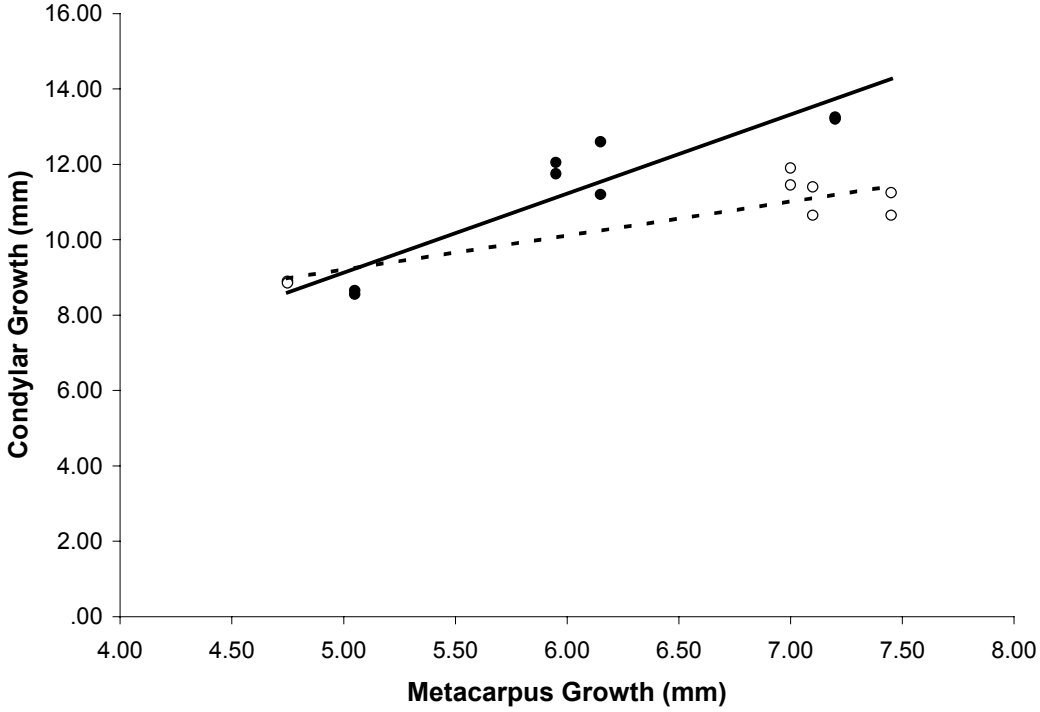


Figure 4.2. Graphic presentation of the condylar growth measured in its largest dimension (variable 2) plotted according to weight gain and metacarpus growth.

○ ····· Control

● ———— Experimental

4.2.2.A. Evaluation of Assumptions

Homogeneity of within-group regression

The proportion of the total variability in dependent variables (DVs) including GROW1, GROW2, GROW3 and GROW4 that is explained by dummy variable (TREATMEN), the covariate (CARPUS, WGTGAIN), and the interaction of dummy variable with the covariate are given by Table 4.2. The interaction of the dummy variables with the covariate is the heterogeneity of the regression slopes, which is not statistically significant; therefore we conclude that the within-group regression is homogeneous.

Table 4.2. Multivariate tests to determine homogeneity within groups.

Effect		F	Sig.	Observed Power
TREATMEN * CARPUS	Pillai's Trace	1.473	0.307	0.261
	Wilks' Lambda	1.473	0.307	0.261
	Hotelling's Trace	1.473	0.307	0.261
	Roy's Largest Root	1.473	0.307	0.261
TREATMEN * WGTGAIN	Pillai's Trace	3.209	0.085	0.528
	Wilks' Lambda	3.209	0.085	0.528
	Hotelling's Trace	3.209	0.085	0.528
	Roy's Largest Root	3.209	0.085	0.528

Model Design: Intercept+ TREATMEN+CARPUS+WGTGAIN+ TREATMEN * CARPUS+TREATMEN *WGTGAIN

Linearity of within-group regression

Since there were no combined effects of independent variable (IV) (TREATMEN) and covariates (CARPUS and WGTGAIN), a new model was written as IV and covariates. Based on “Tests of Between-Subjects Effects” (computed using alpha = 0.05), it was found that the linearity of within-group regression is good for GROW2 and GROW3. The coefficients for GROW1, GROW2, GROW3 and GROW4 are R Squared = 0.199 (Adjusted R Squared = -0.001), R Squared = 0.871 (Adjusted R Squared = 0.838), R Squared =

0.856 (Adjusted R Squared = 0.820), and R Squared = 0.546 (Adjusted R Squared = 0.433). The detail is given on Table 4.3.

Table 4.3. Tests of between-subjects effects to determine linearity within groups

Source	DVs	F	Sig.	Observed Power
CARPUS	GROW1	0.839	0.378	0.135
	GROW2	6.544	0.025	0.652
	GROW3	5.828	0.033	0.602
	GROW4	9.014	0.011	0.787
WGTGAIN	GROW1	0.201	0.662	0.070
	GROW2	12.304	0.004	0.895
	GROW3	10.530	0.007	0.845
	GROW4	0.695	0.421	0.120
TREATMEN	GROW1	1.422	0.256	0.196
	GROW2	32.624	<0.001	0.999
	GROW3	30.452	<0.001	0.999
	GROW4	1.278	0.280	0.181

In the evaluation of assumptions, the null hypothesis that the covariance matrices of the DVs are equal across groups was accepted (p=0.387). The null hypothesis that the residual covariance matrix is proportional to an identity matrix was rejected (p<0.001) and the null hypothesis that the error variance of the dependent variable is equal across groups was accepted (p=0.945). These results indicated that the data set, from its nature, was appropriate for MANCOVA.

4.2.2.B. Multivariate Analysis of Covariance

The four condylar-growth variables- 1,2,3 and 4- were treated as DVs for this analysis. The metacarpal growth and weight gain were treated as covariates. Partial output of omnibus MANCOVA as produced by SPSS appear in Table 4.4. The multivariate results show significant main treatment effects with a high statistical power.

Table 4.4: Multivariate Tests

Effect		F	Sig.	Observed Power
TREATMEN	Pillai's Trace	9.408	0.003	0.977
	Wilks' Lambda	9.408	0.003	0.977
	Hotelling's Trace	9.408	0.003	0.977
	Roy's Largest Root	9.408	0.003	0.977

Model Design: Intercept+CARPUS+WGTGAIN+TREATMEN

Assessing Covariates

The omnibus MANOVA shows a significant relationship between the sets of DVs (GROW1, GROW2, GROW3 and GROW4) and the covariates (CARPUS, WGTGAIN). These relationships can be profitably analysed by looking at the multiple regression analysis of each DVs in turn, with covariate acting as multiple continuous IVs. These analyses were automatically produced by SPSS MANOVA with deck setup, e.g., the interaction between the dummy variable and covariates depicted in Table 4.2. The analysis was done on the pooled within-cell correlation matrix, so that effects of the IV (TREATMEN) are eliminated. The results of the DV-covariate multiple regressions are shown in Table 4.5. Note that for GROW2 and GROW3, both of the covariates (CARPUS, WGTGAIN) were significantly correlated. But for GROW4, only one covariate (CARPUS) was significantly correlated. Neither of the covariate CARPUS nor WGTGAIN was significantly related with GROW1. This indicates that CARPUS and WGTGAIN provided no adjustment for GROW1. WGTGAIN provided no adjustment for GROW4, but the power is low. Additional animals might be required if this type of analysis is intended.

Assessing Dependent Variables

Univariate F's are now reported as adjusted for covariate. The univariate ANCOVA s produced by the SPSS MANOVA run depicted in Table 4.6.

Table 4.5: Parameter Estimates

DVs	Parameter	B	SE	t	Sig.	Observed Power
GROW1	Intercept	0.756	2.139	0.353	0.730	0.062
	CARPUS	0.348	0.380	0.916	0.378	0.135
	WGTGAIN	-0.060	0.135	-0.449	0.662	0.070
	[TREATMEN=.00]	0.664	0.557	1.192	0.256	0.196
	[TREATMEN=1.00]	0	.	.	.	
GROW2	Intercept	6.658	1.488	4.474	0.001	0.984
	CARPUS	0.677	0.264	2.558	0.025	0.652
	WGTGAIN	0.329	0.094	3.508	0.004	0.895
	[TREATMEN=.00]	-2.211	0.387	-5.712	<0.001	0.999
	[TREATMEN=1.00]	0	.	.	.	.
GROW3	Intercept	6.194	1.602	3.866	0.002	0.943
	CARPUS	0.687	0.285	2.414	0.033	0.602
	WGTGAIN	0.327	0.101	3.245	0.007	0.845
	[TREATMEN=.00]	-2.300	0.417	-5.518	<0.001	0.999
	[TREATMEN=1.00]	0	.	.	.	.
GROW4	Intercept	-0.061	2.303	-0.026	0.979	0.050
	CARPUS	1.229	0.409	3.002	0.011	0.787
	WGTGAIN	-0.121	0.145	-0.833	0.421	0.120
	[TREATMEN=.00]	-0.677	0.599	-1.131	0.280	0.181
	[TREATMEN=1.00]	0	.	.	.	.

[TREATMEN=1.00] is set to zero because it is redundant.

Table 4.6 Univariate Tests

DVs		F	Sig.	Observed Power
GROW1	Contrast	1.422	0.256	0.196
GROW2	Contrast	32.624	<0.001	0.999
GROW3	Contrast	30.452	<0.001	0.999
GROW4	Contrast	1.278	0.280	0.181

The F tests the effect of treatment. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

For those DVs associated with significant main treatment effects (i.e., GROW2 and GROW3), interpretation requires associated marginal means (Table 4.7). Table 4.8 contains adjusted marginal means. It was found that the experimental functional appliance induced condylar growth in the postero-superior direction (GROW2) of 2.16 mm and induced growth in posterior direction (GROW3) of 2.35 mm.

Table 4.7: Estimates

		Mean	Std. Error	95% Confidence Interval	
DVs	treatment			Lower Bound	Upper Bound
GROW1	Control	3.407	0.360	2.622	4.192
	Experimental	2.743	0.360	1.958	3.528
GROW2	Control	9.926	0.251	9.380	10.472
	Experimental	12.137	0.251	11.591	12.683
GROW3	Control	9.437	0.270	8.849	10.025
	Experimental	11.738	0.270	11.150	12.326
GROW4	Control	6.611	0.388	5.766	7.457
	Experimental	7.289	0.388	6.443	8.134

Evaluated at covariates appeared in the model: CARPUS = 6.3375, WGTGAIN = 3.6250.

Table 4.8: Pairwise Comparisons

	Mean Difference Between Groups	Std. Error	Sig.	95% Confidence Interval for Difference	
Dependent Variable				Lower Bound	Upper Bound
GROW1	0.664	0.557	0.256	-0.549	1.876
GROW2	2.211	0.387	<0.001	1.368	3.055
GROW3	2.300	0.417	<0.001	1.392	3.208
GROW4	0.677	0.599	0.280	-0.628	1.983

Based on estimated marginal means.

In summary, from multivariate analysis, “left or right side” was found to have no significant effect on condylar growth (F=2.35; P=0.153). No combined effects were found between covariates and treatment (F=3.21; P=0.85 for treatment & weight gain and F= 1.47; P=0.31 for treatment & metacarpus growth respectively). In consequence, data for both sides were pooled for further analysis. Metacarpus growth and weight gain were found to be a covariate of the condylar growth when variables 1 to 4 were treated as a multi-dependent variable (F=9.41; P< 0.01). The coefficients for the growth in the posterosuperior and posterior direction were found to be high (Adjusted r^2 was 0.84 and 0.82 respectively). The results of condylar growth in the four directions adjusted by metacarpus growth and weight gain are given in Table 4.9. The induced growth was estimated to be largest in the posterior direction (variable 3 in Figure 2.7).

Table 4.9: Adjusted Condylar Growth (mm) Using Metacarpus Growth and Weight Gain as Covariates (variables are illustrated in Figure 2.7 in *Chapter 2*).

Variable	Treatment	Pooled Mean (N=8)	Adjusted Mean	Mean Difference (95% CI)	ANCOVA (F value)	P	Power
1	Control	3.40	3.40	0.66	1.422	0.256	0.196
	Experimental	2.75	2.74	(-0.55,1.88)			
2	Control	10.64	9.93	2.21	32.624	<0.001	0.999
	Experimental	11.43	12.14	(1.37, 3.01)			
3	Control	10.15	9.44	2.30	30.452	<0.001	0.999
	Experimental	11.03	11.74	(1.39, 3.21)			
4	Control	6.74	6.61	0.60	1.278	0.280	0.181
	Experimental	7.16	7.29	(-0.63, 1.98)			

4.3. DISCUSSION

In humans, when the growth velocity is measured on the basis of height increment (cm/year), the adolescent growth spurt differs between ethnic groups (Figure 4.3a) (Eveleth & Tanner, 1976). The ascending part of the spurt has been found to cover a period of 2-3 years. In sheep, weight increment is a convenient measure of growth velocity (gm/day) and a pubertal growth spurt becomes evident (Figure 4.3b) (Edey, 1983). In the present study, a 15-week treatment period was chosen to cover the ascending portion of the pubertal growth spurt (Figure 4.2b, marked area). By comparing the pattern of the curves, growth during the treatment period in sheep is deemed to be comparable with a 2 years' growth period in adolescent humans.

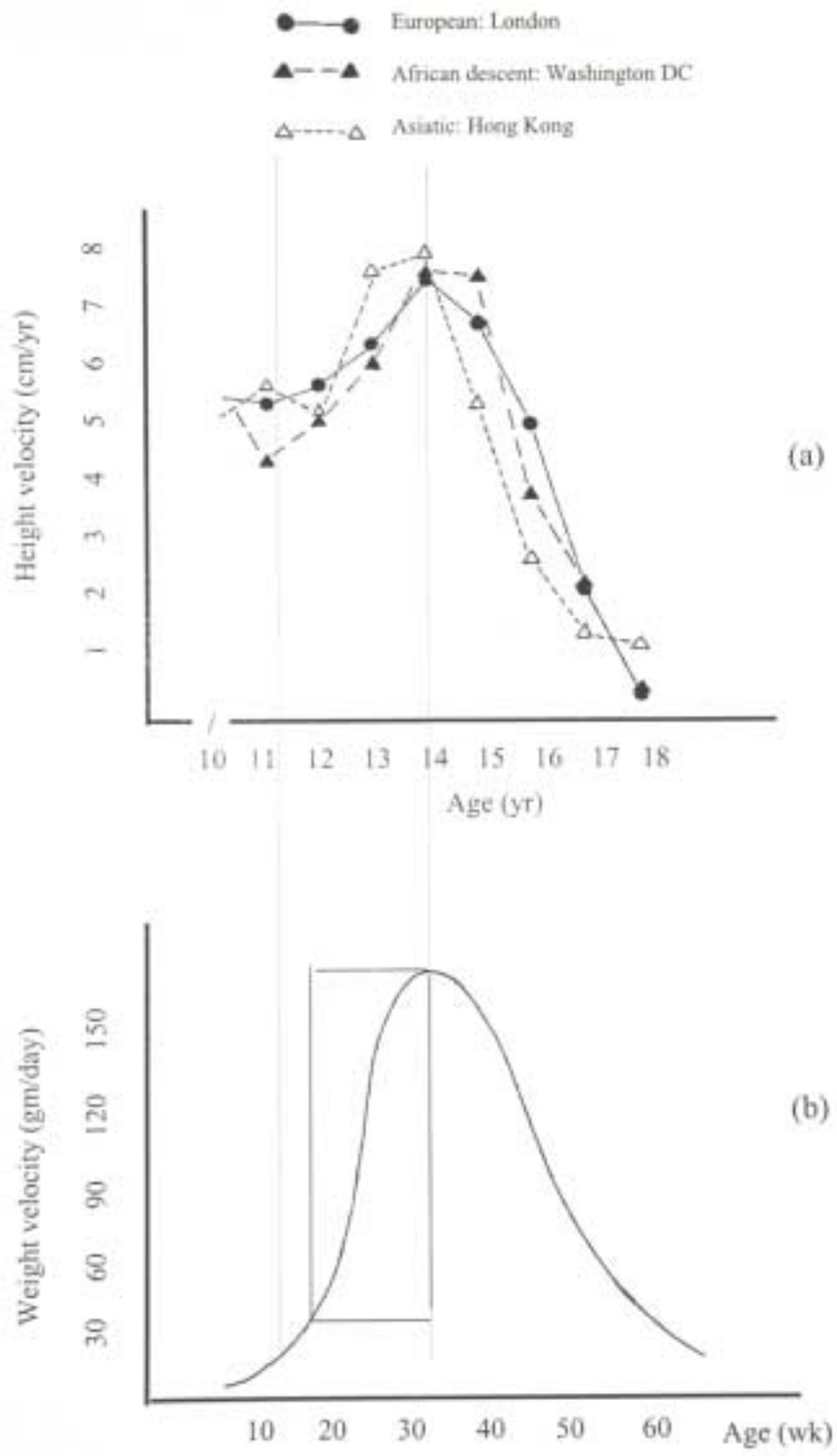


Figure 4.3: Comparison of the growth in sheep to that of humans; European boys (London), Asiatic boys (Hong Kong) and boys of African origin (Washington DC). Between dot lines (a): the adolescent growth spurt (Eveleth & Tanner, 1976) and (b): the pubertal growth spurt in sheep (Edey, 1983). The marked area covers a 15-week period in sheep which can be compared with 2 years' growth in humans.

It was particularly interesting to find that normal condylar growth and induced condylar growth following functional appliance intervention in sheep was comparable with condylar growth in humans. In humans, the annual condylar growth rate during the pubertal maximum was reported by Bjork (1963) as 5.5 mm/year with a range from 4.5 to 8 mm/year. A lesser annual condylar growth rate during the similar growth period was reported by Buschang and co-workers (1999) as 3.1 mm/year, but they also indicated that it was common for individuals to show condylar growth exceeding 5 mm per year. In the sheep model, the total length increment in the control group (the longest distance measured as variable 2, see Figure 2.7) varied from 8.8 to 11.9 mm with the mean being 10.6 mm. Assuming that this growth occurred within a period equivalent to 2 years in humans, the condylar growth rate in the sheep model is 5.3 mm/year which is also comparable with that reported for humans.

With condylar growth apparently comparable in rate with humans, the growth increment in the posterior direction (variable 3) induced by functional appliance treatment in the sheep model ranged from 1.39 mm to 3.21 mm which is comparable with the increased human condylar growth of 1-3 mm suggested by Graber (2000). However, this is slightly smaller than the reported 4 mm of condylar increment induced by the Herbst appliance (Aelbers & Dermaut, 1996). This difference could be explained by the appliances used in the sheep model having a different mode of action; i.e., the forces generated by chewing had an intermittent pattern whereas Herbst appliances create more continuous forces. Also, the measurement method may be different.

Therefore, based on the similarities of human condylar growth reported in the literature and the growth increments in the sheep model observed from this study, it is suggested that the adaptive changes in sheep TMJs after experimental functional appliance intervention, favourably reflect human growth modification effects induced by functional appliance treatment of Class II discrepancy.

The limitations of a sheep model to reflect human structure and function are acknowledged. Sheep, like rodents and many other animal models, have a mandibular condylar growth vector which is posteriorly oriented and more or less parallel to the occlusal plane. One clear disadvantage of the sheep model is that redirection of condylar growth from a superior and anterior vector to a more posterior orientation, which was one possible mechanism of functional appliance treatment in humans (Woodside, 1998), cannot be fully simulated. In the present study, slight and less superior condylar growth (statistically insignificant) associated with additional posterior condylar growth, suggests a redirection of condylar growth from a posterosuperior vector to a more posterior orientation. Any similarity in the mechanism between the redirection of condylar growth in sheep and humans remains equivocal.

4.4. CONCLUSION

Previous limited experimental and clinical studies in animals and humans have investigated the growth modification effects of functional appliance treatment upon the mandibular condyle. The present investigation has

characterised a sheep model that allows quantitative histological analysis in order to study biological responses. In contrast to rodents, this study has determined that sheep mandibular condyles exhibit similar growth in length within a period comparable with human adolescents. Moreover, the induced condylar growth by functional appliance intervention was also similar. The results obtained provide new information regarding condylar growth and growth modification. Using the sheep model, quantitative analysis of the adaptive changes in the TMJ can provide valuable information for understanding the mechanisms of functional appliance treatment in humans.

Chapter 5
Elevated Bone Forming Activity in Condylar Cancellous Bone

5.1. INTRODUCTION

Functional appliances have been reported to achieve correction of Class II discrepancy in human subjects by increasing the length of the mandibule (Pancherz, 1979; Vargervik & Harvold, 1985; Illing et al., 1998) and by rotating the mandible (Williams & Melson, 1982; Birkebaek et al., 1984). It has been suggested that these changes result from stimulation of mandibular condylar growth beyond that which would normally occur in growing children as well as redirection of condylar growth from an upward and forward vector to a more posterior orientation (Woodside, 1998). Modification of the neuromuscular environment of the dentition and associated bones has been demonstrated (Graber et al., 1997). However, the mechanism of bone response to a changed neuromuscular environment is still unclear.

The mandibular condyle is a growth site and an understanding of the subchondral trabecular bone metabolism, following changes in the masticatory musculature activity, will help reveal the mechanisms that operate during functional appliance treatment. Following reduced bite force (induced by feeding rats a soft diet), the mass of mineralised tissue and bone forming activity of the mandibular condyle decreased (revealed by decreased bone matrix-protein expression including; bone sialoprotein, osteopontin, osteocalcin and type I collagen) (Sasaguri et al., 1998). Increases of both bone forming activity (bone formation rate, osteoid volume and osteoid surface) and bone resorbing activity (eroded surface) in the mandibular condyle have also been reported following artificial interference to the

occlusion by unilateral bite raising. In addition, the mass of mineralised tissue remains unchanged (bone volume fraction) (Gazit et al., 1987).

To date, limited investigations into the bony changes in the mandibular condyle subjected to experimental functional appliance treatment have been conducted. The overall results have shown an increase in bone forming activity. In monkeys, it has been reported that newly formed trabecular bone is deposited on the posterior border of the mandibular ramus (McNamara & Carlson, 1979). In rats, additional bone trabeculae have been shown to form in the posterior region of the condyle (Stutzmann & Petrovic, 1997). During this process, increased bone formation throughout the mandible has been revealed by measuring alkaline phosphatase activity and $[^{45}Ca]^{++}$ uptake (Petrovic et al., 1982). The question of whether the tissue response to functional appliance treatment is distributed uniformly throughout the condyle has not been investigated.

Sheep have similar temporomandibular joint (TMJ) anatomical features to humans and the adaptive responses in the TMJ to forward mandibular displacement have been found to be comparable with previous studies conducted on rodents and non-human primates following functional appliance treatment (Ma et al., 2002). Furthermore, the amount of increased longitudinal condylar growth in sheep after forward mandibular displacement has been reported to be similar to that of humans (Ma et al., 2001). The sheep model simulated functional appliance treatment effects and was studied with

particular reference to the growth and development of the mandibular condyle.

The purpose was to investigate whether or not forward mandibular displacement can influence trabecular bone structure and bone formation-resorption dynamics in the mandibular condyle, and whether regional differences exist.

5.2. RESULTS

5.2.1. *Non-parametric statistics*

In growing sheep, the symphysis of the mandible is not fused and the TMJs on both sides function substantially independently. Because no difference between the left and right sides was detected for any variable either by the Mann-Whitney test or by ANCOVA, data for both left and right sides were pooled for analysis (see *Chapter 3, 4*). The median and range (minimum-maximum) for all parameters in the subchondral region, central region and pooled groups, together with the results derived from nonparametric tests, are presented in Tables 5.1 to 5.9.

All the histomorphometric parameters have been defined previously (see Table 2.2 in *Chapter 2*). Differences between the subchondral region and the central region were found for all the bone structural indices. Comparison between groups indicated that in the experimental group, bone structural indices in the subchondral region had a decrease in BV/TV associated with

decreased Tb.Th and increased Tb.Sp. But BS/BV was found to be increased in this region. In the central region, no significant difference was detected for static parameters of bone structural indices (Table 5.1-5.3).

For bone dynamic parameters, MAR was found to differ between the subchondral region and the central region in the control group but not in the experimental group. Comparison between groups indicated increased MAR values in the experimental group subchondral region. Bone dynamic parameters also indicated a significant increase in bone forming activity revealed by the increased BFRs in both the subchondral region and the central region. The data from the experimental group show, the MLT was shorter in the subchondral region indicating a faster rate of bone matrix mineralisation (Table 5.4-5.6). The increase was most pronounced in the subchondral region when BFR was calculated with respect to bone mineral surface (Figure 5.1).

For static indices of bone turnover, no difference between the subchondral region and the central region was found only for ES/BS in both the control group and the experimental group. Regional differences within the condyle were variably presented in all the other variables between the control and the experimental groups. Indeed, except for OSW, no difference between the subchondral region and the central region was found in the control group. However, differences between the subchondral region and the central region were found for OS/BS, QS/BS, OV/BV and OV/TV in the experimental group. The comparison between groups indicated decreased QS/BS in the central

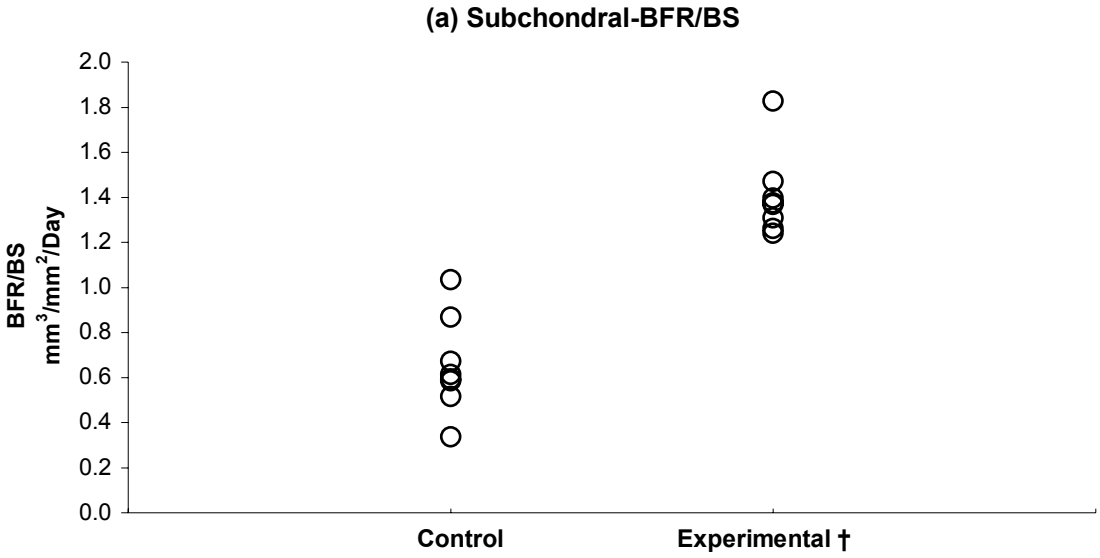
region of the experimental group. The difference for other static indices of bone turnover in the subchondral region was not statistically significant between groups (Table 5.7-5.9).

Table 5.1. Median and range (minimum-maximum) of bone structural indices for the subchondral and the central regions in the control group, experimental group and pooled data for both groups.

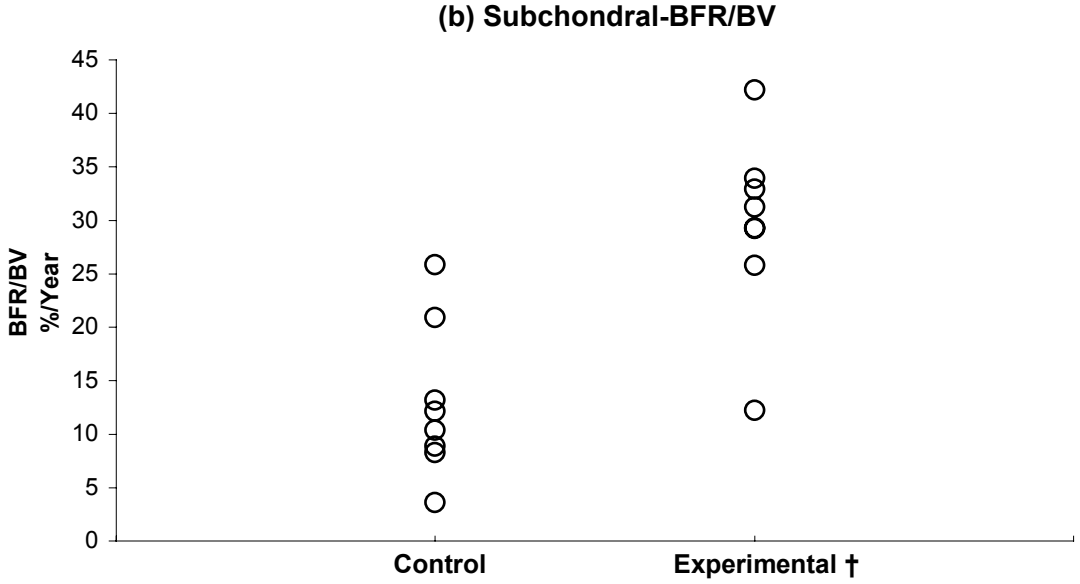
	Control Group		**Experimental Group**		**Pooled Groups**	
	Subchondral Region	Central Region	Subchondral Region	Central Region	Subchondral Region	Central Region
BV/TV	60.3* (54.1-66.5)	52.8 (39.2-61.3)	52.4 * † (47.7-60.1)	41.3 (34.2-53.1)	55.6* (47.7-66.5)	43.5 (34.2-61.3)
BS/TV	5.77* (5.38-6.38)	4.04 (3.20-4.77)	5.66* (5.19-6.31)	3.91 (3.16-4.21)	5.71* (5.19-6.38)	3.95 (3.16-4.77)
BS/BV	9.46* (8.97-10.71)	7.92 (6.20-9.80)	10.93 * † (9.47-13.05)	9.15 (7.97-10.26)	10.45* (8.97-13.05)	8.60 (6.20-10.26)
Tb.Th	0.212* (0.191-0.221)	0.253 (0.201-0.321)	0.184 * † (0.151-0.211)	0.221 (0.20-0.261)	0.194* (0.151-0.221)	0.233 (0.201-0.321)
Tb.Sp	0.132* (0.111-0.161)	0.259 (0.171-0.331)	0.163 * † (0.141-0.201)	0.299 (0.221-0.421)	0.160* (0.111-0.201)	0.298 (0.17-0.421)
Tb.N	2.89* (2.69-3.19)	2.02 (1.61-2.38)	2.83* (2.59-3.15)	1.96 (1.58-2.11)	2.85* (2.59-3.19)	1.97 (1.58-2.38)

- "*"indicates a significant difference within each group between the subchondral region and the central region revealed by Wilcoxon signed ranks test ($p<0.023$).
- "†"indicates a significant difference in the experimental group subchondral region or central region compared to the controls revealed by Mann-Whitney test ($p<0.028$).

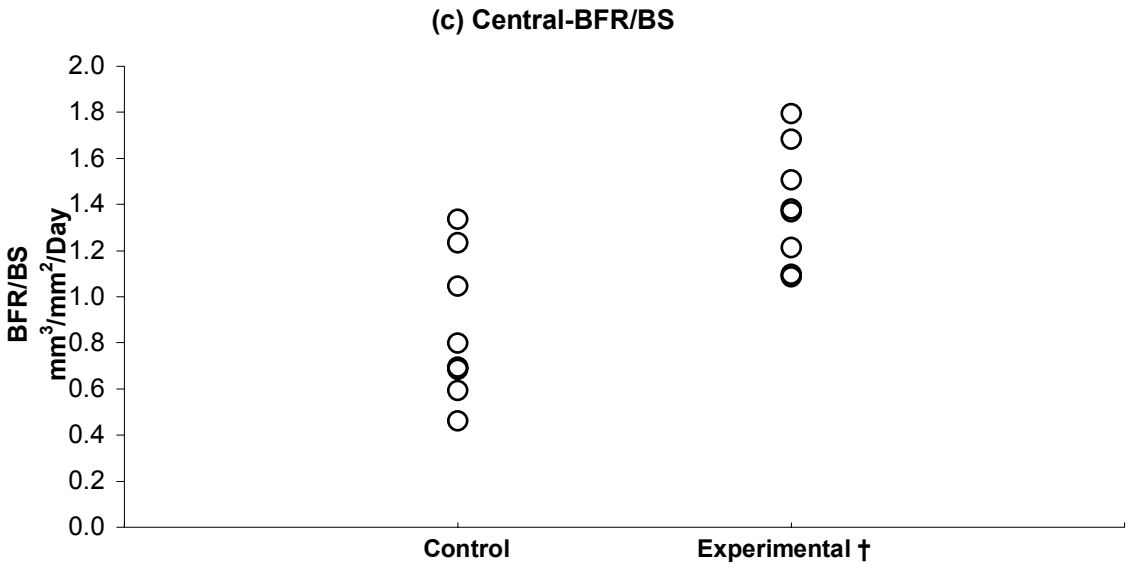
Figure 5.1: Bone formation rate in the control and the experimental condyles.



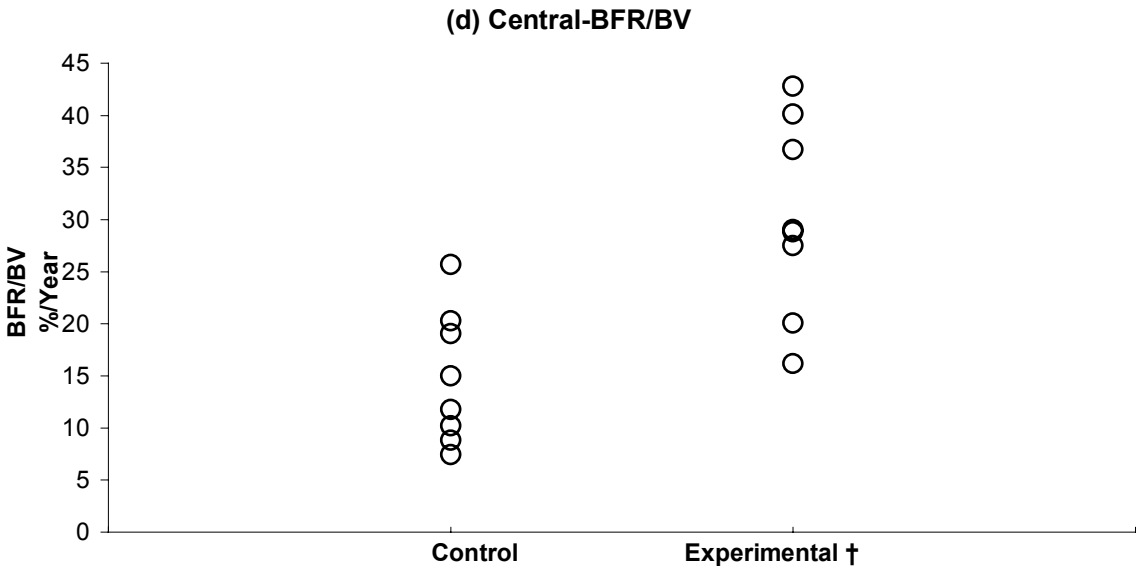
(a): Bone formation rate with respect to bone mineral surface in the subchondral region (N=8 in each group).



(b): Bone formation rate with respect to bone mineral volume in the subchondral region (N=8 in each group).



(c): Bone formation rate with respect to bone mineral surface in the central region (N=8 in each group).



(d): Bone formation rate with respect to bone mineral volume in the central region (N=8 in each group).
"†" in the experimental group data indicates a significant difference compared to controls revealed by the Mann-Whitney test.

Table 5.2. P-value of Mann-Whitney test of bone structural indices between the control group and the experimental group in the subchondral region and central region ($p < 0.05$ is printed in bold).

	Subchondral Region	Central Region
BV/TV	**0.015**	0.083
BS/TV	0.645	0.574
BS/BV	**0.010**	0.105
Tb.Th	**0.015**	0.105
Tb.Sp	**0.028**	0.234
Tb.N	0.645	0.574

Table 5.3. P-value of Wilcoxon signed ranks test of bone structural indices between the subchondral region and the central region in the control group, experimental group and pooled data for both groups ($p < 0.05$ is printed in bold).

	Control Group	Experimental Group	Pooled Groups
BV/TV	**0.008**	**0.016**	**<0.001**
BS/TV	**0.008**	**0.008**	**<0.001**
BS/BV	**0.023**	**0.008**	**<0.001**
Tb.Th	**0.016**	**0.008**	**<0.001**
Tb.Sp	**0.008**	**0.008**	**<0.001**
Tb.N	**0.008**	**0.008**	**<0.001**

5.2.2. *Analysis of co-variance*

In order to explain the decreased QS/BS in the central region in the experimental group, ANCOVA analysis was used to compare OS/BS between groups with ES/BS treated as covariate.

Before proceeding with the ANCOVA, the variables were assessed with respect to practical limitations of the technique. In the central region, the proportion of the total variability in the dependent variable (DV) OS/BS that was explained by independent variable (IV) “control or treatment”, the covariate ES/BS, and the interaction of IV with the covariate were analysed. The interaction of the IV with the covariate was the heterogeneity of the regression slopes, which was not statistically significant ($F=0.442$, $p=0.519$). Therefore it was concluded that the within-group regression was homogeneous. There was no combined effect of the IV with the covariate.

Table 5.4. Median and range (minimum-maximum) of bone dynamic indices for the subchondral and the central regions in the control group, experimental group and pooled data for both groups.

	Control Group		**Experimental Group**		**Pooled Groups**	
	Subchondral Region	Central Region	Subchondral Region	Central Region	Subchondral Region	Central Region
MAR	1.79* (1.46-2.01)	2.18 (1.71-2.58)	2.14 † (1.96-2.65)	2.32 (2.07-2.82)	2.01* (1.46-2.65)	2.26 (1.71-2.82)
BFR/BS	0.61 (0.34-1.03)	0.75 (0.46-1.34)	1.37 † (1.24-1.83)	1.37 † (1.09-1.79)	1.14 (0.34-1.83)	1.15 (0.46-1.79)
BFR/BV	11.2 (8.3-25.9)	13.4 (7.4-25.7)	30.4 † (25.8-42.2)	28.8 † (16.2-42.8)	25.8 (8.3-42.2)	20.2 (7.4-42.8)
MLT	3.61 (2.82-4.63)	3.45 (2.85-4.03)	3.04† (1.36-3.53)	3.09 (2.70-4.03)	3.17 (1.36-4.63)	3.31 (2.70-4.03)

- "*"indicates a significant difference within each group between the subchondral region and the central region revealed by Wilcoxon signed ranks test ($p<0.023$).
- "†"indicates a significant difference in the experimental group subchondral region or central region compared to the controls revealed by Mann-Whitney test ($p<0.028$).

Table 5.5. P-value of Mann-Whitney test of bone dynamic indices between the control group and the experimental group in the subchondral region and central region ($p < 0.05$ is printed in bold).

	Subchondral Region	**Central Region**
MAR	**0.001**	0.195
BFR/BS	**0.000**	**0.005**
BFR/BV	**0.000**	**0.003**
MLT	**0.028**	0.382

Table 5.6. P-value of Wilcoxon signed ranks test of bone dynamic indices between the subchondral region and the central region in the control group, experimental group and pooled data for both groups ($p < 0.05$ is printed in bold).

	Control Group	**Experimental Group**	**Pooled Groups**
MAR	**0.023**	0.461	**0.016**
BFR/BS	0.078	0.641	0.252
BFR/BV	1.000	0.547	0.597
MLT	0.383	0.148	0.669

Table 5.7. Median and range (minimum-maximum) of bone forming and resorbing indices for the subchondral and the central regions in the control group, experimental group and pooled data for both groups.

	Control Group		Experimental Group		Pooled Groups	
	Subchondral Region	Central Region	Subchondral Region	Central Region	Subchondral Region	Central Region
OSW	1.05 *	1.20	1.05	1.28	1.05 *	1.22
	(0.95-1.15)	(1.15-1.30)	(0.60-1.26)	(0.93-1.56)	(0.60-1.26)	(0.93-1.56)
OS/BS	19.6	38.4	18.2 *	50.3	19.6 *	41.6
	(11.8-57.7)	(16.7-56.1)	(0.1-34.9)	(20.6-66.9)	(0.1-57.7)	(16.7-66.9)
ES/BS	20.9	21.2	24.9	20.9	23.2	21.2
	(8.9-33.1)	(7.6-30.3)	(15.9-46.7)	(10.2-40.1)	(8.9-46.7)	(7.6-40.1)
QS/BS	53.6	49.6	55.3 *	29.1 †	54.1*	37.5
	(33.4-73.8)	(30.1-53.8)	(37.2-70.3)	(22.9-53.5)	(33.4-73.8)	(22.9-53.8)
OV/BV	0.79	2.04	0.96 *	3.85	0.82 *	2.31
	(0.15-5.09)	(1.07-3.74)	(0.01-0.27)	(1.05-5.14)	(0.01-5.09)	(1.05-5.14)
OV/TV	0.46	0.87	0.45 *	1.43	0.46 *	0.96
	(0.09-2.30)	(0.49-1.38)	(0.01-1.10)	(0.36-1.97)	(0.01-2.30)	(0.36-1.97)

- "*"indicates a significant difference within each group between the subchondral region and the central region revealed by Wilcoxon signed ranks test ($p<0.023$).
- "†"indicates a significant difference in the experimental group subchondral region or central region compared to the controls revealed by Mann-Whitney test ($p<0.005$).

Table 5.8. P-value of Mann-Whitney test of bone forming and resorbing indices between the control group and the experimental group in the subchondral region and central region ($p < 0.05$ is printed in bold).

	Subchondral Region	**Central Region**
OSW	0.983	0.554
OS/BS	0.574	0.130
ES/BS	0.382	0.721
QS/BS	0.721	**0.028**
OV/BV	1.000	0.130
OV/TV	0.941	0.328

Table 5.9. P-value of Wilcoxon signed ranks test of bone forming and resorbing indices between the subchondral region and the central region in the control group, experimental group and pooled data for both groups ($p < 0.05$ is printed in bold).

	Control Group	Experimental Group	Pooled Groups
OSW	**0.008**	0.078	**0.001**
OS/BS	0.148	**0.008**	**0.001**
ES/BS	0.313	0.148	0.105
QS/BS	0.078	**0.023**	**0.003**
OV/BV	0.109	**0.008**	**0.001**
OV/TV	0.250	**0.023**	**0.008**

Since there were no combined effects of IV "control or treatment" and the covariate ES/BS, a new model was written as IV and covariates. Based on "Tests of Between-Subjects Effects" (computed using alpha = 0.05), it was found that the linearity of within-group regression was good. The coefficient for OS/BS and ES/BS was $r^2 = 0.705$ (Adjusted $r^2 = 0.660$). The detail is given in Table 5.10.

Table 5.10. Tests of Between-Subjects Effects in the Central Region: Dependent Variable was OS/BS

Source	F	Sig.	Observed Power
ES/BS	26.484	0.000	0.997
Treatment	9.268	0.009	0.803

Also in the evaluation of assumptions, the null hypothesis that the error variance of the dependent variable was equal across the group was accepted ($p=0.783$). These results indicated that the data set, from its nature, was appropriate for ANCOVA. The omnibus ANOVA shows a significant relationship between the DV (OS/BS) and the covariate (ES/BS) (t=-5.146, $p<0.001$) which indicated ES/BS was a covariate of OS/BS.

Univariate testing was then used to compare OS/BS between the control and the experimental groups, with ES/BS taken into consideration. Univariate

testing reported a significant increase of OS/BS in the experimental group in the central region (F= 9.268, p=0.009) and the observed power was high at 0.803.

In the subchondral region, the proportion of the total variability in OS/BS that was explained by "control or treatment", the covariate ES/BS, and the interaction of IV with the covariate were also analysed. The interaction of the IV with the covariate was not statistically significant (F=0.268, p=0.614); therefore it was concluded that the within-group regression was homogeneous. There was no combined effect of the IV with the covariate.

The new model was written as IV and covariates. Unlike the finding in the central region, based on "Tests of Between-Subjects Effects" (computed using alpha = 0.05), it was found that the linearity of within-group regression was poor. The coefficients for OS/BS and ES/BS was r^2 = 0.293 (Adjusted r^2 = 0.184). The detail is given in Table 5.11. The analysis was taken no further in the subchondral region.

Table 5.11. Tests of Between-Subjects Effects in the Subchondral region: Dependent Variable was OS/BS

Source	F	Sig.	Observed Power
ES/BS	3.899	0.070	0.448
Treatment	0.233	0.637	0.073

In summary, with ANCOVA analysis, no interaction of "control or treatment" was found with the covariate ES/BS in the central region. Therefore, it was concluded that the within-group regression is homogeneous implying that no interaction existed between the treatment effect and the covariate. The

linearity of within-group regression is highly significant ($p<0.001$) with r^2 = 0.705. A significant increase of OS/BS was found in the central region ($p=0.009$) in the experimental group. No such correlation was found in the subchondral region (Figure 5.2). These results obtained from ANCOVA were in accordance with the nonparametric tests; in the experimental central region QS/BS was decreased due to increased OS/BS with ES/BS unchanged. The increased OS/BS resulted in the difference between the subchondral region and the central region in the experimental group.

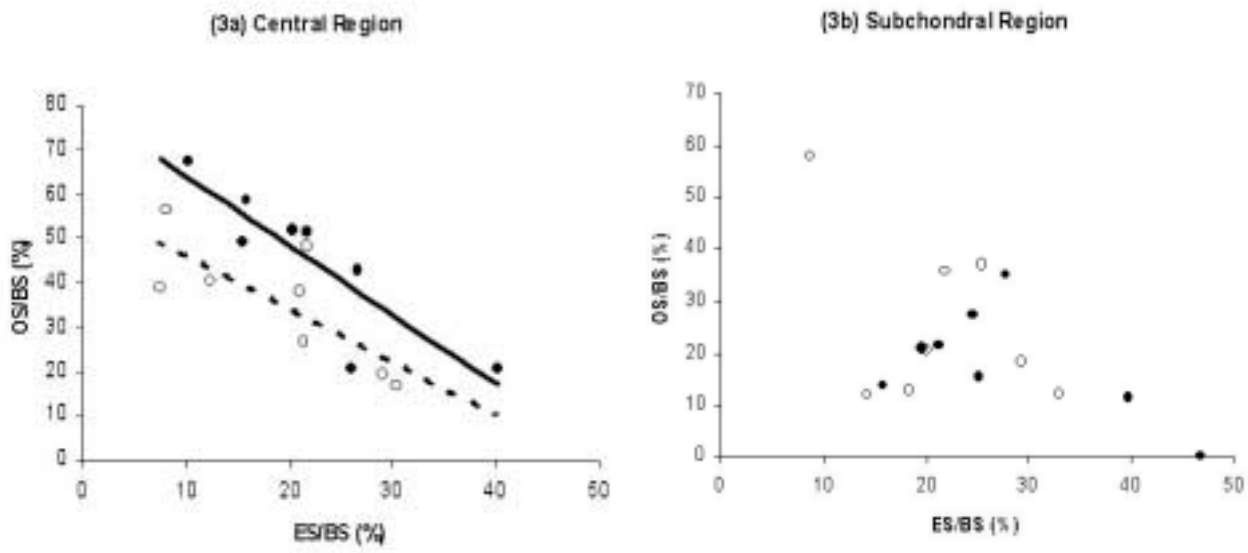


Figure 5.2. Plot of OS/BS versus ES/BS in the central region (3a) and the subchondral region (3b). In the central region, no interaction of "control or treatment" was found with the covariate ES/BS ($p=0.519$). The linearity of within-group regression is significant ($p<0.001$) with r^2 = 0.705. The straight lines are those that best fit the data for the control and the experimental groups. A significant increase of OS/BS was found ($p=0.009$) in experimental group (3a). No such difference was found in subchondral region (3b).

○ ····· Control

● ——— Experimental

5.3. DISCUSSION

5.3.1. Regional differences of bone structure and bone adaptation

The architecture of trabeculae within the condyle has been reported to differ between regions (Teng & Herring, 1995). From the mechanical point of view, Gieson & van Eijden (2000) have suggested that the subchondral region of the condyle is subject to multidirectional forces whereas mechanical forces applied to the central region are more or less unidirectional. As with previous studies, all the bone structural indices were found to be different between the subchondral region and the central region. Considering these structural differences further suggested the mechanical environment within the condyle differs regionally (Teng & Herring, 1996).

In the present study, the adaptive responses to forward mandibular displacement were not found to be uniform throughout the condyle. The subchondral region showed a more pronounced change than the central region. From the condylar growth point of view, the subchondral region was newly developed during the experiment and the central region consisted of pre-existing bone. This regional difference could be attributed to the growth modification having a great impact on bone modelling in the newly formed bone in contrast to that on bone remodelling in the pre-existing bone. This regional difference could also be attributed to the change of mechanical environment induced by forward mandibular displacement. Condylar growth has been found to be affected by, not only the magnitude of the force, but also the pattern of the force, i.e., small intermittent compressive force induces

condylar growth whereas large intermittent compressive and continuous compressive force inhibits condylar growth (Copray et al., 1985a,b).

5.3.2. The uniqueness of bone adaptation in the mandibular condyle

The present study indicated adaptive responses of the TMJ modified by the functional appliance were unique to other modifications. For example, in the findings from the central region of the condyle, similar to those of Gazit et al. (1987) obtained from an artificial interference to occlusion, an increased bone formation rate, osteoid surface and unchanged bone volume fraction were found. But differing from that study, eroded surface was found to be unchanged. Instead, a significant decrease of quiescent surface associated with a significant increase of osteoid surface tends to support the notion that only bone forming activity was increased in the present study. Another example is provided in the subchondral region of the condyle. Different from the study of Sasaguri et al. (1998) which showed that the mass of mineralised tissue and bone forming activity decreased in association with feeding animals with a soft diet, a decreased bone volume fraction was found associated with an increased bone forming activity in the present study.

One possible factor causing these differences might be the result of a different time frame in the study design. The present study used a longer study period which avoided study of the initial transient remodelling responses but focused on the new steady state, even though the completion of the remodelling may require an even longer time.

It was more likely that these differences were caused by the unique loading pattern on the condyle modified by the functional appliance. Bone adaptation was induced by a dynamic, rather than static, loading pattern (Turner, 1998). This dynamic pattern consisted of the changed magnitude and frequency of the loading. Therefore, a reduced bone volume fraction observed in this study was not necessarily the result of decreased mechanical loading. The associated increasing of bone forming activity was possibly the result of increased frequency of the mechanical loading.

5.3.3. Bone matrix formation and mineralisation

According to Parfitt (1985), histomorphometric data indicate the following the physiological significance. Bone formation occurs in two stages, matrix formation and mineralisation. Each is separate both in time and in space. The volume of the newly formed bone is determined by bone matrix formation, the matrix mineralisation does not alter this volume. The newly formed bone can be divided into mineralised bone and un-mineralised bone (osteoid). The total volume of mineralised bone was measured on von Kossa stained sections and the total volume of osteoid was measured on von Kossa/H.E. stained sections. Osteoid volume was determined by the volume-based bone formation rate (BFR/BV) and the mean osteoid life span. The osteoid life span is the same as MLT.

In the newly developed subchondral region, the mineralised bone matrix was found to be less in the experimental group when measured as BV/TV, whereas un-mineralised bone matrix remained un-changed (measured as

OV/TV), associated with an increase of the BFR/BV and a decrease of the MLT. Therefore, the increased bone formation rate observed in the subchondral region was most likely only the result of increased matrix mineralisation, but the matrix formation was not increased accordingly. As the result that the condylar dimension in the experimental animals was larger than that of the controls (*Chapter 4*), the increased bone formation was indeed spreading the unchanged volume of bone matrix into a larger space within the same time period.

The bone matrix mineralisation is under the control of the local cells, both the osteoblasts on the surface and the osteoblasts which have become buried within the osteoid. In the present study, the most pronounced difference between the two groups was identified for increased bone formation rate with respect to bone mineral surface in the subchondral region. Osteoblast and osteoclast cellular activity occurs on the bone mineral surface. Furthermore, a significant increase of BS/BV was also found in the same region suggesting the amount of bone mineral surface for a given amount of bone mineral has increased, thus increasing the available surface for bone cell activity. This difference suggests that the increased bone forming activity is associated with increased bone mineral surface providing a basis for increased activity of osteoblasts.

5.3.4. Mechanical forces and the mandibular condylar adaptation

Modification of the muscle activity during chewing has been reported after forward mandibular displacement (Graber et al., 1997), but the effect on the

mechanical environment of the TMJ is not known. Mechanical factors are known to play an important role in the differentiation, growth, modelling and remodelling of the skeleton. An overall decrease in functional loading produces bone loss and osteoporosis in patients (Krolner & Toft 1983). In rats, mechanical unloading results in a diminished growth rate of long bone growth plate with an associated reduced mass of mineralised tissue, decreased number of osteoblasts and increased number of osteoclasts (Wronski & Morey 1982).

The importance of mechanical stimulation on bone metabolism has also been described by *in vitro* studies. Studies of cultured foetal mouse calvariae or long bones have shown that mechanical stimulation by intermittent compressive force increases bone formation (Klein-Nulend et al., 1987) and decreases bone resorption (Klein-Nulend et al., 1987, 1990). It has been suggested that mechanical forces might modulate skeletal modelling and remodelling by affecting the production of local growth factors (Klein-Nulend et al., 1993).

Mechanical loading augments skeleton mass during growth, e.g., growing animals subjected to weight-bearing exercise develop thicker, denser long bones compared with non-exercised controls (Biewener & Bertram, 1994). Reduced bone volume and bone formation in the mandibular condyle is believed to be the result of reduced functional forces (Sasaguri et al., 1998). In the experimental animals of the present study, reduced bone volume fraction in the subchondral region was found associated with increased bone

formation rates, which did not indicate the functional loading on the condyle was reduced. In fact, bone adaptation not only depends on the magnitude of the force but also the frequency of the force (Turner, 1998). Furthermore, after mechanical stimulation by intermittent compressive force, bone formation increases (Klein-Nulend et al., 1987). The differences in bone formation in the newly formed subchondral bone tend to support the notion that the frequency of the functional force increased during the period of experimental functional appliance treatment.

5.4. CONCLUSION

The decreased bone volume fraction might be the result of distributing an unchanged amount of mineralised tissue into a larger total tissue volume, associated with increased condylar growth.

The regional differences in adaptive response within the mandibular condyle were also reported in this study and indicated a complicated alteration of the mechanical environment (Teng & Herring, 1996). The causal factor which increased bone formation with forward mandibular displacement was most likely to be the increased frequency of the mechanical forces of the mandibular condyle during chewing, whereas the magnitude of the forces remain unaffected. Further discussions will be made in *Chapter 7* considering the magnitude, orientation and pattern of the mechanical forces as causal factors in inducing the adaptation of the mandibular condyle.

Chapter 6
Assessment of the Normality of Bone Histomorphometric Data

6.1. INTRODUCTION

6.1.1. *Normal distribution*

In the previous chapter, non-parametric statistics were used. The limitation of non-parametric statistics is that their ability to detect a significant difference between two sets of data is lower than that of the parametric statistics. Non-parametric tests simply take account of the rank or order of the data, whereas the equivalent parametric tests make use of all the information in the data.

The distribution of much biological data follows normal distribution. With the assumption of a normal distribution, a number of important procedures can be applied to help test an hypothesis. First, the sample size can be estimated and provide guidance for the study design. Second, the confidence interval for a set of data can be calculated. This will help to define the normal range of the variable and will help to deal with the extremes in the collected data. Third, after hypothesis testing, the power of the tests and Type I/Type II error can be determined. These goals cannot be achieved with non-parametric statistics.

The purpose of this chapter was to assess the normality of the distribution of the histomorphometric data.

6.1.2. *Assessment of the normal distribution*

According to Zar (1996), the departure from normality of the data can be assessed by goodness-of-fit tests, such as chi-square or Kolmogorov-

Smionov goodness-of-fit procedures. However, these methods perform poorly and possess very low power and are not recommended.

The most desirable procedure for testing a hypotheses about normality is Symmetry and Kurtosis Measures described by D'Agostino and Pearson (1973). This test works well for sample size larger than 20.

Graphical assessment of normality involves plots of the observed data value against a predicted value. It is instructive and aids in visually assessing departures from normality.

6.2. METHODS

6.2.1. *Graphical assessment of normality*

For each variable, the sample size was 8 in each group after the left and right sides data were pooled. This small sample size did not allow good performance of Symmetry and Kurtosis Measures. Therefore graphical assessment was used.

A Normal Q-Q chart was first generated for each variable using the SPSS software package. In the chart, observed values of a single numeric variable were plotted against the expected values determined by the software based on probability theory when a normal distribution is assumed. If the sample is from a normal distribution, points will cluster around a straight line (Figure 6.1). The assessment of normality was made thereafter.

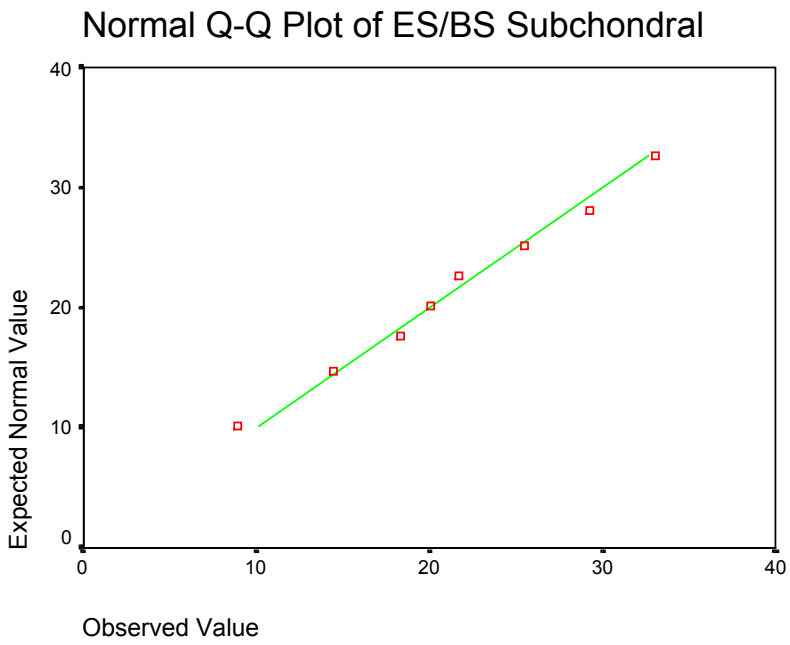


Figure 6.1. A Normal Q-Q chart showing a good correlation of the observed value (horizontal axis) and the expected normal value (vertical axis) along the straight line.

6.2.2. *Student t-test versus non-parametric test*

Based on the data in the previous chapter, a pilot study was performed to re-examine the data using parametric statistics; namely student's *t-test*. The calculation was also performed with SPSS software package.

6.3. RESULTS

The Normal Q-Q charts of the control group data and the experimental group data are presented in Appendix 1 and Appendix 2 respectively. The author concluded that all the histomorphometric variables fitted well with normal distribution since no obvious deviation was observed.

The results showed that the paired sample *t*-test and the Wilcoxon test provided the same results when a p-value of < 0.05 indicated statistical significance (Table 6.1).

Table 6.1. P-value of Wilcoxon signed ranks test and paired t-test of bone histomorphometric data between the subchondral region and the central region in the control group and experimental group (p<0.05 is printed in bold).

	Wilcoxon signed ranks test		**Paired t-test**	
	Control Group	**Experimental Group**	**Control Group**	**Experimental Group**
BV/TV	**0.008**	**0.016**	**0.010**	**0.001**
BS/TV	**0.008**	**0.008**	**<0.001**	**<0.001**
BS/BV	**0.023**	**0.008**	**0.011**	**0.001**
Tb.Th	**0.016**	**0.008**	**0.012**	**0.001**
Tb.Sp	**0.008**	**0.008**	**<0.001**	**<0.001**
Tb.N	**0.008**	**0.008**	**<0.001**	**<0.001**
MAR	**0.023**	0.461	**0.020**	0.297
BFR/BS	0.078	0.641	0.060	0.863
BFR/BV	1.000	0.547	0.538	0.609
MLT	0.383	0.148	0.479	0.131
OSW	**0.008**	0.078	**<0.001**	0.059
OS/BS	0.148	**0.008**	0.111	**0.001**
ES/BS	0.313	0.148	0.454	0.108
QS/BS	0.078	**0.023**	0.125	**0.008**
OV/BV	0.109	**0.008**	0.097	**0.004**
OV/TV	0.250	**0.023**	0.284	**0.013**

The results showed that the independent sample *t*-test and the Mann-Whitney test provided very similar results. For example, all the significant differences between the control and the experimental groups detected by the Mann-Whitney test were also detected by the independent sample *t*-test. The only differences were BV/TV and Tb.Th in the central region which were also found to be significantly different (indicated by *) when the independent sample *t*-test was used whereas they were not when the Mann-Whitney test was used.

Table 6.2. P-value of the Mann-Whitney test and independent sample t-test of bone histomorphometric data between the control group and the experimental group in the subchondral region and central region (p<0.05 is printed in bold).

	Mann-Whitney test		**Independent sample t-test**	
	Subchondral Region	**Central Region**	**Subchondral Region**	**Central Region**
BV/TV	**0.015**	0.083	**0.007**	**0.046** *
BS/TV	0.645	0.574	0.826	0.526
BS/BV	**0.010**	0.105	**0.018**	0.057
Tb.Th	**0.015**	0.105	**0.020**	**0.049** *
Tb.Sp	**0.028**	0.234	**0.021**	0.127
Tb.N	0.645	0.574	0.826	0.526
MAR	**0.001**	0.195	**0.001**	0.175
BFR/BS	**<0.001**	**0.005**	**<0.001**	**0.002**
BFR/BV	**<0.001**	**0.003**	**<0.001**	**0.002**
MLT	**0.028**	0.382	**0.022**	0.477
OSW	0.983	0.554	0.655	0.465
OS/BS	0.574	0.130	0.286	0.222
ES/BS	0.382	0.721	0.203	0.500
QS/BS	0.721	**0.028**	0.814	**0.017**
OV/BV	1.000	0.130	0.782	0.115
OV/TV	0.941	0.328	0.666	0.168

6.4. DISCUSSION

Without reliable information on the distribution of the bone histomorphometric data of the mandibular condyle, the appropriate statistical procedure is difficult to determine. Although many biological data sets follow normal distribution, other distributional pattern cannot be completely ruled out. For example, many biological data follow exponential distribution, like a growth curve, and the bone histomorphometric data analysed in this study are indeed derived to describe the growth and growth modification effects of the mandibular condyle. If the data follow a distribution other than normal, other statistical procedures need to be applied.

Based on the graphical assessment of normality, a normal distribution was not rejected. The interpretation of the statistical results does not conflict with current knowledge on the inter-relations among those bone histomorphometric parameters that have been evaluated. Therefore, normal distribution was assumed for further analysis of the histomorphometric data. Further study to increase the sample is desirable, so as to validate the normality of the data.

A statistical difference between the control and the experimental group was detected for BV/TV and Tb.Th in the central region by independent sample *t*-test, which was not detected by the Mann-Whitney test. The possible reason was that the ability of non-parametric statistics in detecting a significant difference between two sets of data is lower than the parametric statistics. Since these differences did not affect the conclusion made in the previous chapter, the statistical procedure was not repeated using parametric tests.

6.5. CONCLUSION

In the statistical analysis, the assumption that data came from the control and the experimental group at random and from a normal population with equal variances was accepted. Therefore, parametric statistics were used in *Chapter 7*.

Chapter 7
Reduced Variation of Bone Structure in the Mandibular Condyle

7.1. INTRODUCTION

The purpose of this investigation was to characterise the bone structure of the mandibular condyle during functional appliance treatment in a sheep model, in order to explore the mechanical environment of the temporomandibular joint (TMJ) modified by functional appliances using an alternative approach.

The clinical management of functional appliance treatment has focussed on modifying the action of the TMJ through the neuromuscular responses produced by altering unfavourable posturing behaviours of the mandible. In addition, removal of the restraining muscle forces, aims to re-condition the patient's musculature to sustain an altered favourable forward mandibular posture (Hicks, 1994). Ultimately, the goal is to alter the mandibular position from a muscle-controlled forward position to a structure-controlled forward position achieved by growth and remodelling of the TMJ (Chintakanon et al., 2000 (a)).

In human subjects, functional appliances have been reported to achieve correction of Class II discrepancy during growth through increasing the mandibular length (Pancherz, 1979; Vargervik & Harvold, 1985; Illing et al., 1998) and rotating the mandible (Williams & Melson, 1982; Birbekaek et al., 1984). Although it is possible in experimental animals that increased activity of the masticatory muscles increases the proliferation of condylar tissue (Oudet & Petrovic; 1978), the correlation between growth modification in the TMJ and

the mechanical environment of the TMJ modified by functional appliances has not been characterised.

The mechanical environment of the TMJ is modified by masticatory muscles, but it is unclear how the activities of masticatory muscles change following functional appliance treatment. Increased, decreased and unchanged muscle activities have been reported. McNamara (1980), using electromyography (EMG) measured with bipolar implanted hook electrodes, has reported an increased activity of lateral pterygoid muscle (LPM) and masseter muscle in non-human primates. A decreased activity in those muscles has also been reported using a very similar method (Sessel et al., 1990; Yamin-Lacouture et al., 1997). In human subjects, Aggarwal et al. (1999), using EMG with bipolar surface disk electrodes has reported an increased activity of anterior temporalis and masseter muscles. No significant change was identified using maximum muscle protrusive force and fatigue time measures (Chintakanon et al., 2000 (a)).

Inconsistency in the results may be due to the technical difficulty in measuring the muscle activity. Previous studies have focussed on the jaw protrusive muscles such as the LPM. The LPM is difficult to access beneath the overlaying structures and it is not possible to directly palpate clinically (Wanman & Agerbery, 1986). Any attempt to do so leads the possibility of confusion with the sensitivity of the medial pterygoid or temporalis muscles (Johnstone & Templeton, 1980; White, 1985). Using EMG is generally invasive with minor to severe consequences (Koole et al., 1990), which limits

its widespread use. When an alternative approach is used to assess a combination of all the protrusive muscles using maximum protrusive force and fatigue time, the possible change of muscle fibre type with the treatment makes the maximum protrusive force and fatigue time less representative of the muscle activity.

The best time to measure the muscle activity may be another issue related to the inconsistency. All the researchers have reported that the change in activity of masticatory muscles diminished shortly after appliance insertion and before correction of the jaw relationship was achieved (Auf der Maur, 1980; Sessle et al., 1990; Hiyama 1996; Yamin-Lacouture et al., 1997). Differences in timing of the measurements would contribute to the variation in the results.

With the correlation between functional appliance treatment and the activity of masticatory muscles unestablished, its correlation with bone structure and formation in TMJ tissues has been reported (Petrovic et al., 1969; 1982; Ma et al., 2002a). The correlation between the structure and the mechanical environment of bone during growth is well known. The magnitude of the force is correlated with the density of both the cortical bone and the trabecular bone. The orientation of the force is correlated with the orientation of the trabeculae. It is now generally believed that during growth, trabeculae are formed in response to principle strain magnitude and direction resulting in the trabecular structural alignment (Bertrman & Swartz, 1991). Once the trabecular alignment is established, it is not altered when functional strains are removed (Biewener et al., 1996).

The correction of Class II discrepancy was simulated in our newly developed sheep model. The morphological changes in the TMJ after the experimental functional appliance treatment was reported as a thickening of the condylar cartilage together with increased bone deposition both on the posterior border of the ramus and on the anterior surface of the postglenoid spine (Ma et al., 2002b). These findings were comparable to those in non-human primates (Stockli & Willert, 1971, McNamara & Carlson, 1979; McNamara et al., 1982; Hinton & McNamara, 1984; Woodside et al., 1987). Also in the sheep model, increased condylar dimension in the treated animals was found to be similar to that reported in the human subjects (Ma et al., 2001).

In this investigation, the bone structure in the mandibular condyle after functional appliance treatment in our sheep model was characterised and the mechanical environment of the TMJ associated with the functional appliance treatment was extrapolated.

7.2. RESULTS

In the cortical bone, the mean and standard deviation of Ct.Th, 1-CtV/TV and Ct.Th×(CtV/TV/100%) for the anterior and posterior regions in the control group, experimental group and pooled data for both groups are given in Table 7.1. No significant difference was detected between the control and the experimental group. However, the variation of Ct.Th was found to be larger in the control compared with the experimental group (Table 7.2). In the paired comparison between the anterior region and the posterior region, the 1-

CtV/TV of the cortical bone was found to be consistently lower in the anterior region than the posterior region (Table 7.3).

In the trabecular bone, *Et*, *Ec*, *Ec-Et*, maxima and the minima of Tb.An in the control group, the experimental group and the pooled data for both groups are given in Table 7.4. No significant difference was detected between the control and the experimental group (Table 7.5). However, in the experimental group, *Et* in the subchondral region was found to be significantly different from that in the central region. No such difference was found in the control group (Table 7.6).

Table 7.1. Mean and standard deviation of cortical bone structural indices for the anterior and posterior regions in control group, experimental group and pooled data for both groups.

	Control Group		**Experimental Group**		**Pooled Group**	
	Anterior	Posterior	Anterior	Posterior	Anterior	Posterior
Ct.Th	1428.6 (377.0)	3482.0 (1337.9)	1318.0† (135.8)	2481.0 (705.9)	1373.3 (279.6)	2981.5 (1155.4)
1-CtV/TV	47.3 * (4.6)	67.4 (6.9)	47.3 * (7.4)	67.2 (8.0)	47.3 * (6.0)	67.3 (7.2)
Ct.Th× (CtV/TV/ 100)	747.3 (173.2)	1130.6 (521.8)	690.1 (83.8)	801.7 (269.3)	718.7 (134.7)	966.1 (435.6)

- "*"indicates a significant difference within each group between the anterior region and the posterior region revealed by *t*-test for paired samples ($p<0.05$).
- "†"indicates a significant difference between the variance in the experimental group compared to the variance in the control group in the anterior region or posterior region revealed by Levene's test for equal variance ($p<0.05$).
- No significant difference was found between the means in the experimental group compared to those in the control group in the anterior region or posterior region revealed by *t*-test for independent samples.

Table 7.2. P-value of 2 independent sample *t*-test between the control group and the experimental group in the anterior region and posterior region without assuming equal variances between groups.

	Levene's Test for Equality of Variances	**Anterior Region**	**Levene's Test for Equality of Variances**	**Posterior Region**
Ct.Th	**0.035**	0.455	**0.020**	0.089
1-CtV/TV	0.319	0.996	0.915	0.955
Ct.Th×(CtV/TV/100)	0.098	0.415	0.197	0.135

Table 7.3. P-value of paired sample *t*-test between the anterior region and the posterior region.

	Control Group	**Experimental Group**	**Pooled Groups**
1-CtV/TV	**<0.001**	**0.003**	**<0.001**

Table 7.4. Mean and standard deviation of trabecular bone anisotropy (Tb.An) in the central and subchondral regions in control group, experimental group and difference between the two regions.

	Control group		**Experimental group**		**Pooled groups**	
	Subchondral region	Central region	Subchondral region	Central region	Subchondral region	Central region
Et	33.97 (12.80)	33.08 (13.95)	37.45 * (3.17)	36.61 (3.19)	35.71 (9.19)	34.85 (9.94)
Ec	139.35 (9.84)	137.51 (10.55)	140.00 (2.92)	139.48 (2.41)	139.68 (7.02)	138.50 (7.46)
Ec-Et	101.62 (0.96)	102.57 (3.42)	101.81 (1.18)	101.50 (0.67)	101.72 (1.04)	102.03 (2.44)
Minima	0.74 (0.05)	0.77 (0.06)	0.68 (0.09)	0.73 (0.03)	0.71 (0.08)	0.75 (0.05)
Maxima	1.31 (0.07)	1.26 (0.07)	1.39 (0.13)	1.32 (0.05)	1.35 (0.11)	1.29 (0.06)

- "*"indicates a significant difference within each group between the subchondral region and the central region revealed by *t*-test for paired samples ($p<0.05$).
- No significant difference between the variance in the experimental group compared to the variance in the control group in the subchondral region or the central region revealed by Levene's test for equal variance.
- No significant difference was found between the means in the experimental group compared to those in the control group in the subchondral region or central region revealed by *t*-test for independent samples.

In the comparison of ratios of Tb.An-min and Tb.An-max describing the trabeculae alignment of the subchondral and the central regions (see *Chapter 2*), the trabecular alignment during growth along the nominal

compressive force based on the nominal tensile force (III/II) was found to be significantly larger and the trabecular alignment during growth along the nominal tensile force based on the nominal compressive force (IV/I) was found to be significantly smaller in the experimental group (Fig. 7.1).

Table 7.5. P-value of *t*-test for 2 independent samples without assuming equal variances between the control group and the experimental group in the subchondral region and central region.

	Levene's Test for Equality of Variances	Subchondral Region	Levene's Test for Equality of Variances	Central Region
Et	0.155	0.468	0.088	0.497
Ec	0.192	0.861	0.125	0.616
Ec-Et	0.557	0.723	0.118	0.400
Minima	0.185	0.150	0.232	0.073
Maxima	0.136	0.141	0.386	0.080

Table 7.6. P-value of *t*-test for 2 related samples between the subchondral region and the central region.

	Control Group	Experimental Group	Pooled Groups
Et	0.482	**0.018**	0.167
Ec	0.381	0.294	0.251
Ec-Et	0.487	0.550	0.652
Minima	0.242	0.238	0.082
Maxima	0.223	0.189	0.063

7.3. Discussion

7.3.1. *The orientation of the mechanical forces*

From the mechanical point of view, stimulation can be characterised as

magnitude, orientation and pattern. Among them, as stated previously in this chapter, the orientation of the mechanical stimulation is closely represented by the trabecular orientation. Among the parameters of trabecular orientation, *Et* was found to be significantly larger in the subchondral region when compared with that in the central region in the experimental group, indicating a posterior rotation of *Et* during growth. In other terms, trabeculae aligned with the direction of principle tensile ε angle were more posteriorly oriented. No such difference was identified in the control group. No difference was identified for *Ec* either.

Similar to these findings, a posterior rotation of the trabeculae was also found following functional appliance treatment in rats (Charlier et al., 1969). Differing from the present approach, the trabecular orientation was measured as the major axis of the overall trabeculae in their study; neither *Et n*or *Ec* was specified.

Recent bone remodelling simulations in idealised, concave incongruous joint models have suggested that due to bending, the distribution of subchondral bone density deviates considerably from the contact pressure at the joint surface and follows more closely the distribution of tensile stress (tangential to the articular surface) than that of the compressive stress (Eckstein et al., 1997; Jacobs & Eckstein 1997). Although suggested by the authors, these idealised models have the limitation that they were not specifically adapted to a real joint; in particular, the action of muscle forces and the amount of support by the surrounding trabecular and compact bone (Eckstein et al.,

1999). These models may be correct since *Et* was found to be the only parameter modified by the functional appliance treatment in the present study.

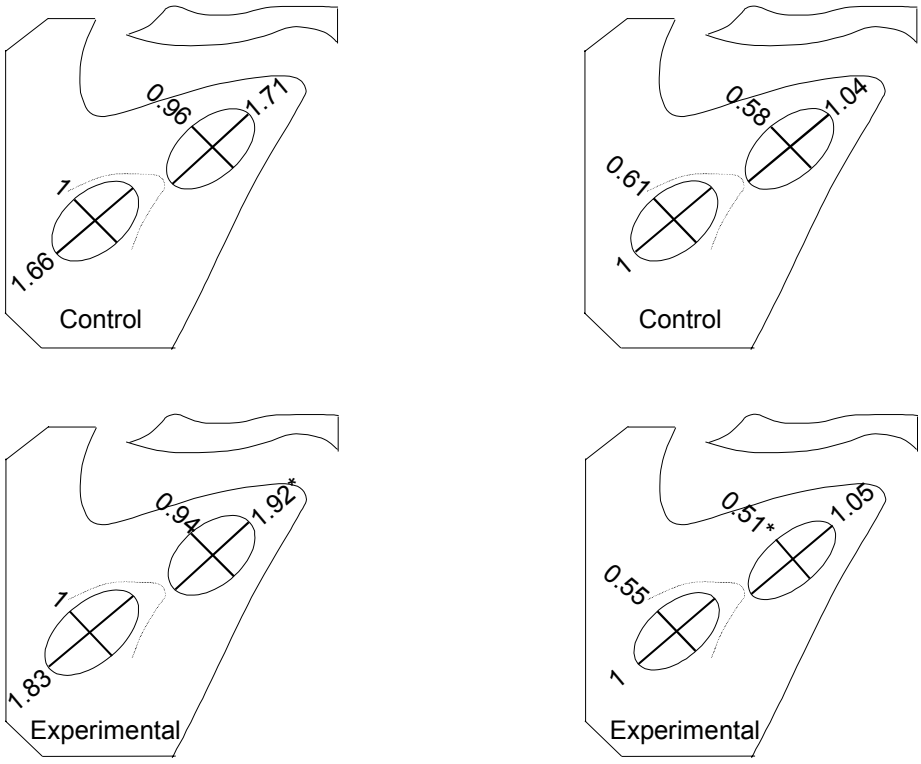


Figure 7.1. The comparison of ratios of Tb.An-min and Tb.An-max describing the trabeculae alignment of the subchondral and the central regions (see **Figure 2.21** in *Chapter 2*).
Left: within each condyle, Tb.An-min of the central region (II) is standardised and set as "1". Tb.An-max of the central region (I), Tb.An-max of the subchondral region (III) or Tb.An-min of the subchondral region (IV) are compared as numerator against it. The numbers are means of those ratios for the 8 animals of each group. "*" indicating a statistical significance $p<0.05$.
Right: same comparison. But Tb.An-max of the central region (I) is standardised and set as "1". Tb.An-min of the central region (II), Tb.An-max of the subchondral region (III) or Tb.An-min of the subchondral region (IV) are compared as numerator against it.

7.3.2. *Mechanical forces*

Mechanical stimulation augments skeleton mass during growth above the genetic baseline (Turner, 1999). BV/TV in the trabecular bone and 1-CtV/TV in the cortical bone, are close measures of the bone mass. In *Chapter 5*, BV/TV was found to be significantly lower in trabecular bone in the newly developed subchondral region in the experimental group compared to the control group. In this chapter, 1-CtV/TV was not found to be significantly different either in the anterior region or in the posterior region between the control and experimental groups. These data showing the bone mass did not increase indicated the mechanical stimulation did not increase during the experimental period.

When the ratios of Tb.An-min and Tb.An-max describing the trabecular alignment of the subchondral and the central regions were compared with the Tb.An-min or Tb.An-max of the central region set as “1” (see Figure 7.1), Tb.An-max of the subchondral region (III/II) was found to be significantly larger and Tb.An-min of the subchondral region (IV/I) was found to be significantly smaller in the experimental group. These results indicated that more trabeculae aligned with the compressive strain and fewer trabeculae aligned with the tensile strain during growth in the mandibular condyle following functional appliance treatment. The combination of the decreased BV/TV in the trabecular bone and unchanged 1-CtV/TV in the cortical bone has led the author to conclude that the magnitude of the mechanical stimuli was not necessarily increased or decreased, but that the pattern of the mechanical stimuli was more uniform in the experimental group. In particular,

a smaller standard deviation was found in the experimental group for Ct.Th (statistically significant), *Et*, *Ec* and *Ec-Et* (statistically in-significant).

Turner (1998) has stated that bone adaptation is driven by dynamic, rather than static loading. In the mandibular condyle of sheep during functional appliance treatment, more uniform mechanical stimuli were created in the experimental group by the appliance compared with those in the control group created by the occlusion. This explains the smaller variance of bone structure of the mandibular condyle in the experimental group compared with those in the controls. The lower BV/TV in the subchondral trabecular bone was more likely the result of faster growth rather than decreased loading.

7.3.3. *Similarity between human and sheep condylar structure*

This study shows lower porosity (1-CtV/TV) in the anterior region of the condyle in sheep. This is another similarity between sheep and other species including humans. Generally, the anterior regions of the condyle were stiffer and stronger than the posterior regions. In pigs the anterior joint surface appears to be the major bearing area of the TMJ (Teng & Herring, 1996). Anatomic and computer studies of human TMJ have also suggested that anterior areas are more stressed than posterior areas (Tanaka et al., 1994). TMJ erosion occurs more frequently on the anterior and lateral areas of the condylar joint surface than elsewhere (Oberg et al., 1971; Takamura & Maruyama, 1980; Mongini, 1984).

In this study, viewed from the sagittal aspect, the trabeculae in the sheep mandibular condyle aligned with the principle compressive strain angle were found to follow a postero-superior direction. Measured from anterior to posterior and referring to the occlusal plane, the angle was 139.35° to 140.00° in the subchondral region and 137.51° to 139.48° in the central region. These angles were slightly larger than those in pigs when measured by Teng and Herring (1996) from posterior to anterior and referring to the occlusal plane, which were 51.8° in the superior region and 51.5° in the inferior region respectively. If these angles in pigs were measured from anterior to posterior, these angles would be 128.2° in the superior region and 128.5° in the inferior region, respectively.

In the TMJ, the trabecular patterns of the bone reflect its functional loading patterns (O'Ryan & Epker, 1984). Computer models suggested that mechanical loading on the mandibular condyle differs between the working-side and the balancing-side; the loading also varies according to the site of tooth contact and the clenching task (Korioth & Hannam, 1994). Nonetheless, the largest loads on the TMJ are thought to be vertically oriented (Hylander, 1979, DuBrul, 1988; Ferrario & Sforza, 1994; Tanaka et al., 1994; Teng & Herring, 1996). Further studies are recommended to analyse trabecular patterns in the condyle from the frontal aspect and to measure the stiffness and strength of the mandibular condyle in sheep, so as to clarify the associations between the trabecular orientation and loading axis.

7.4. Conclusion

There are close inter-relationships between the mandibular condylar bone structure and its mechanical environment (Teng & Herring, 1996). Although the mechanical forces applied to the mandibular condyle was not directly measured in this study, based on the structure of the mandibular condyle, the mechanical environment of the TMJ during functional appliance treatment was extrapolated as follows. (1) Since the porosity of the cortical bone was not different between the experimental group and the control group, the magnitude of the mechanical forces applied to the TMJ was not necessarily changed during the treatment. (2) Since the tensile principle ε angle was posteriorly rotated, the orientation of the mechanical forces applied to the TMJ was changed, most likely to a more posterior orientation. (3) Since the variation of bone structure was smaller in the experimental group, the pattern of the mechanical forces applied to the TMJ (both the magnitude and the orientation) was more constrained by the functional appliances.

Chapter 8
Changed Mandibular Position During Functional Appliance Treatment and Related Histological Changes in the Mandibular Condyle

8.1. INTRODUCTION

The changes in the mandibular condyle induced by functional appliances have been reported in previous chapters: Increased thickness of the condylar cartilage was reported in *Chapter 3*. Increased bone forming activity in the mandibular condyle was reported in *Chapter 5* and the change of bone structure was reported in *Chapter 5* and *Chapter 7*. These changes were all associated with an increase of the condylar dimension as reported in *Chapter 4*. All these changes in the temporomandibular joint (TMJ) have been thought to be the result of the growth modification effects of the functional appliance treatment.

It is of great importance to understand how these changes are associated with the functional appliance treatment. Technically, functional appliances induce the changes in the TMJ through changing the position of the mandible. Analysis of the association between mandibular position and the histology of the TMJ will help to understand the mechanisms involved with functional appliance treatment. The aim of this investigation was to evaluate the effect of the functional appliance on the position of the mandible, and its association with histological changes in the TMJ.

In this investigation, the mandibular position was measured using 3-D cephalometry with the assistance of the eight metal implant markers in each animal. The detailed method was described in *Chapter 2*. The linear relationships between those implant markers were measured and the angular

relationships between those implant markers were calculated. The linear relationships included the distance between the zygomatic process implant and the condylar implant (Zy-Co), the distance between the zygomatic process implant and the gonion implant (Zy-Go), the distance between the zygomatic process implant and the chin implant (Zy-Ch).

The angular relationships between the implant markers referred to the position of the condylar, gonion and chin implants relative to the zygomatic implant. The line Go-Ch was used to represent the mandibular plane. The purpose of this measurement was to analyse the rotation of the mandible. If the mandible underwent forward translation without any rotation, an increase of angle ∠Zy-Go-Ch or ∠Zy-Co-Ch would be found associated with the increased distances between Zy-Co, Zy-Go or Zy-Ch. If the mandible underwent forward rotation, an increase of the angle ∠Zy-Ch-Go or ∠Zy-Ch-Co would be associated with increased distances between Zy-Co, Zy-Go or Zy-Ch (Figure 8.1). To facilitate calculating the angles, the distances between those implants were first measured. The relative angles were then calculated based on those distances using trigonometrical formulae for the solution of oblique triangles when three sides are given. The following angles were calculated: ∠Zy-Ch-Co, ∠Zy-Ch-Go, ∠Zy-Co-Ch and ∠Zy-Go-Ch.

Because in sheep the mandible moves somewhat independently on both sides as described in *Chapter 3*, the mandibular positions were measured separately for each side.

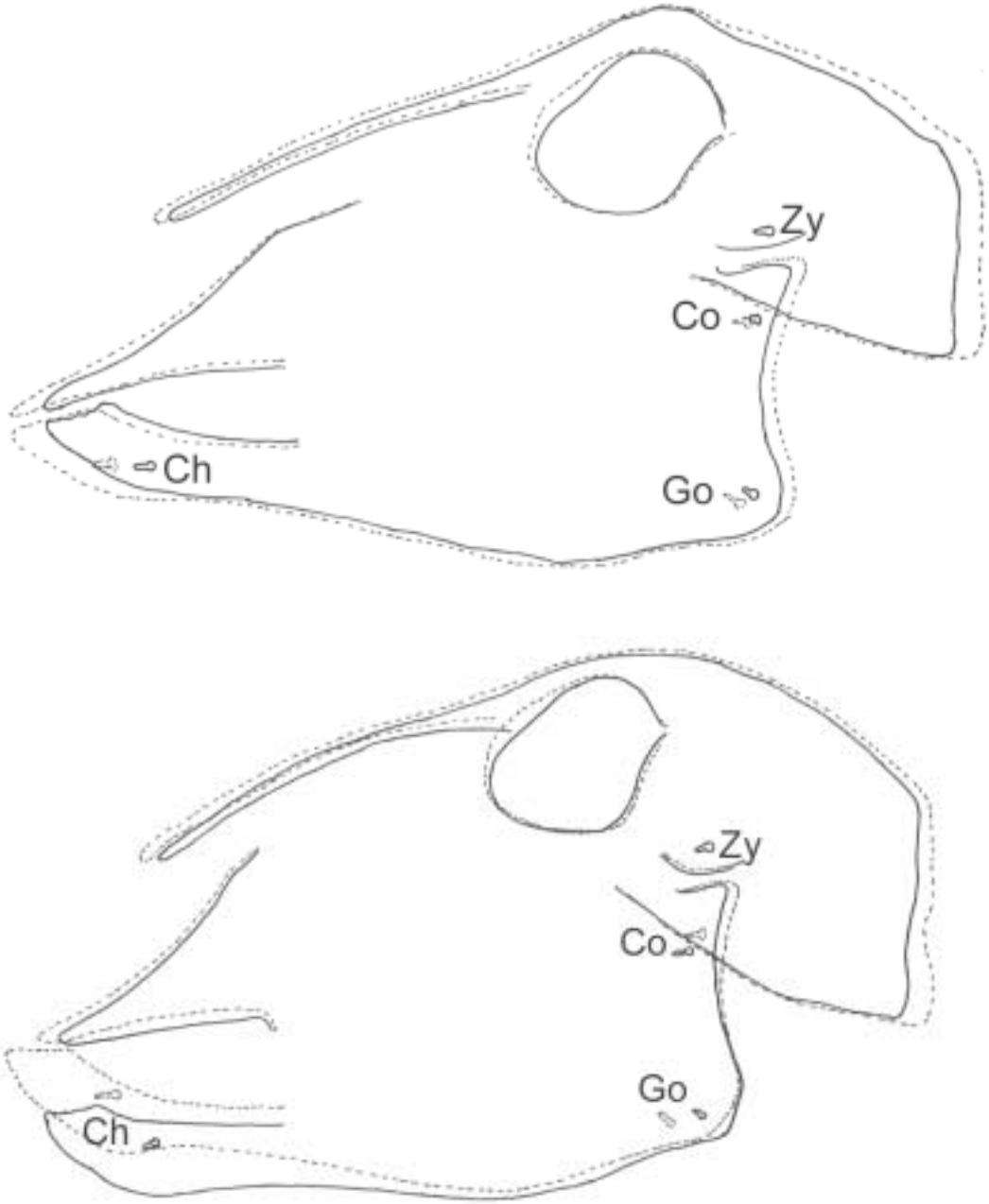


Figure 8.1. Schematic representation showing changes in the position of the implants which indicate the possible positional changes of the mandible. Upper: translation of the mandible. The angle ∠Zy-Go-Ch or ∠Zy-Co-Ch increases could be associated with increased distances between Zy-Co, Zy-Go or Zy-Ch. Lower: forward rotation of the mandible. The angle ∠Zy-Ch-Go or ∠Zy-Ch-Co increases could be associated with increased distances between Zy-Co, Zy-Go or Zy-Ch.
Solid line: mandibular position at an early stage of the experiment.
Dotted line: mandibular position at a late stage of the experiment.

Because the location of the implant markers varied among the animals, the changes in these distances or angles were calculated for comparison. Two methods were adopted to present the changes: (i) changes by month and (ii) changes throughout the experiment. There were four sets of serial cephalograms taken for each animal. The changes by month were calculated based upon the measurements performed on the 2nd, 3rd and 4th sets of cephalograms against the 1st set of cephalograms. Among the seven variables (three distances and four angles), only the variable(s) consistently different between the control group and the experimental group during the experimental period was (were) selected to analyse the correlation with the histological difference between the groups.

When analysing the correlation between the changes of mandibular position with the histological difference between the groups, the changes of mandibular position throughout the experimental period were used. This involved calculating the change of the mandibular position between the last and the first set of cephalograms. The difference between these two sets of cephalograms chronologically coincided with the injection of the first and the third fluorochrome. Therefore, the adaptation of the TMJ observed in previous chapters occurred chronologically coincident with the displacement of the mandible.

The histological variables found to differ between the control and the experimental groups were examined using Pearson Correlation analysis with respect to the mandibular position. They included cartilage thickness in the

anterior, intermediate and posterior regions of the condyle [mm]. Structural indices including bone volume fraction (BV/TV, [%]), specific bone surface (BS/BV, [mm^2/mm^3]), trabecular thickness (Tb.Th, [mm]) and trabecular separation (Tb.Sp, [mm]) in the subchondral region. Bone dynamic indices including mineral apposition rate (MAR, [μm/day]), bone formation rate with respect to bone mineral surface (BFR/BS, [mm^3/mm^2/day]) and bone formation rate with respect to bone mineral volume (BFR/BV, [%/year]) both in the central and the subchondral region.

8.2. RESULTS

Because the construction of the head frame was not complete when the experiment started one animal in each group did not have the 1st month cephalograms. The other three out of the four animals in each of the control and the experimental groups were subsequently recruited for the analysis. There were 10 sets of cephalograms for the 3 control animals and 12 sets for the 3 experimental animals. Since the mandibular displacement was treated separately between both the left and the right sides, the sample size for the control and the experimental groups was 20 and 24 respectively.

The distances represent the displacement of the mandible. When the changed mandibular position was measured by month, all the distances were found significantly correlated with time as the experiment proceeded. These results were thought to be the result of increased craniofacial size during growth. In the control group, The angles ∠Zy-Co-Ch and ∠Zy-Go-Ch were

found to be correlated significantly with time whereas ∠Zy-Ch-Co and ∠Zy-Ch-Go were not. These angle changes represented the translation of the mandible in the control group. In the experimental group, none of the angles were found to be significantly correlated with time as the experiment proceeded (Table 8.1). Therefore, only the distances were selected for further analysis.

Among the three distances, only the distance that showed consistent difference between the control and the experimental groups was subjected to correlation analysis. In an attempt to identify any variable which may have differed throughout the entire experimental period, analysis of covariance (ANCOVA) was used treating time (day) as the covariate.

Table 8. 1. The correlation between mandibular position and time (day) in the experiment.

		Control (N=20)	**Experimental (N=24)**
ZY-CO (mm)	Pearson Correlation	**0.817**	**0.743**
	Sig. (2-tailed)	**<0.001**	**<0.001**
ZY-GO (mm)	Pearson Correlation	**0.898**	**0.562**
	Sig. (2-tailed)	**<0.001**	**0.004**
ZY-CH (mm)	Pearson Correlation	**0.938**	**0.811**
	Sig. (2-tailed)	**<0.001**	**<0.001**
∠Zy-Ch-Co (°)	Pearson Correlation	0.347	-0.213
	Sig. (2-tailed)	0.134	0.317
∠Zy-Ch-Go (°)	Pearson Correlation	0.318	-0.254
	Sig. (2-tailed)	0.171	0.232
∠Zy-Co-Ch (°)	Pearson Correlation	**0.462**	-0.111
	Sig. (2-tailed)	**0.040**	0.607
∠Zy-Go-Ch (°)	Pearson Correlation	**0.490**	0.047
	Sig. (2-tailed)	**0.028**	0.827

Before proceeding with the ANCOVA, the variables were assessed with respect to practical limitations of the technique. First, the proportion of the total variability in the dependent variable (DV) Zy-Co, Zy-Go or Zy-Ch that

was explained by independent variable (IV) “control or treatment”, the covariate time (day), and the interaction of IV with the covariate were analysed. The interaction of the IV with the covariate was the heterogeneity of the regression slopes, which was not statistically significant for two of the three measurements (F= 0.241, p= 0.626 for Zy-Co and F= 2.027, p= 0.162 for Zy-Go) but significant for Zy-Ch (F= 4.407, p= 0.042), therefore it was concluded that the within-group regression was homogeneous for Zy-Co and Zy-Go and there was no combined effect of the IV with the covariate for these two variables. On the contrary, the within-group regression was heterogeneous for Zy-Ch. The difference of Zy-Ch was not consistent between the groups during the experimental period. ANCOVA was taken no further for Zy-Ch.

Since there were no combined effects of IV “control or treatment” and the covariate for Zy-Co and Zy-Go, a new model was written as IV and covariates. Based on “Tests of Between-Subjects Effects” (computed using alpha = 0.05), it was found that the linearity of within-group regression was high for Zy-Co. The coefficients for Zy-Co and time was r^2 = 0.657 (Adjusted r^2 = 0.640). The detail is given in Table 8.2.

Table 8.2. Tests of Between-Subjects Effects: Dependent Variable was Zy-Co

Source	F	Sig.	Observed Power
Time	61.549	<0.001	1.000
Treatment	18.609	<0.001	0.988

The linearity of within-group regression was found not significant with the IV for Zy-Go. The coefficients for Zy-Go and time was r^2 = 0.564 (Adjusted r^2 =

0.543). The detail is given in Table 8.3. Without significant difference between the control and the experimental groups, functional appliance treatment was not thought to be the factor causing the difference between the two groups. ANCOVA was taken no further for Zy-Go as well.

Table 8.3. Tests of Between-Subjects Effects: Dependent Variable was Zy-Go

Source	F	Sig.	Observed Power
Time	50.162	<0.001	1.000
Treatment	3.461	0.070	0.443

In the evaluation of assumptions for the only remaining distance Zy-Co, the null hypothesis that the error variance of the dependent variable was equal across groups was accepted (p= 0.347). The results indicated that the data set for Zy-Co, from its nature, was appropriate for ANCOVA. The ANOVA shows a significant relationship between the DV (Zy-Co) and the covariate (Time) (*t*= 7.845, p<0.001), which indicated Time was a covariate of Zy-Co.

Univariate testing was then used to compare Zy-Co between the control and the experimental groups, taking time into consideration. Univariate testing reported a significant increase of Zy-Co in the experimental group (F= 18.609, p< 0.001) and the observed power was high at 0.988. Pairwise comparisons indicated Zy-Co was 2.40 mm (95% CI: 1.28, 3.52) larger in the experimental group (p<0.001). Graphic representation is provided in Figure 8.1. This difference in Zy-Co was thought to be achieved by the functional appliances, which were designed to displace the mandible anteriorly by 4 mm.

When the changes throughout the experiment were used to present the changes of mandibular position, the correlation between Zy-Co and cartilage thickness, bone structural indices and bone dynamic indices were analysed and the results are presented in Table 8.4. Significant correlation was found only for the cartilage thickness and most interestingly, a negative correlation was found in the control group whereas a positive correlation was found in the experimental group.

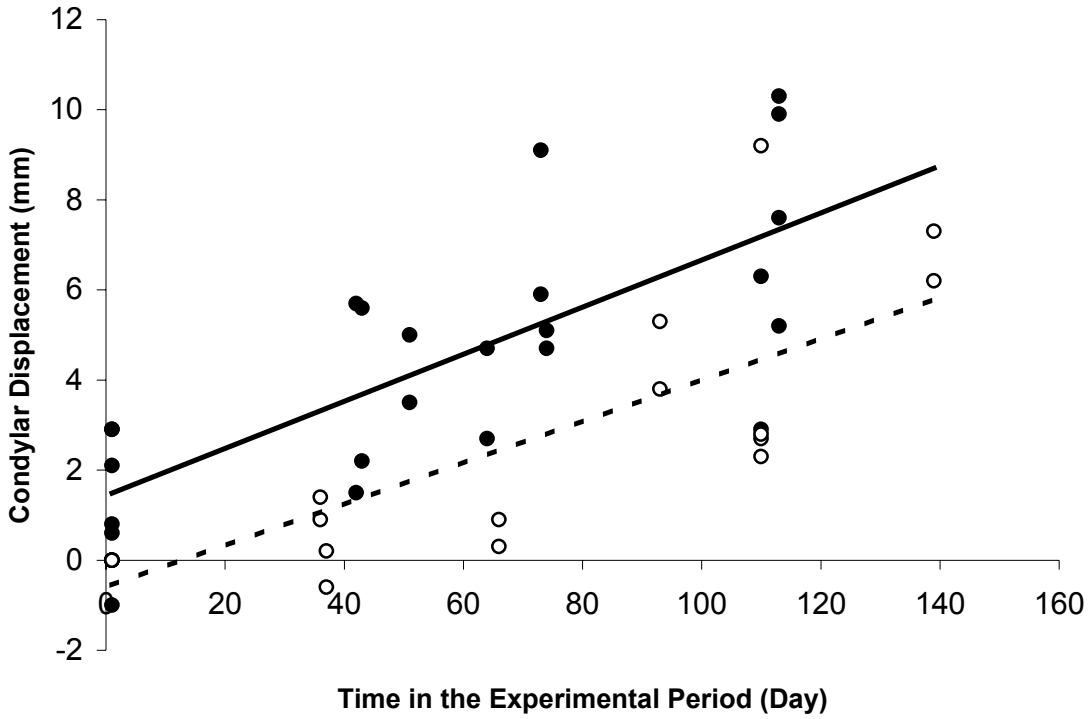


Figure 8.2. Plot of condylar displacement (Zy-Co) versus time. No interaction of "control or treatment" was found with the covariate Time (p=0.626). The linearity of within-group regression was significant (p<0.001) with r^2 = 0.657. The straight lines are those that best fit the data for the control and the experimental groups. A significant increase of Zy-Co was found (p<0.001) in the experimental group.

○ ····· Control (N=20)
● ——— Experimental (N=24)

Table 8. 4: The correlation between Zy-Co and time (day).

		Control (N=8)	Experimental (N=8)
Cartilage thickness-Anterior	Pearson Correlation	-0.568	**0.852**
	Sig. (2-tailed)	0.240	**0.031**
Cartilage thickness-Intermediate	Pearson Correlation	**-0.915**	**0.921**
	Sig. (2-tailed)	**0.010**	**0.009**
Cartilage thickness-Posterior	Pearson Correlation	**-0.868**	**0.875**
	Sig. (2-tailed)	**0.025**	**0.022**
BV/TV-Subchondral Region	Pearson Correlation	-0.546	0.734
	Sig. (2-tailed)	0.262	0.096
BS/BV-Subchondral Region	Pearson Correlation	-0.121	-0.564
	Sig. (2-tailed)	0.820	0.244
Tb.Th. -Subchondral Region	Pearson Correlation	0.063	0.617
	Sig. (2-tailed)	0.906	0.192
Tb.Sp. -Subchondral Region	Pearson Correlation	0.742	-0.627
	Sig. (2-tailed)	0.092	0.183
MAR-Central Region	Pearson Correlation	-0.282	0.579
	Sig. (2-tailed)	0.589	0.229
BFR/BS-Central Region	Pearson Correlation	-0.523	0.425
	Sig. (2-tailed)	0.287	0.401
BFR/BV-Central Region	Pearson Correlation	-0.399	0.152
	Sig. (2-tailed)	0.433	0.774
MAR-Subchondral Region	Pearson Correlation	0.220	0.513
	Sig. (2-tailed)	0.675	0.298
BFR/BS-Subchondral Region	Pearson Correlation	0.259	0.532
	Sig. (2-tailed)	0.620	0.277
BFR/BV-Subchondral Region	Pearson Correlation	0.723	-0.268
	Sig. (2-tailed)	0.104	0.608

8.3. Discussion and Conclusion

In the experimental functional appliance treatment group, it was found that the condylar position relative to the zygomatic process of the temporal bone was the only variable showing a consistent difference throughout the experimental period. The association between the magnitude of the condylar displacement and the increased condylar dimension was also noticed when comparison was made between the control and the experimental groups. The 4 mm forward positioning of the lower components of the appliances designed and

constructed on the semi-adjustable articulator resulted in 2.40 mm forward condylar displacement in the experimental group when compared with the controls. This displacement was associated with an increased condylar dimension in the experimental group of 2.21 mm in the postero-superior direction and 2.30 mm in the posterior direction.

The achieved displacement in the mandibular condyle was smaller than that designed on the articulator when the appliance was constructed. The possible reason was that the articulator did not fully simulate the jaw motion in sheep. The mode of the action of this functional appliance was to guide the mandible to a protruded position by the ramps on its upper and lower components when they engaged during molar contact. The articulator could simulate the protrusion of the mandible, but not rotation or lateral shifting. A smaller protrusion in the condyle suggested that the molar contact in sheep was achieved by combined translation and rotation of the mandible, or shifting of the mandible. A detailed study of the jaw motion in sheep would help to validate this suggestion.

Assuming the implant markers in the zygomatic process of the temporal bone and in the mandibular condyle did not move during growth, the displacement of the implant marker in the mandibular condyle closely represented the displacement of the condyle. The cause of condylar displacement has been discussed, and tooth eruption as well as condylar growth were thought to be the important factors. In this experiment, the appliances were not removed from the mouth while the serial cephalograms were taken. The condylar

displacement observed in this investigation was the combined effect of the functional appliances and the tooth eruption as well as the wearing of the teeth together with condylar growth. With the limitation of using 3-D cephalometry, which only provided a static snapshot of a continuous process, the condylar displacement did not fully represent the effects created by the functional appliances. Those histological variables did not found to be correlate with the condylar displacement from this investigation do not necessarily fail to correlate with the functional appliance treatment in sheep.

The only histological variable found to be correlated to the condylar displacement was cartilage thickness. In the control group, the larger the condylar displacement caused by growth, the thinner the condylar cartilage. As described by Kantomaa (1994), decreased functional loading on the mandibular condyle results in thinning of the condylar cartilage. Following the same theory, when the condyle was displaced further from the fossa, less functional loading would be expected to apply to the condyle. The result was the thinning of the condylar cartilage. In the experimental group, to the contrary, the larger the condylar displacement, the thicker the condylar cartilage. This result can only be explained by the increased functional loading on the TMJ, since condylar cartilage was always found to be thicker in functionally loaded joints before excessive loading caused cartilage degradation (Kantomaa & Pirttiniemi, 1998). In the present study, it was suggested that in the experimental group, the mandibular condyle was displaced further away from the fossa. This larger displacement changed the pattern of TMJ movement to an exaggerated pattern. In seeking a balance

during TMJ movement, more frequent contact of the condyle and the fossa would have resulted, most likely in the anterior region of the condyle.

In conclusion, the condylar displacement (Zy-Co) was the only measurement found to be associated with the functional appliance treatment, whereas the vertical mandibular displacement (Zy-Go) or the chin displacement (Zy-Ch) was not. As stated by Sondhi (1997), absolute and abstract measurement of condylar position do not prove to be clinically reliable or significant. He recommended tomography to measure the changes in condylar position with corrected axis tomography. The current investigation also suggested that the change of condylar position is an important measure of the effect of functional appliance treatment.

Chapter 9
Concluding Remarks

9.1. The Answers to the Study Questions

In *Section 1.8.4* of *Chapter 1*, two out of the 6 study questions involving the investigation of the bone responses to the functional appliance treatment are defined. This project involved the analysis and interpretation of the bone histomorphometric data in the mandibular condyle following functional appliance treatment.

One study question answered by this project was whether the treatment increases the quantity of bone matrix formation. What is the impact on bone resorption? This question was based on the reported increase of new bone formation in the human temporomandibular joint (TMJ) during functional appliance treatment observed from medical imaging techniques, and increased bone matrix mineralisation in animal experiments observed with histological techniques.

The answer to this question was given in detail in *Section 5.3.2* and *Section 5.3.3*. In brief, the quantity of bone matrix formation within the mandibular condyle was not increased following experimental functional appliance treatment. There is no evidence showing the treatment has any impact on bone resorption within the mandibular condyle. Therefore, in the first null hypothesis, functional appliance treatment has no effect on bone matrix mineralisation is rejected; but there is not enough evidence to reject that functional appliance treatment has no effect on bone matrix formation or resorption.

The other question answered by this project was whether functional appliances have any impact on the mineralisation lag time within the TMJ?

The answer to this question was given in detail in *Section 5.3.3*. The second null hypothesis, functional appliance treatment has no effect on the mineralisation lag time, was rejected. This result suggests that the life-span of the active osteoblasts was reduced. In brief, because the bone matrix mineralisation is under the control of osteoblasts on the bone surface and osteoblasts became buried within the osteoid, and the osteoid life-span is numerically the same as mineralisation lag time, it is deduced that the life-span of the osteoblasts buried within the osteoid was reduced because the mineralisation lag time was shorter in the experimental group.

In this project, increased bone matrix mineralisation was found. This increase was most likely the result of osteoblasts (both BS/BV and BFR/BS increased). The systemic calcitropic hormones or local autocrine/paracrine factors may be responsible for this increased osteoblastic activity. From the clinical viewpoint, the systemic calcitropic hormones can be measured from serum or urinary samples, but local autocrine/paracrine factors cannot be easily measured. The contribution of cartilage to those local autocrine/paracrine factors, which may also be important, is even more difficult to determine. This was the reason for raising the questions regarding the chondrocyte cell lineage.

Recent studies have demonstrated that cells within areas of mineralised cartilage share many molecular characteristics with osteoblasts and express a

subset of common gene products including alkaline phosphatase, bone sialoprotein, osteopontin and osteocalcin. An explanation for the presence of homologous proteins in these very different tissues is probably related to similarity in their functions at the molecular level within the extracellular matrix in both the process of mineralisation and resorption (Gerstenfeld & Shapiro, 1996). The major question related to the expression of osteopontin, bone sialoprotein, and osteocalcin by cells within the condylar cartilage is whether genes for these proteins are regulated in the same fashion, in both cartilage and bone, in response to the systemic calcitropic hormones or to the local autocrine/paracrine factors. This would an interesting field of study.

9.2. The Conclusion Drawn From Bone Histomorphometric Data

To link the study questions to clinical practice, the questions can be summarised as whether functional appliance treatment increased the quantity of the bone formed during treatment, or the treatment changed the distribution of the bone during the treatment, Or both? The former suggests that functional appliance treatment creates additional growth, whereas the later suggests that the treatment only changes the shape of a structure. The detailed answer to these questions on the responses of TMJ tissue to functional appliances can only be derived from animal experiments.

The conclusion drawn from the bone histomorphometric data regarding experimental bone matrix formation and mineralisation after comparison with the control group data is summarised in Table 9.1. The conclusion regarding

the mechanical factors influencing bone matrix formation and mineralisation is summarised in Table 9.2 with the location of these mechanical factors indicated on Figure 9.1.

Table 9.1. The Biology of Growth Modification

Evidence	Related Knowledge	Conclusion
MAR, BFR/BS and BFR/BV increased, MLT decreased	**MAR** is the thickness of the layer of new mineralised bone laid down per unit time. **BFRs** are the volume of mineralised new bone formed per unit area of osteoid surface, or per unit volume of pre-existing bone, per unit time. **MLT** is the time during which newly formed osteoid remains un-mineralised.	Bone matrix mineralisation became faster
BV/TV and Tb.Th decreased, Tb.Sp increased. OV/BV and OV/TV unchanged.	**OVs** are the volume occupied by un-mineralised bone expressed as a fraction of the volume occupied by bone, or bone plus marrow. **BV/TV** is the volume occupied by mineralised bone expressed as a fraction of the volume occupied by bone plus marrow. **Tb.Th** is trabecular thickness. **Tb.Sp** is trabecular separation.	Bone matrix formation was not increased

Table 9.2. The Mechanobiology of Growth Modification

Evidence	Related Knowledge	Conclusion
The cartilage thickness increased anteriorly	Increased compressive force increase the cartilage thickness	The compressive force on the anterior part of condylar cartilage increased
The standard deviation of Ct.Th was less in the experiemtnal group	Mechanical loading augments skeleton mass during growth	The mechanical loading on the anterior and posterior cortical bone follows a more uniform pattern
Et rotated posteriorly	Trabecular orientation follows closely the orientation of the mechanical loading	The orientation of mechanical loading on the subchondral region rotated posteriorly
BV/TV, Tb.Th decreased; Tb.Sp increased	Mechanical loading augments skeleton mass during growth	The magnitude of mechanical loading on the subchondral region was not increased
BFR/BS and BFR/BV increased	Mechanical stimulation by intermittent compressive force increases bone formation	The frequency of the mechanical loading on the subchondral region was increased

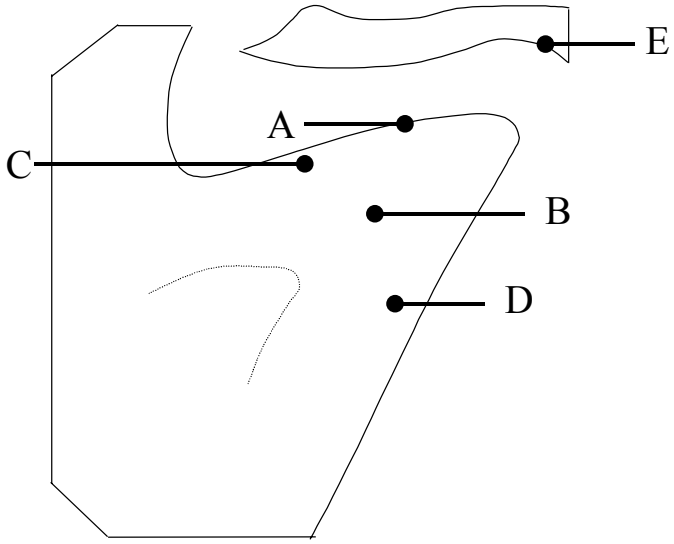


Figure 9.1. The regions showing significant differences between the control and the experimental groups within the mandibular condyle. A: Anterior region of the condylar cartilage; B: Subchondral region of the condylar trabecular bone; C: Anterior region of the cortical bone; D: Posterior region of the cortical bone and E: Anterior spine of the post-glenoid fossa.

9.3. Summary

The purpose of this project was to investigate the controlling mechanisms of functional appliance treatment in order to further develop understanding of their effects and to guide their use in clinical practice. The conclusions derived from this animal experiment using sheep may not fully represent the mechanisms of functional appliance treatment in human subjects. However, with regard to the biological basis of growth and development of the TMJ, this study provided important quantitative data on the growth modification effects of functional appliance treatment. The details are provided as follows:

The experiment was conducted over a period equivalent to 2 years in human subjects during their pubertal growth and found the total condylar growth was between 8.8 mm and 11.9 mm in the untreated control group. The functional appliance used in this project was worn full-time and created an initial condylar displacement from 1.28 mm to 3.52 mm with the average being 2.40 mm measured based on 3-D cephalometry.

This change of the condylar position probably led to changes in the TMJ motion. On one hand, the changed condylar position was the result of changed occlusion induced by the appliances, and this occlusion was un-stable. This un-stable occlusion may have led to more frequent contact between the condylar cartilage and the disc as well as the fossa, probably in the anterior region of the TMJ. The result was the thickened condylar cartilage anteriorly. On the other hand, the appliances may have modified the orientations of the chewing muscle contraction forces, leading to less variable anterior contraction forces. The results were less variation in the condylar bone structure and posteriorly rotated trabeculae. The magnitude of the forces were not measured but might not be affected.

In the mandibular condyle, it was found that bone formation rates increased and mineralisation lag-time decreased whereas bone resorption seemed to be un-affected. Therefore, the increased mandibular condylar length or changed condylar growth orientation was deemed to be the result changed distribution of bone during growth, i.e., more bone distributed into the posterior region of the mandibular condyle, rather than the result of extra bone.

As the results of the re-distributed bone matrix, the condylar dimension was increased from 1.39 mm to 3.21 mm with the mean being 2.30 mm. Also because of the anterior displacement, bone deposition increased in the anterior region of the posterior glenoid spine.

The results obtained from this project indicated the treatment effect of functional appliances is the reorganisation of the TMJ through bone modelling and remodelling. In patient selection of functional appliance treatment, only those who have the potential of bone modelling and remodelling in the TMJ should be selected. The treatment effect of functional appliance treatment needs to be evaluated based on the induced bone modelling and remodelling in the TMJ. This would be an important field for future study.

Epilogue

In order to develop a method to detect the skeletal changes induced by functional appliance treatment and further develop it to help with patient selection, the mechanism of the functional appliance treatment, in this project, is viewed as the reorganisation of the temporomadibular joint (TMJ) during growth through bone modelling and remodelling.

The growth potential of the skeleton, which determines the ability of TMJ reorganisation during growth, has been thought to be important in dentofacial orthopaedic treatment and it was suggested to be most beneficial when the orthopaedic treatment was conducted during the adolescent growth spurt. It is also generally believed that in most facial dimensions the timing of the growth spurt occur at about the same time as in stature, although the timing varies slightly in different parts of the body (Nanda, 1955; Bergersen, 1972; Björk, 1972).

In order to evaluate the skeletal growth potential of a patient, two methods, appropriate for orthodontics, have been devised. One method is to predict the skeletal growth spurt by detecting peak height velocity using repeated standing height measurements. The other method is to assess the skeletal developmental stage using hand-wrist radiographs. However, there are problems associated with these methods. For the former, when using the repeated height measurements technique, the large number of pre-treatment visits required, make this method impracticable (Barton et al., 1997). For the later, it is not possible from a single radiograph to determine with any degree

of reliability whether a stage is early or late in its development (Houston, 1980) and repeated radiography is also impracticable.

Furthermore, to assess growth potential of the facial skeleton only considering the skeletal growth during the adolescent growth spurt may not be sufficient. Behrents (1985) has demonstrated that craniofacial growth may continue to a considerable extent in both males and females after the age of thirty years. It has been suggested by Pancherz (2000) that growth adaptation to dentofacial orthopaedic treatments still have clinical significance even after the age of twenty years. For the patient in this age group, the two methods mentioned above provide little information on their skeletal growth potential.

Additional attempts have been made to assess growth potential of the facial skeleton in orthodontic patients using biochemical markers. Markers mean measurable and quantifiable biological parameters, which serve as indices for physiology related assessment. The serum levels of the markers for bone formation, including osteocalcin (OC), type I collagen carboxy-terminal propeptide (PICP) and bone specific alkaline phosphatase (BALP), and the markers of bone resorption, including N-telopeptides (NTX), free pyridinoline (Pyr), free deoxypyridindine (Dpyr) and C-telopeptides (CTX) are found to be correlated with bone growth. The following markers have been investigated in orthodontic patients: (1) serum levels of hormone dehydroepiandrosterone sulfate (DHEAS) and osteocalcin have been measured in a small adolescent population (Ghafari et al, 1995) and (2) urine levels N-telopeptide of type I collagen have been measured in a small group with the age range up to

twenty years old (Oh et al., 2000). Although the usefulness of these markers in assessing the growth potential of a patient is still questionable, significant correlations between those biochemical markers and physical measures (including height and skeletal maturation) have been reported. These results indicated the possibility of assessing the skeletal growth potential of a patient using bone markers.

The fundamental basis of bone growth and development is bone matrix formation and resorption. Those activities change during growth. The assessment of bone growth potential based upon bone forming and resorbing activity would have direct clinical relevance for orthodontic treatment planning, especially for the patients in their late developmental stage when the physical measures are no longer effective.

In human subjects, the bone formation activities can be measured using metabolic markers from serum or urine. These tests are comparatively simple and largely non-invasive. More importantly, information in normal untreated subjects is currently being collected for the study of bone growth and development (Blumsohn et al., 1994). With the inter-disciplinary collaborations in collection of the information, we will eventually be able to develop a method to select only those patients who can present the skeletal changes to best respond to functional appliance treatment before the commencement of the treatment.

The assessment of skeletal growth potential using those metabolic bone markers starts with the understanding of the influence of dentofacial orthopaedic treatment on those markers. The functional appliance treatment was found to influence the TMJ with bone modelling and remodelling which are predominant in the process of bone mineralisation. To examine the association between the condylar adaptive responses and the presence of the following proteins may help to understand how the mineralisation process was affected. These proteins include transforming growth factor beta (TGF-β), bone morphogenic protein IV (BMP-IV), interleukin-6 (IL-6), bone sialoprotein (BSP), osteopontin (OPN), osteocalcin (OC), type I collagen (COL-I) and type X collagen (COL-X). They can be detected by immunohistochemical markers.

Further studies need the combined assessment of the above local markers with those metabolic bone markers during growth as stated previously. If simple and non-invasive tests can be developed, monitoring those markers before, during and after functional appliance treatment can help to identify the characters of the successful treatment when the clinical assessment is performed simultaneously. Such knowledge may finally help us to formulate a protocol in patient selection.

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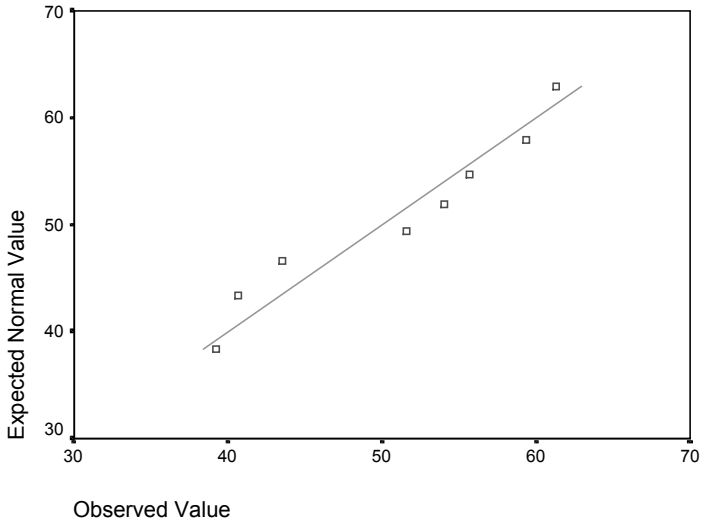
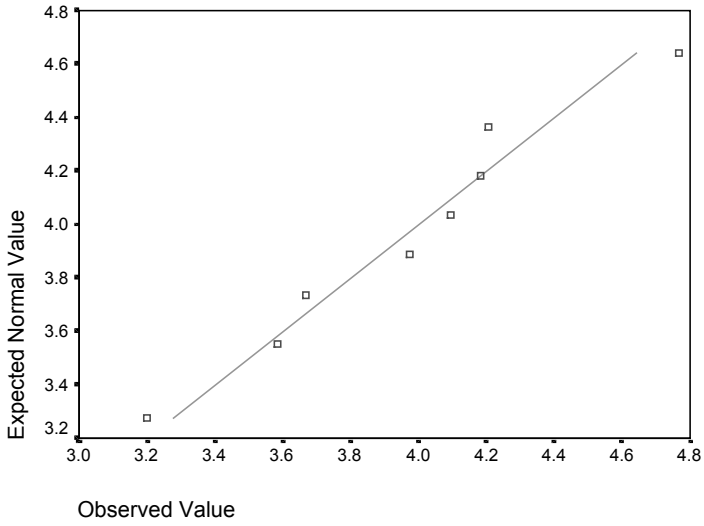
Appendix 1
Graphic Assessment of Normality-
Control Group Data

Trabecular Bone Structural Indices: Central Region

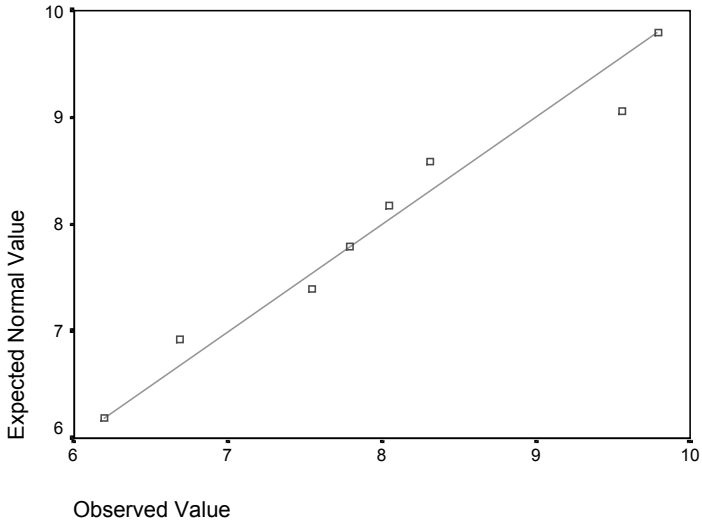
Normal Q-Q Plot of BV/TV Central

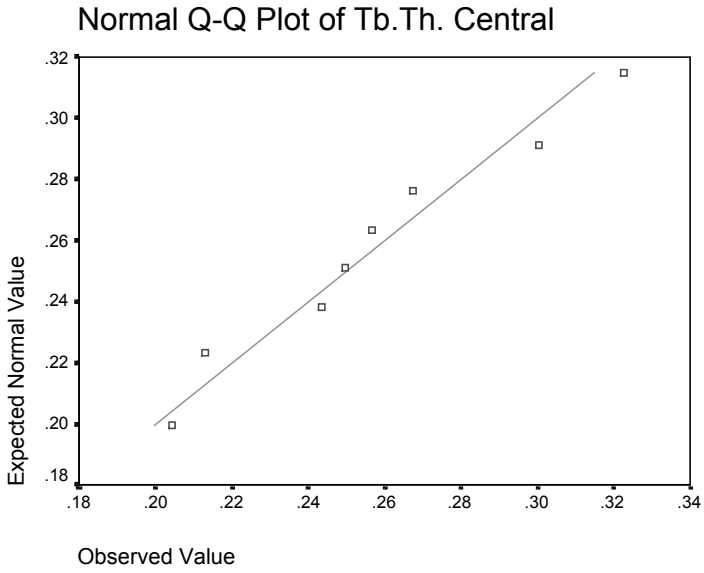


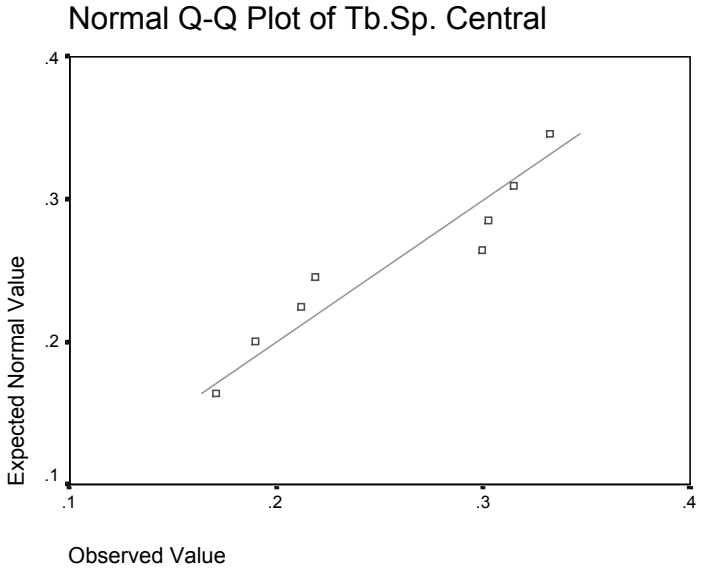
Normal Q-Q Plot of BS/TV Central

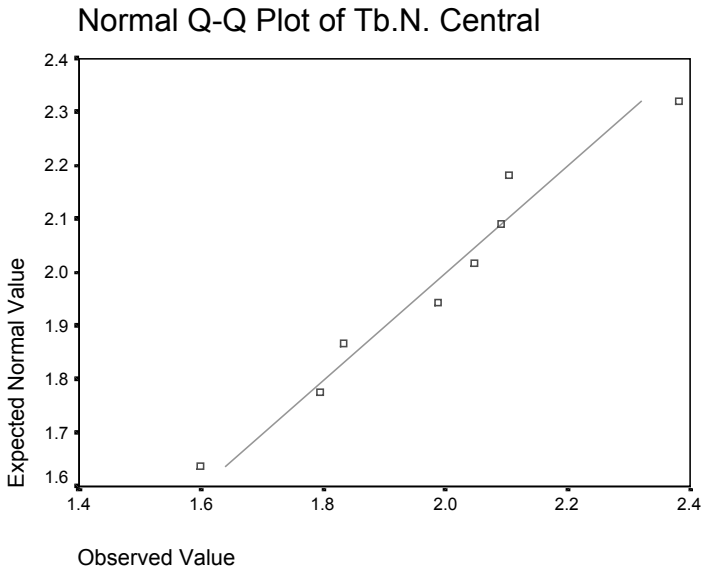


Normal Q-Q Plot of BS/BV Central



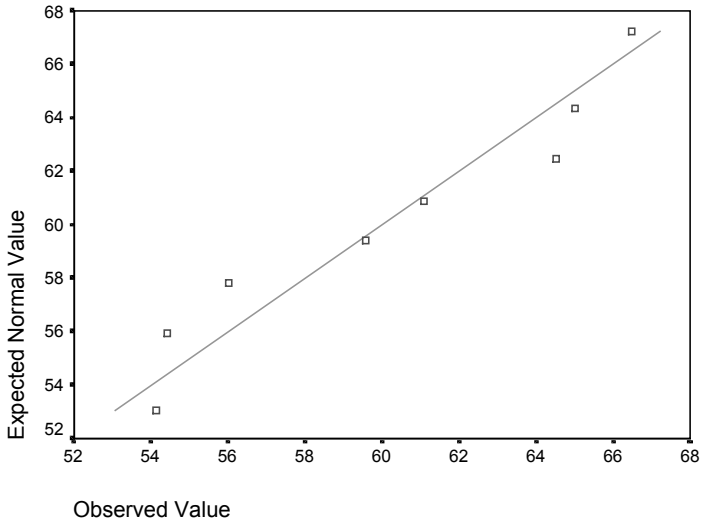

Normal Q-Q Plot of Tb.Th. Central
Expected Normal Value
Observed Value
.18
.20
.22
.24
.26
.28
.30
.32
.34


Normal Q-Q Plot of Tb.Sp. Central
Expected Normal Value
Observed Value
.1
.2
.3
.4

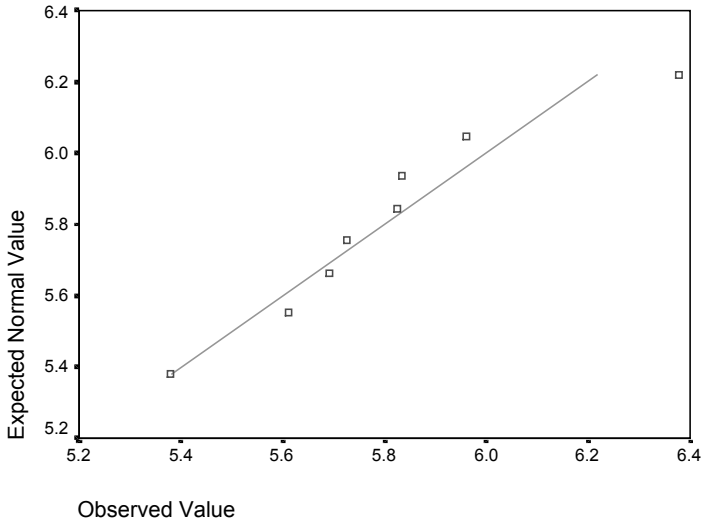

Normal Q-Q Plot of Tb.N. Central
Expected Normal Value
Observed Value
1.4
1.6
1.8
2.0
2.2
2.4

Trabecular Bone Structural Indices: Subchondral Region

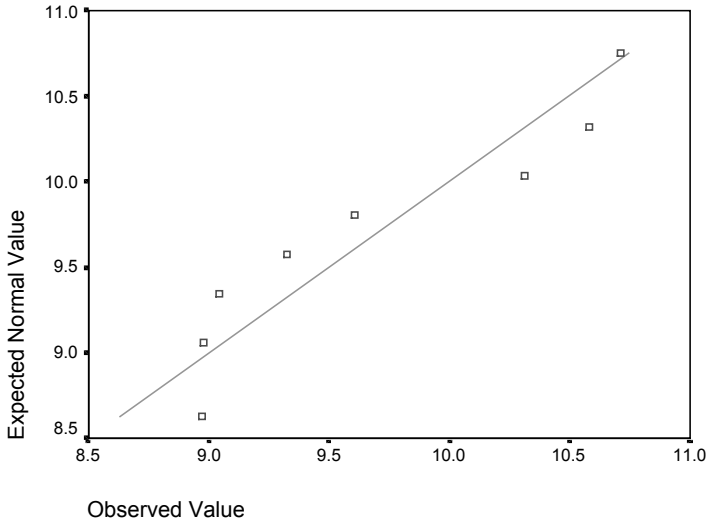
Normal Q-Q Plot of BV/TV Subchondral



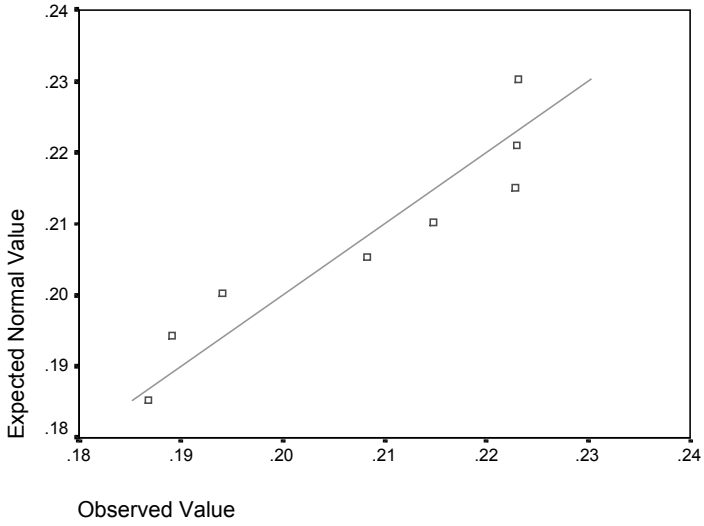
Normal Q-Q Plot of BS/TV Subchondral



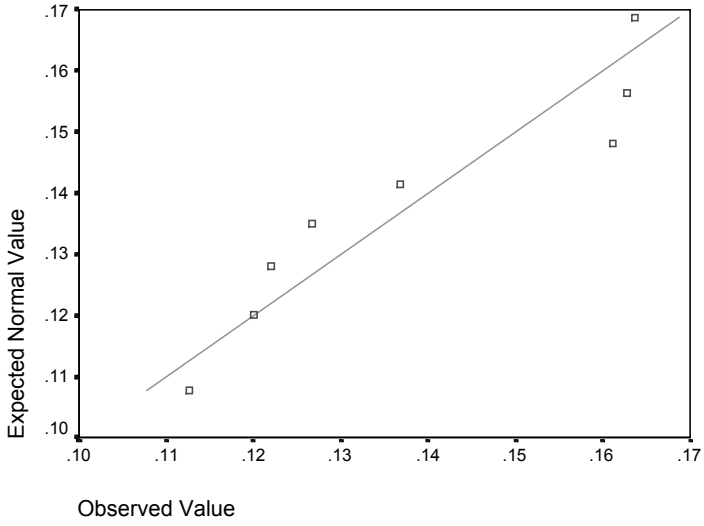
Normal Q-Q Plot of BS/BV Subchondral



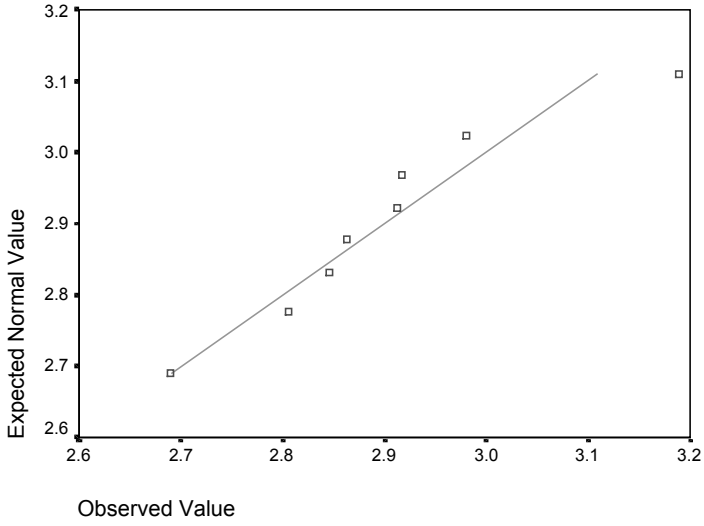
Normal Q-Q Plot of Tb.Th. Subchondral



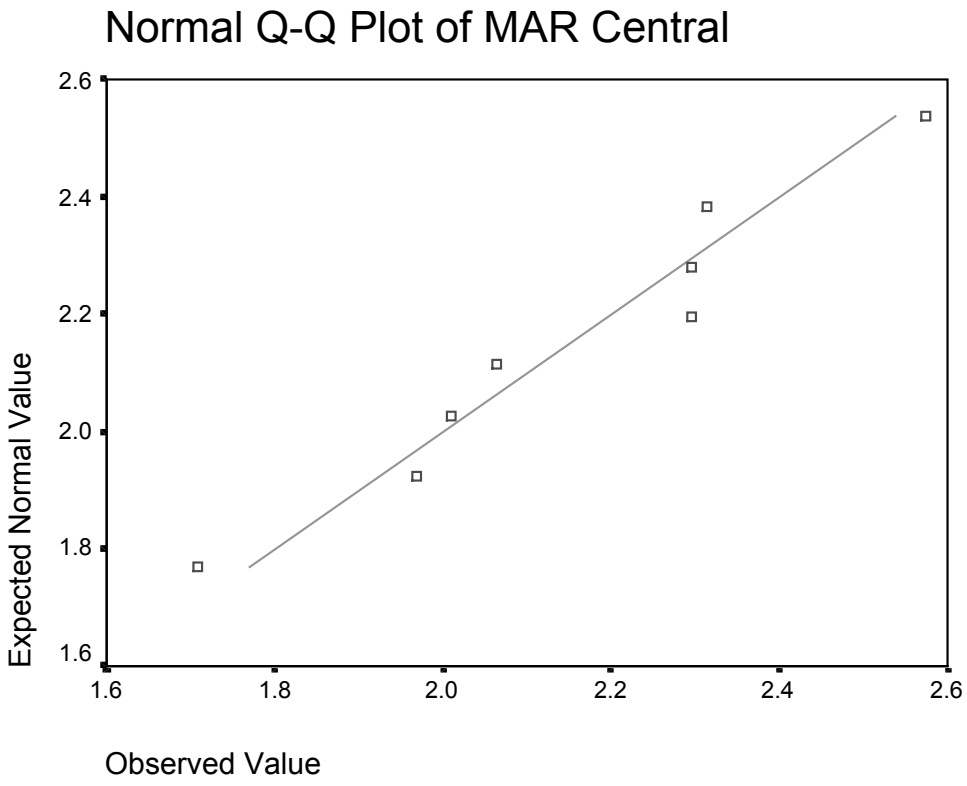
Normal Q-Q Plot of Tb.Sp. Subchondral

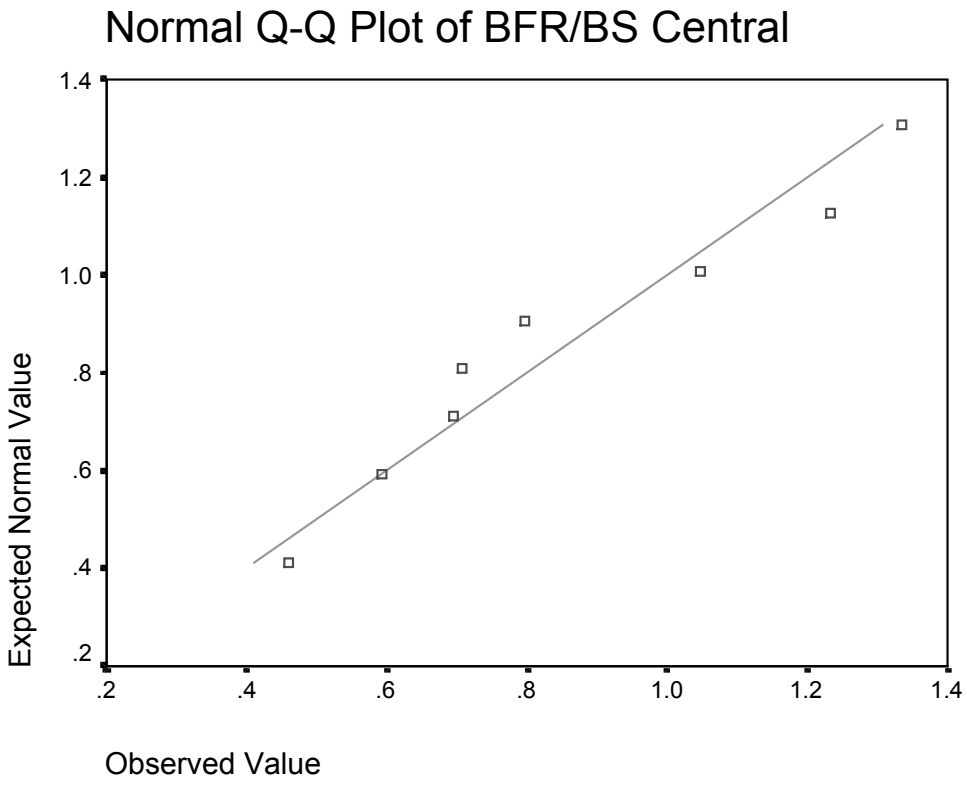


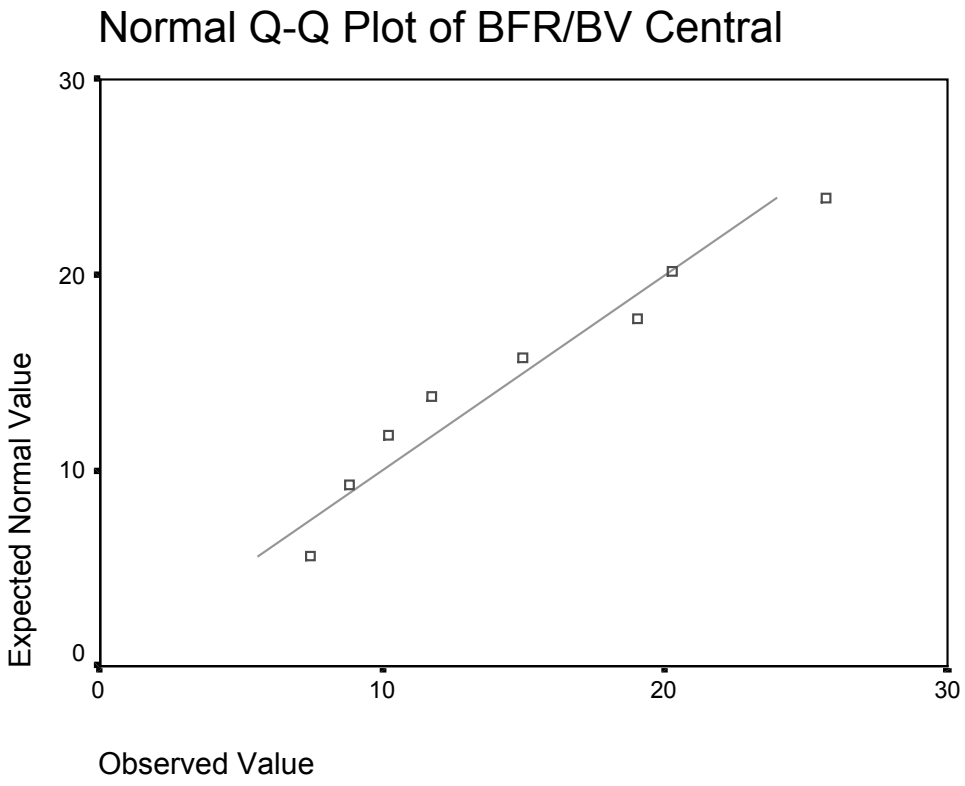
Normal Q-Q Plot of Tb.N. Subchondral

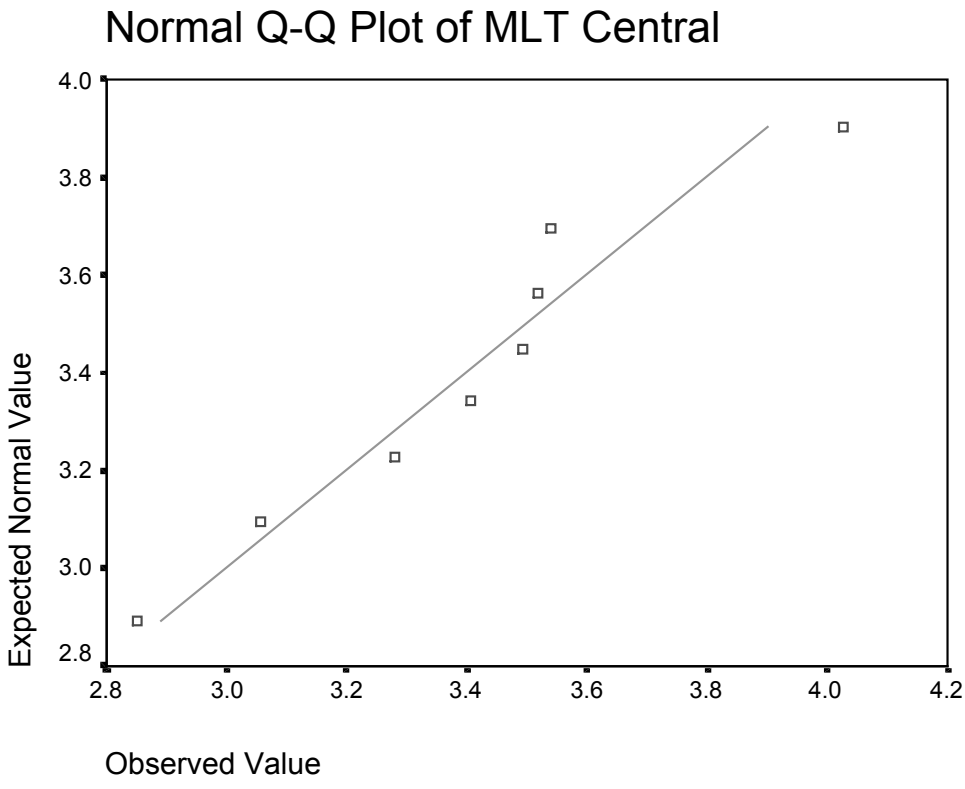


Trabecular Bone Dynamic Indices: Central Region

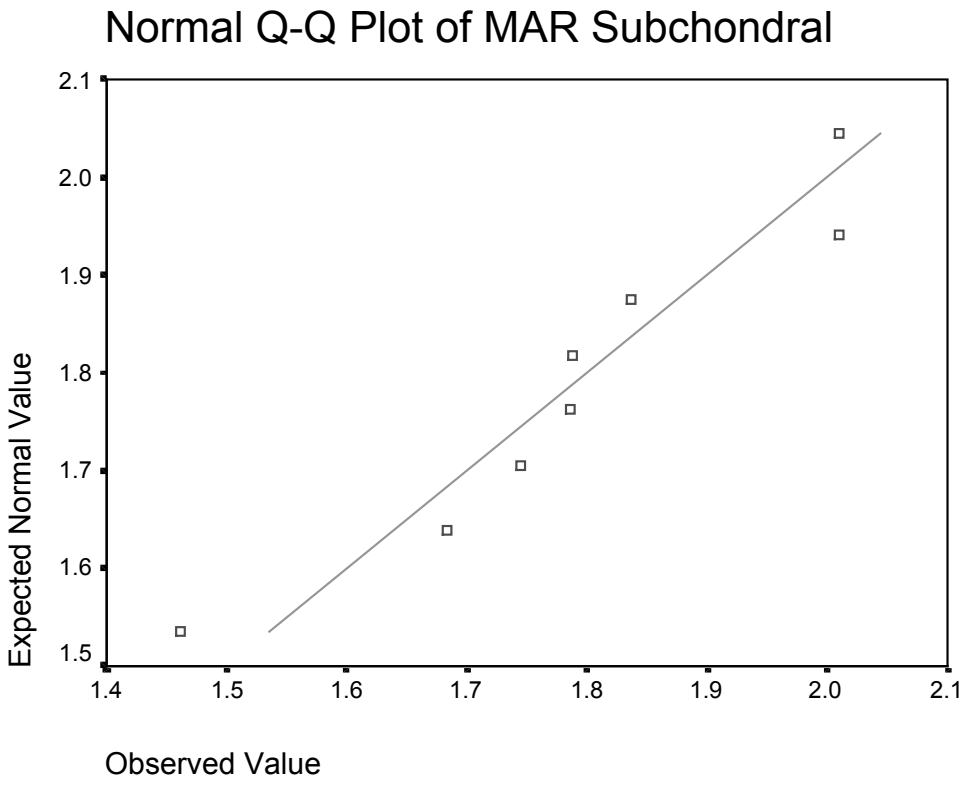


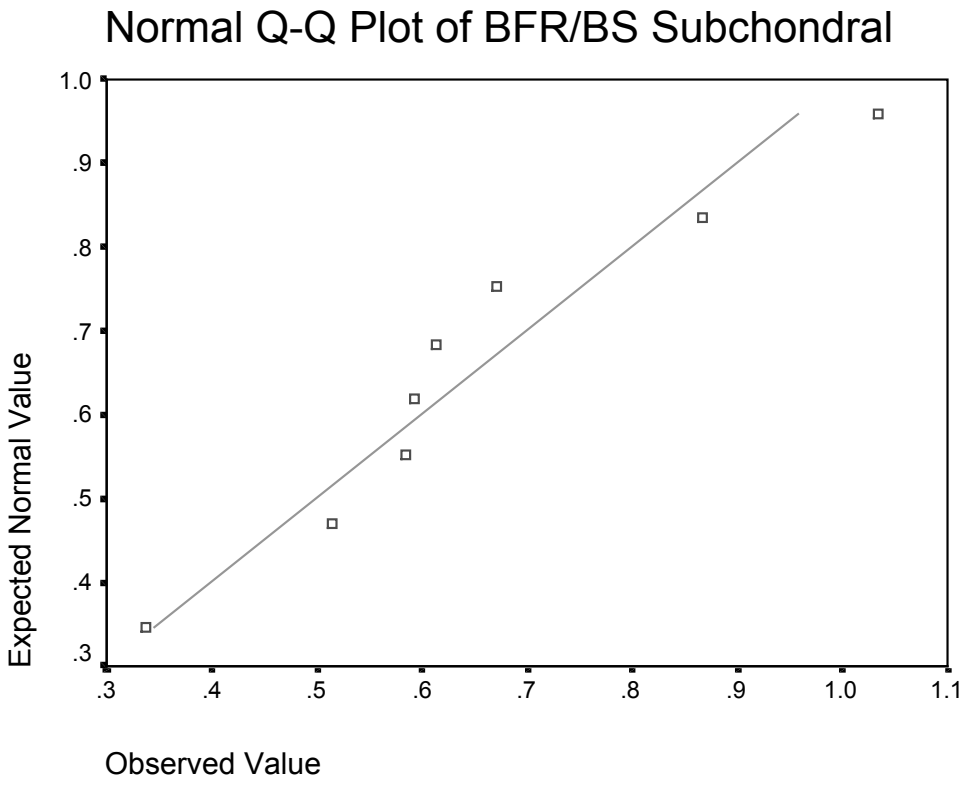


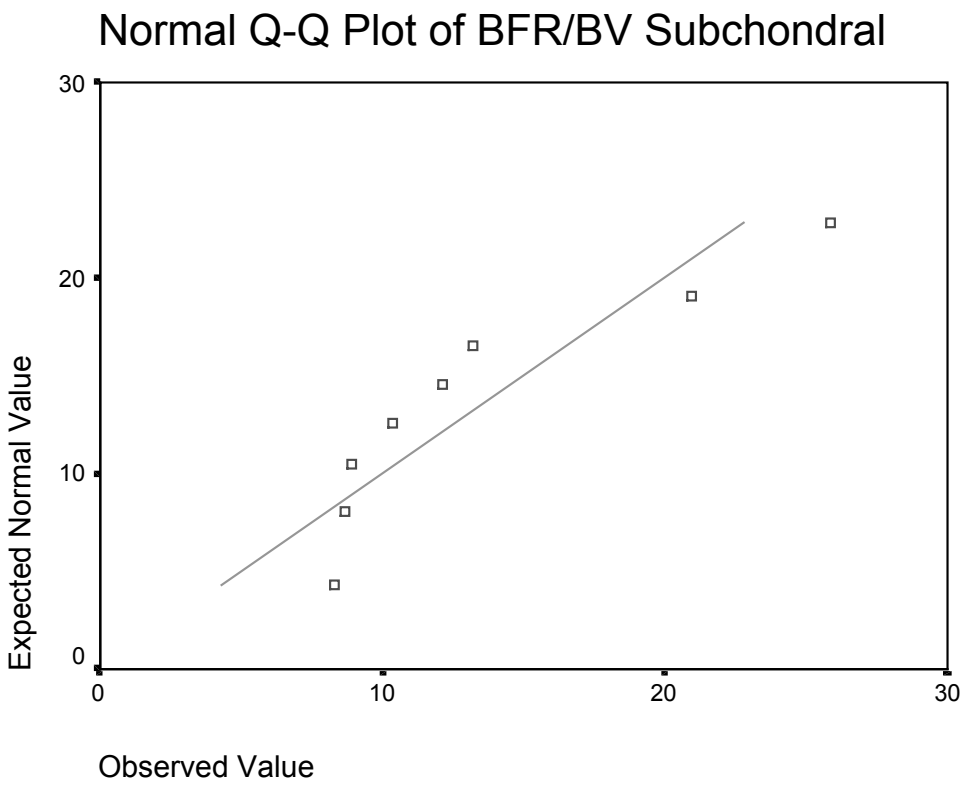

Normal Q-Q Plot of BFR/BV Central
Expected Normal Value
Observed Value
0
10
20
30

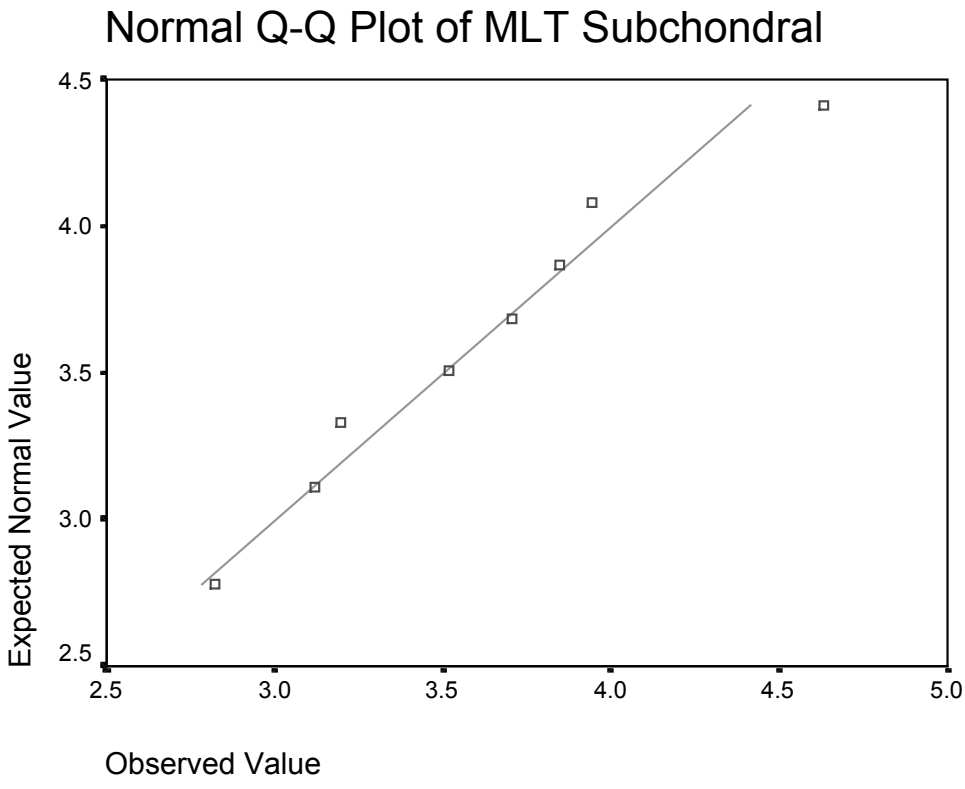

Normal Q-Q Plot of MLT Central
Expected Normal Value
Observed Value
2.8
3.0
3.2
3.4
3.6
3.8
4.0
4.2

Trabecular Bone Dynamic Indices: Subchondral Region

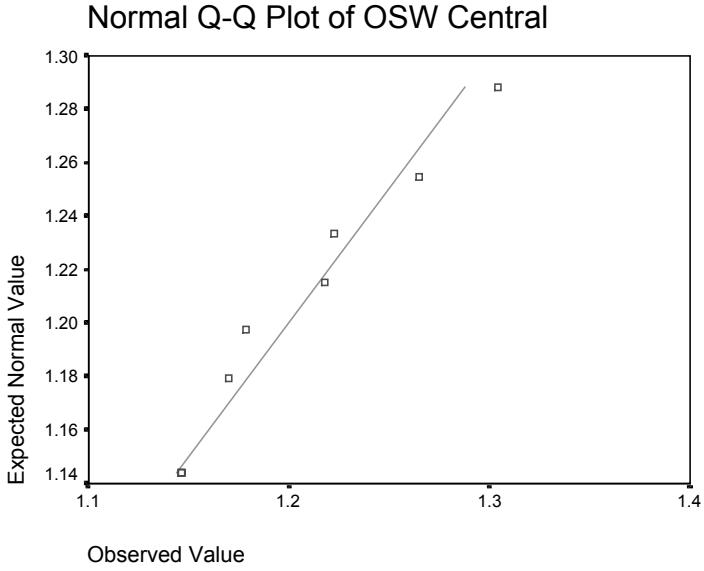


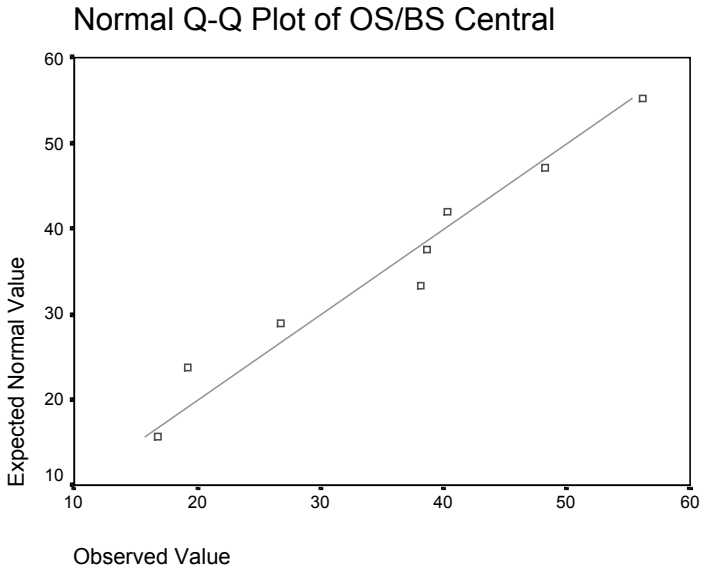


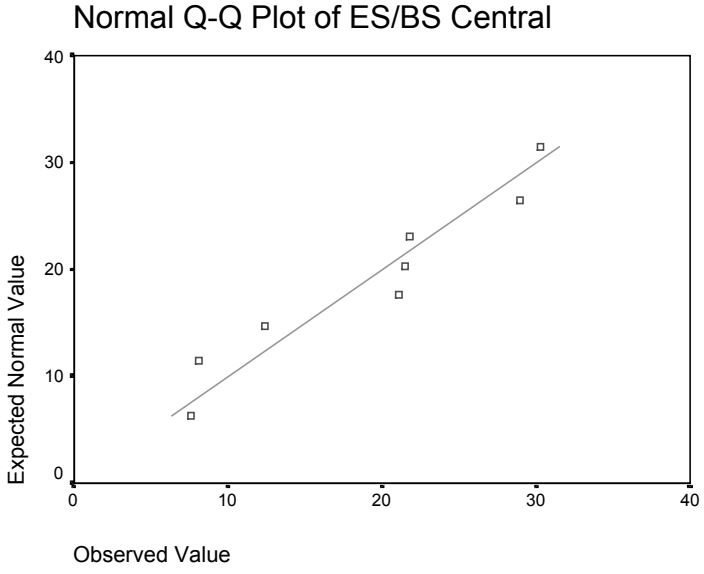

Normal Q-Q Plot of BFR/BV Subchondral
Expected Normal Value
Observed Value
0
10
20
30

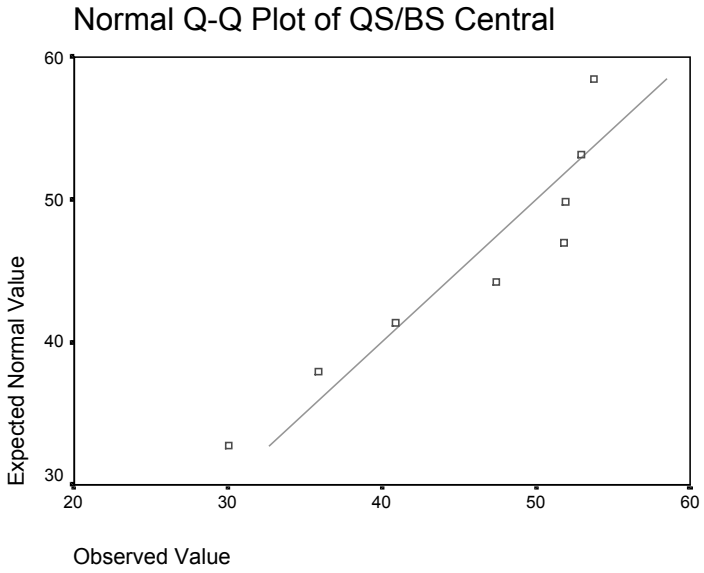

Normal Q-Q Plot of MLT Subchondral
Expected Normal Value
Observed Value
2.5
3.0
3.5
4.0
4.5
5.0

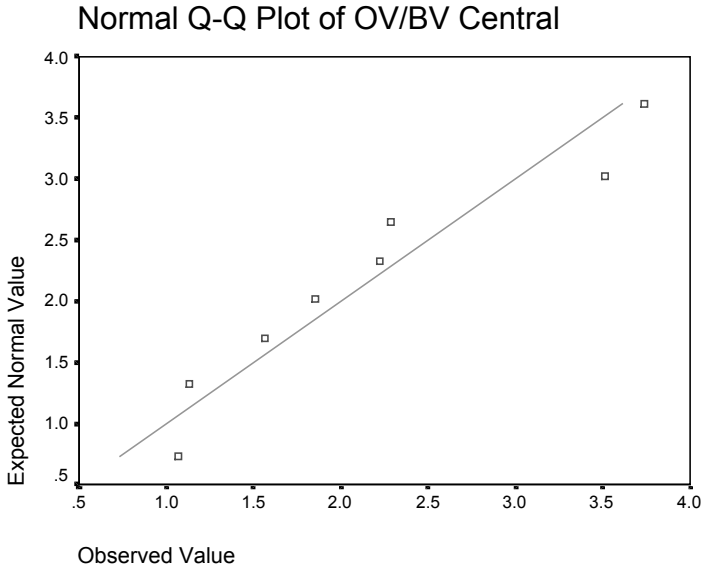
Trabecular Bone Forming and Resorbing Indices: Central Region

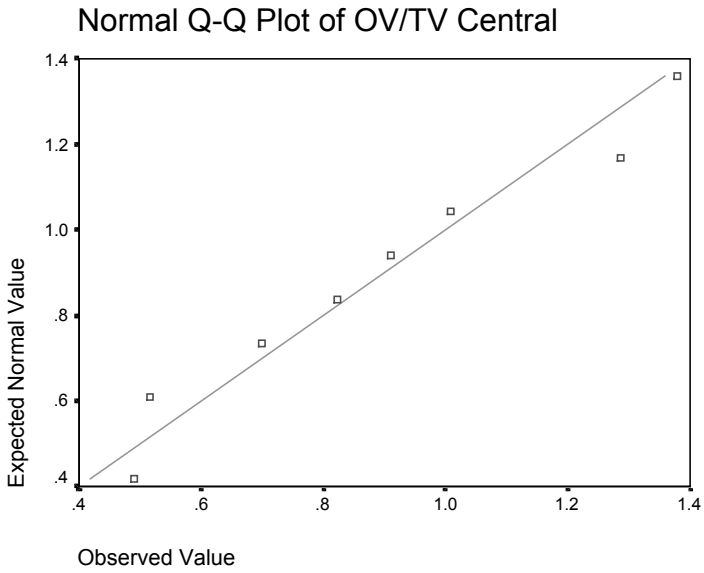




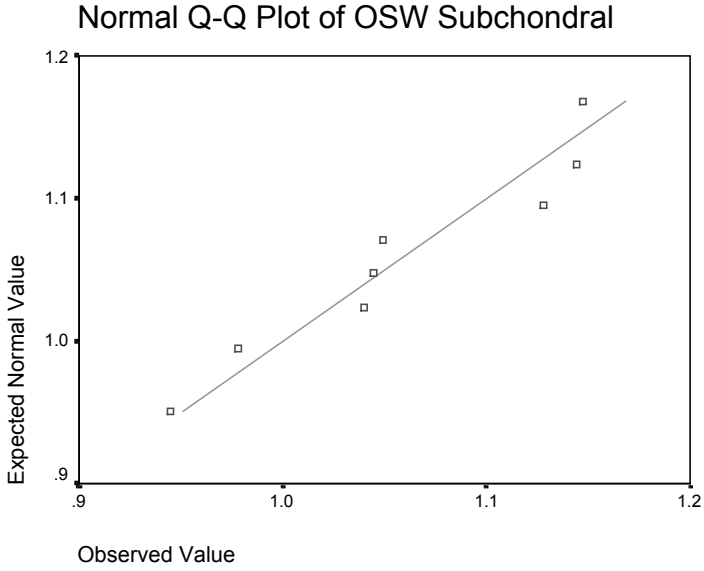


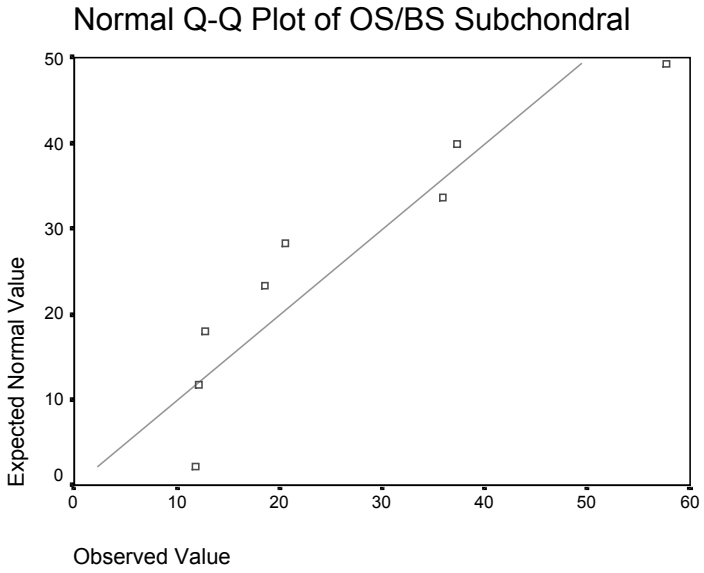

Normal Q-Q Plot of QS/BS Central
Expected Normal Value
Observed Value
60
50
40
30
20
30
40
50
60

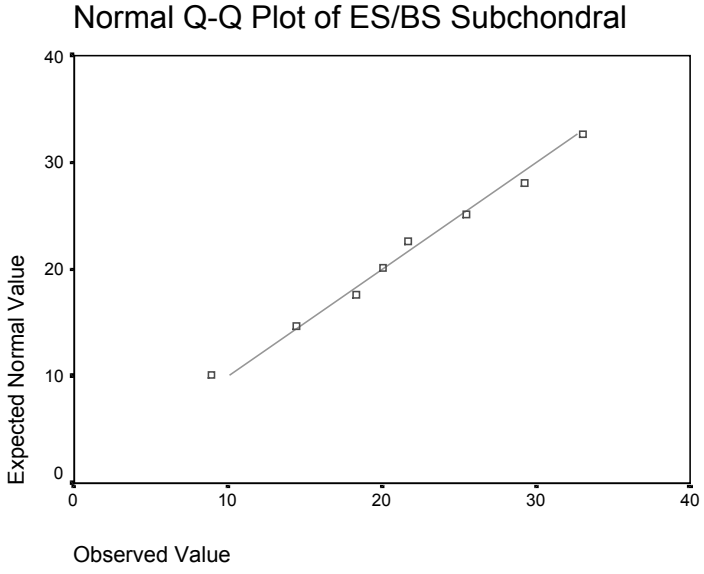

Normal Q-Q Plot of OV/BV Central
Expected Normal Value
Observed Value
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0

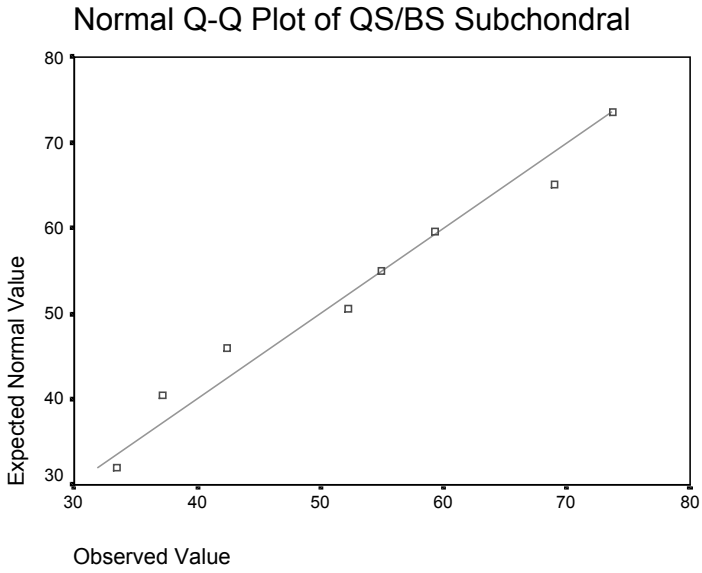

Normal Q-Q Plot of OV/TV Central
Expected Normal Value
Observed Value
1.4
1.2
1.0
.8
.6
.4
.4
.6
.8
1.0
1.2
1.4

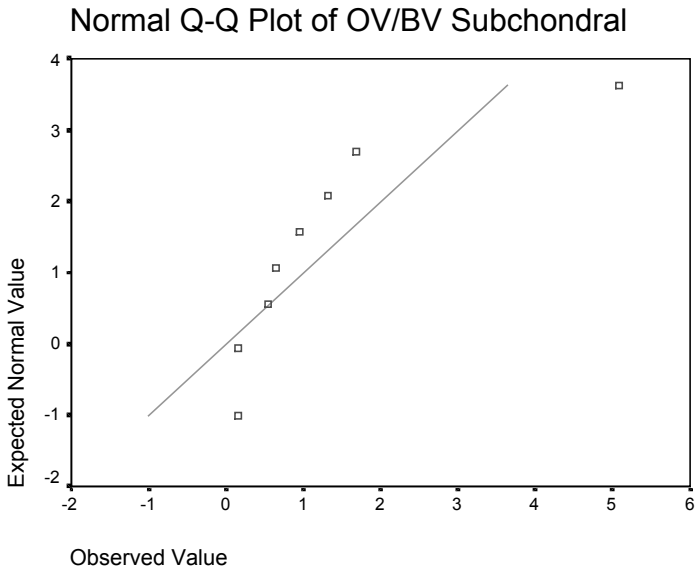
Trabecular Bone Forming and Resorbing Indices: Subchondral Region

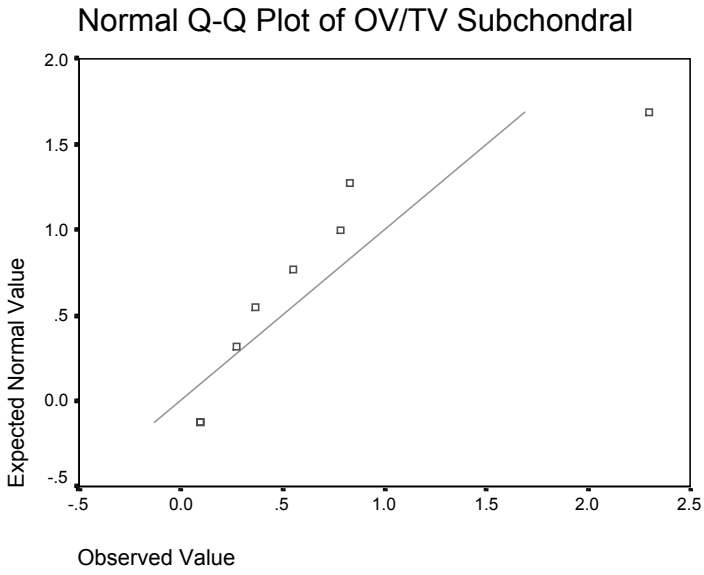




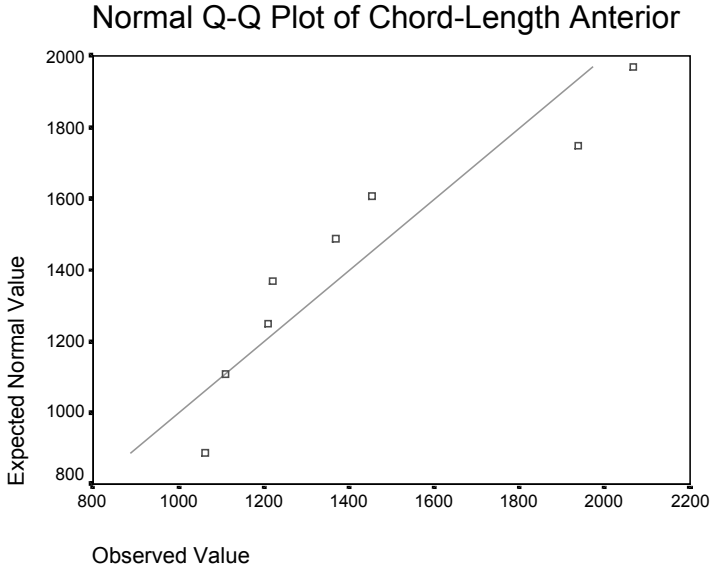


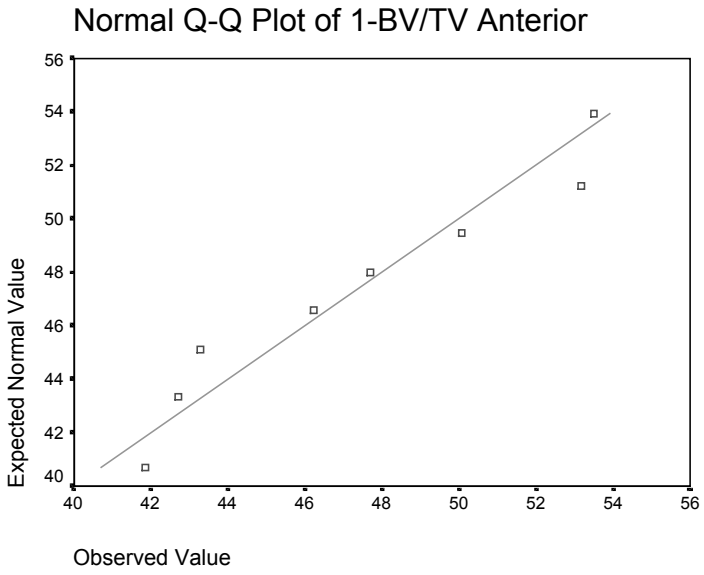

Normal Q-Q Plot of QS/BS Subchondral
Expected Normal Value
Observed Value
80
70
60
50
40
30
30
40
50
60
70
80

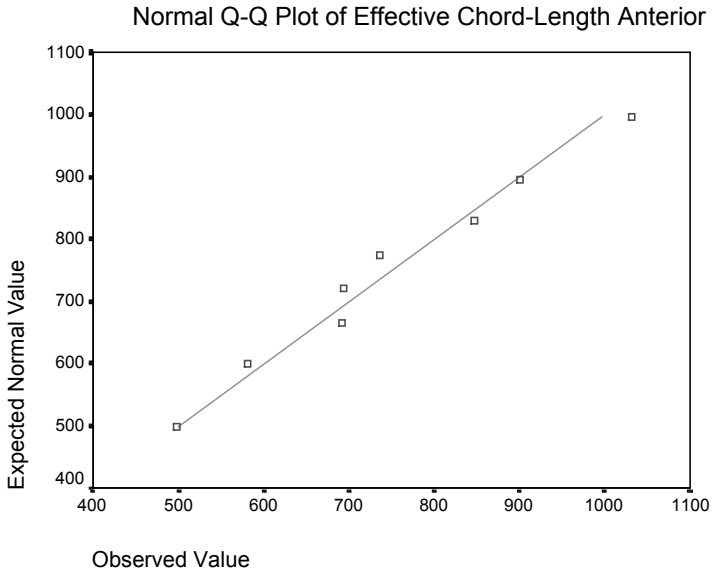

Normal Q-Q Plot of OV/BV Subchondral
Expected Normal Value
Observed Value
4
3
2
1
0
-1
-2
-2
-1
0
1
2
3
4
5
6


Normal Q-Q Plot of OV/TV Subchondral
Expected Normal Value
Observed Value
2.0
1.5
1.0
.5
0.0
-.5
-.5
0.0
.5
1.0
1.5
2.0
2.5

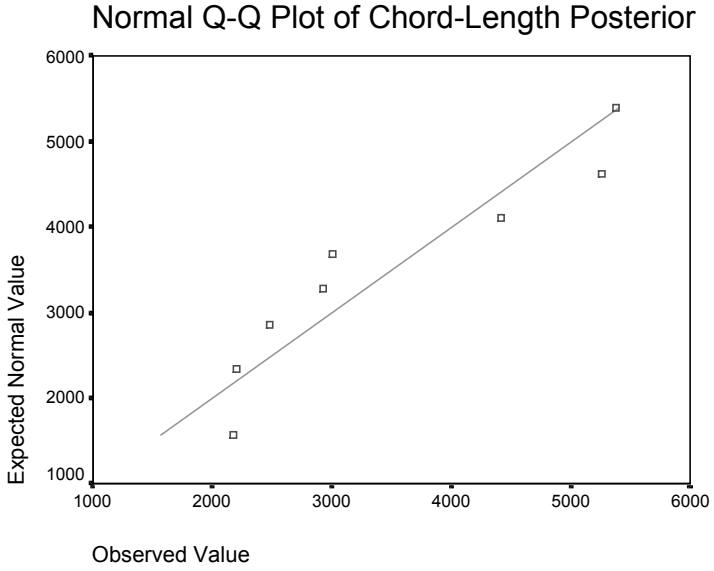
Cortical Bone Structural Indices: Anterior Region

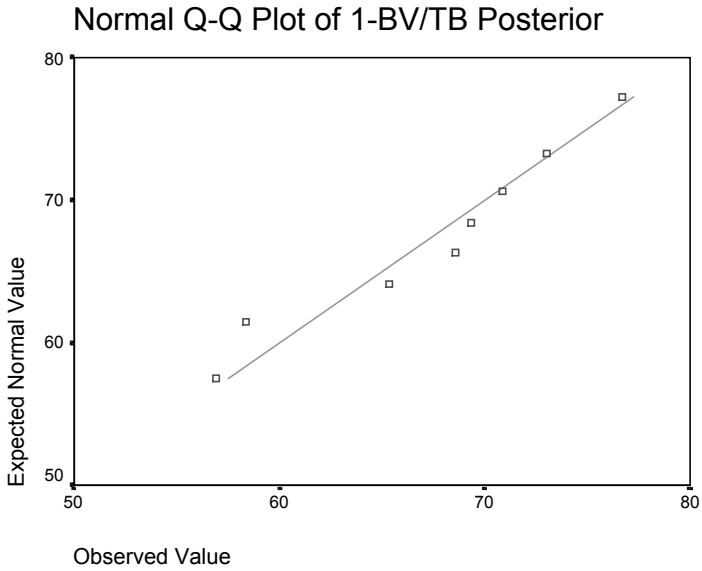


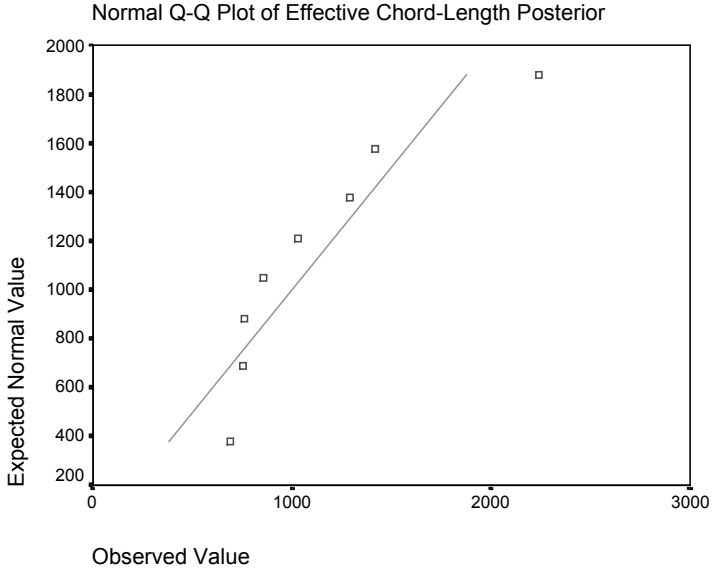




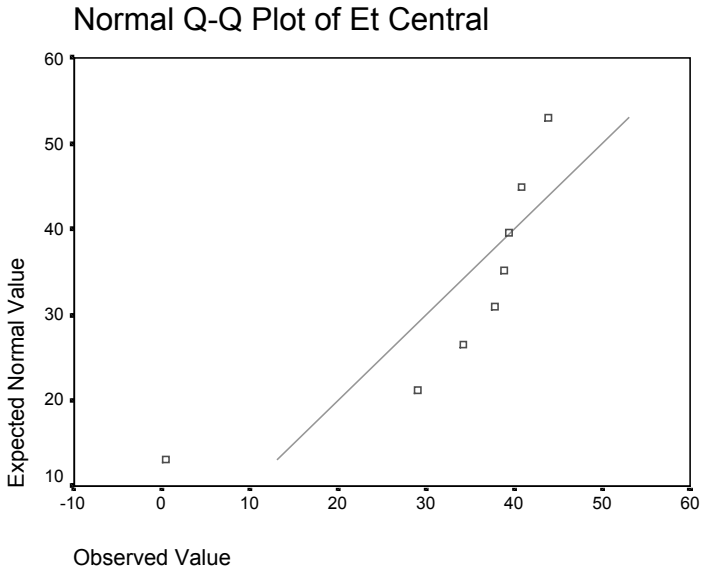
Cortical Bone Structural Indices: Posterior Region

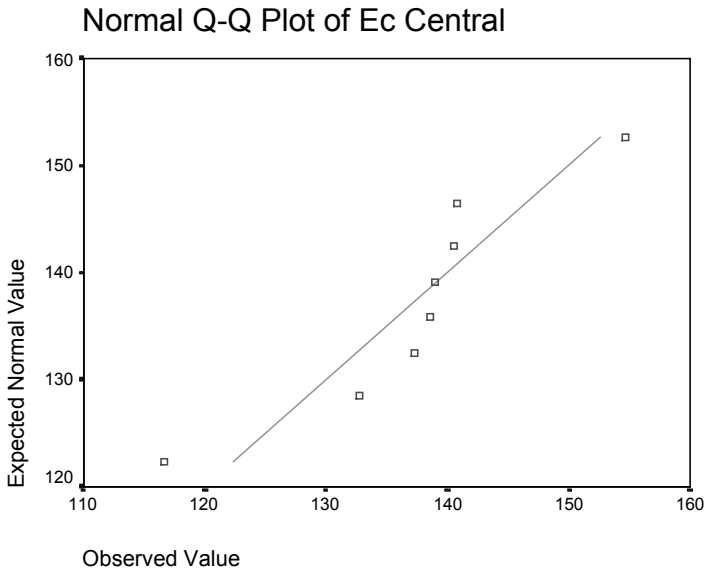


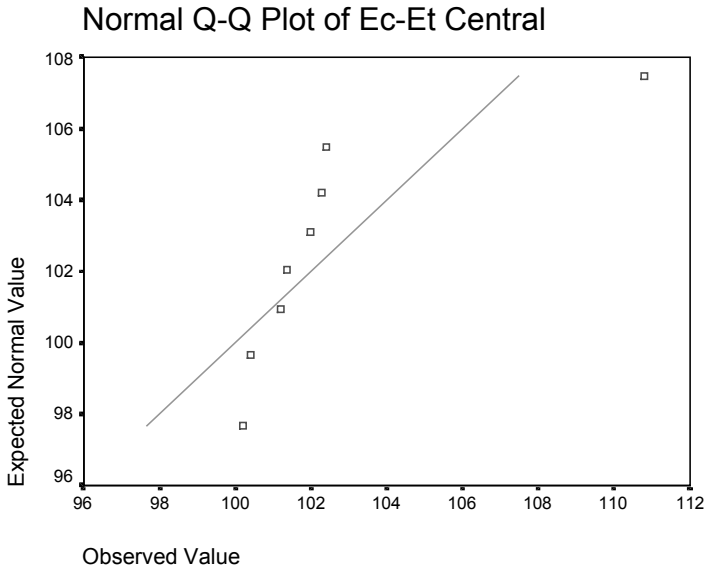


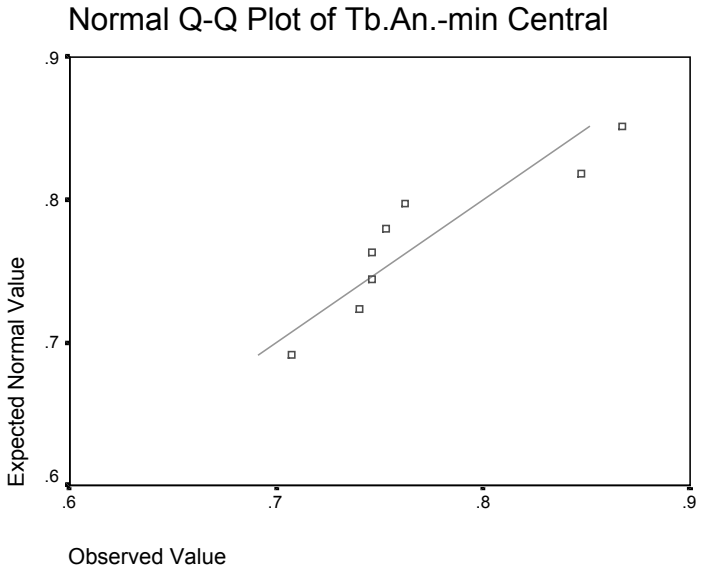


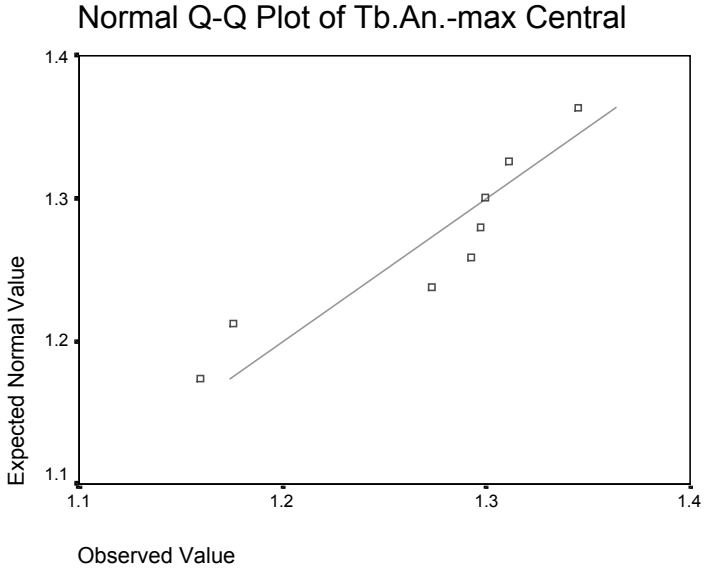
Trabecular Bone Anisotropy: Central Region



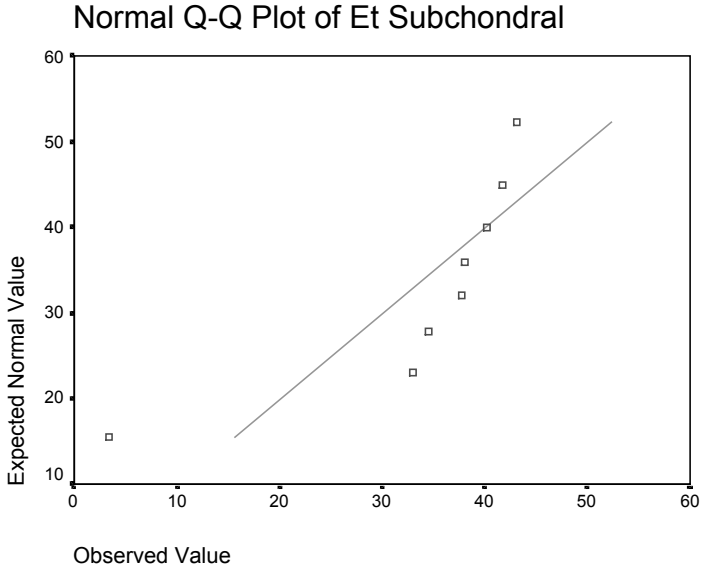


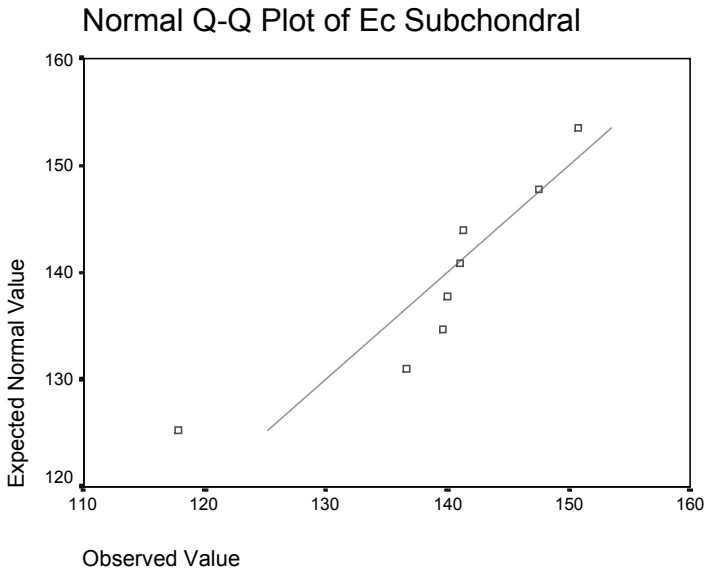


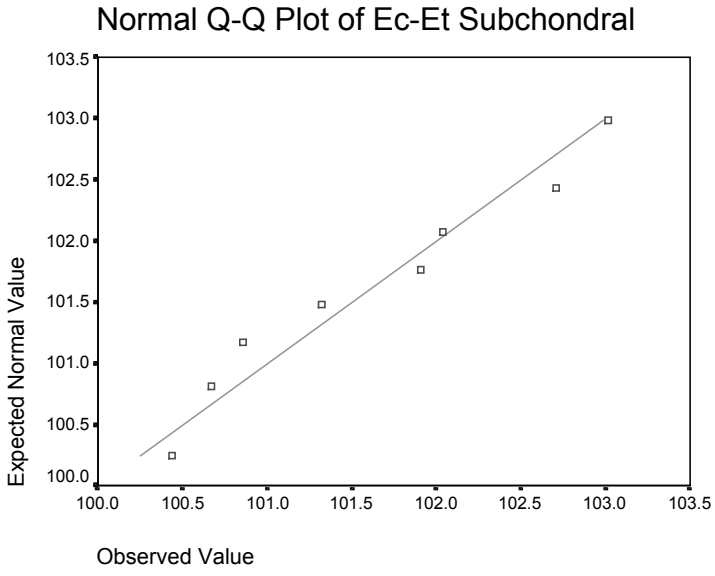

Normal Q-Q Plot of Tb.An.-min Central
Expected Normal Value
.9
.8
.7
.6
.6
.7
.8
.9
Observed Value

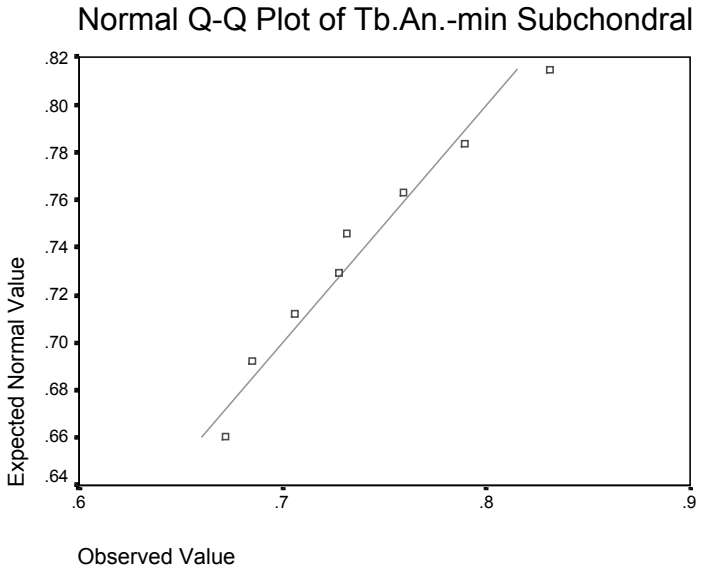

Normal Q-Q Plot of Tb.An.-max Central
Expected Normal Value
1.4
1.3
1.2
1.1
1.1
1.2
1.3
1.4
Observed Value

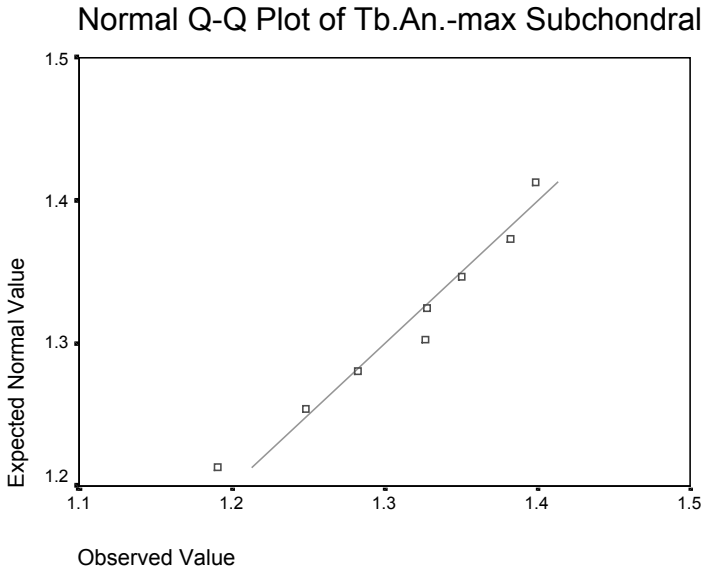
Trabecular Bone Anisotropy: Subchondral Region





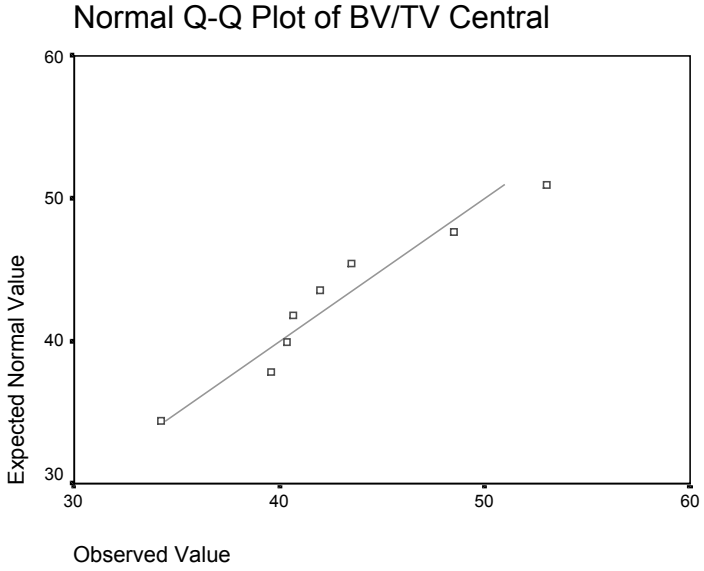


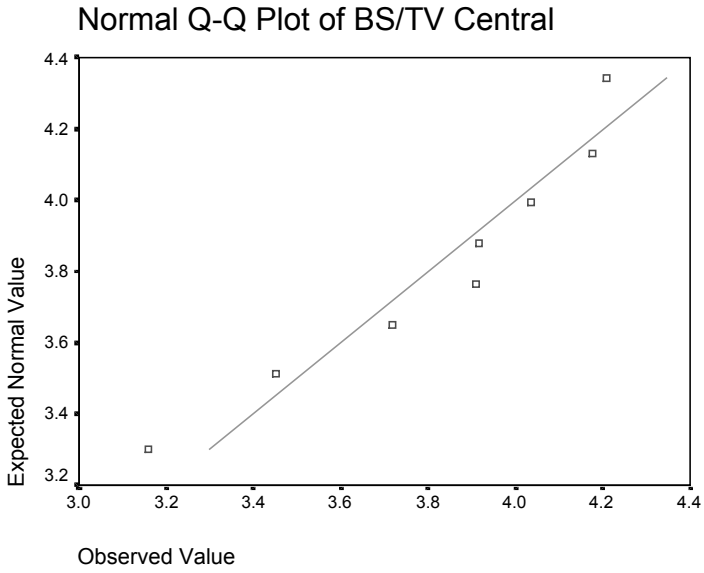

Normal Q-Q Plot of Tb.An.-min Subchondral
Expected Normal Value
.82
.80
.78
.76
.74
.72
.70
.68
.66
.64
.6
.7
.8
.9
Observed Value

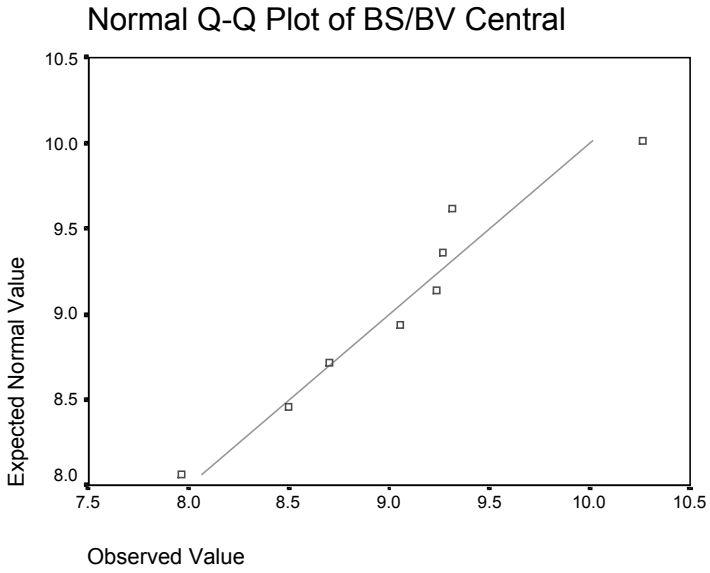

Normal Q-Q Plot of Tb.An.-max Subchondral
Expected Normal Value
1.5
1.4
1.3
1.2
1.1
1.2
1.3
1.4
1.5
Observed Value

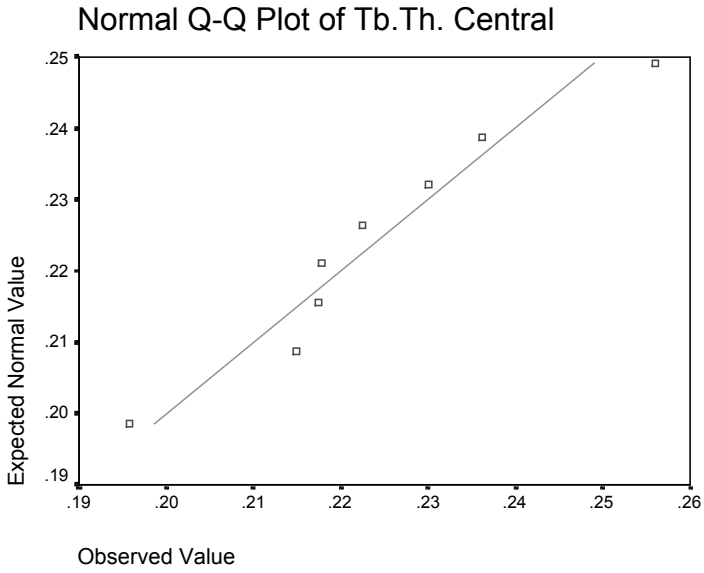
Appendix 2
Graphic Assessment of Normality-
Experimental Group Data

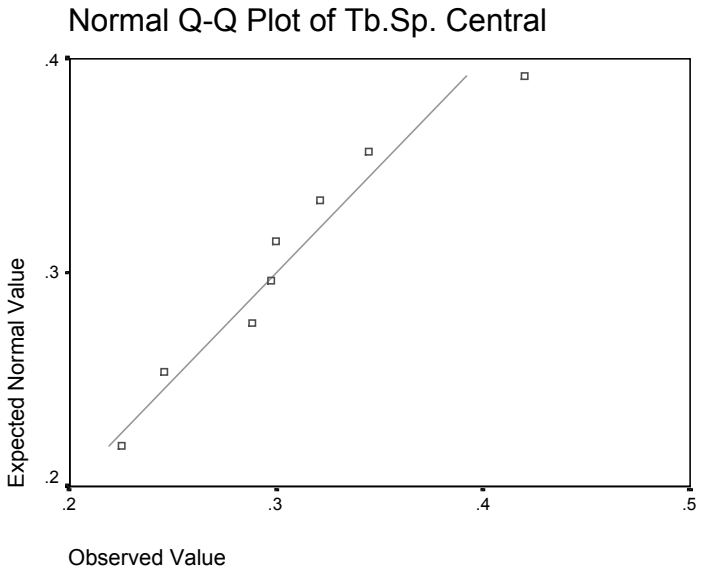
Trabecular Bone Structural Indices: Central Region

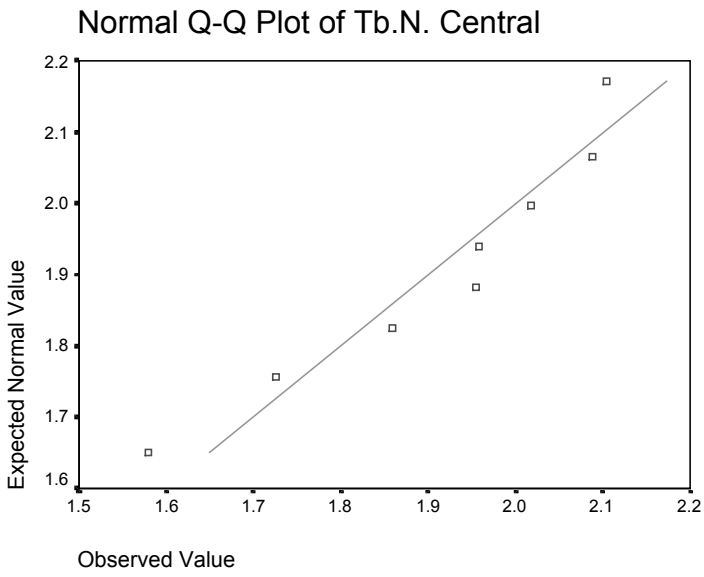




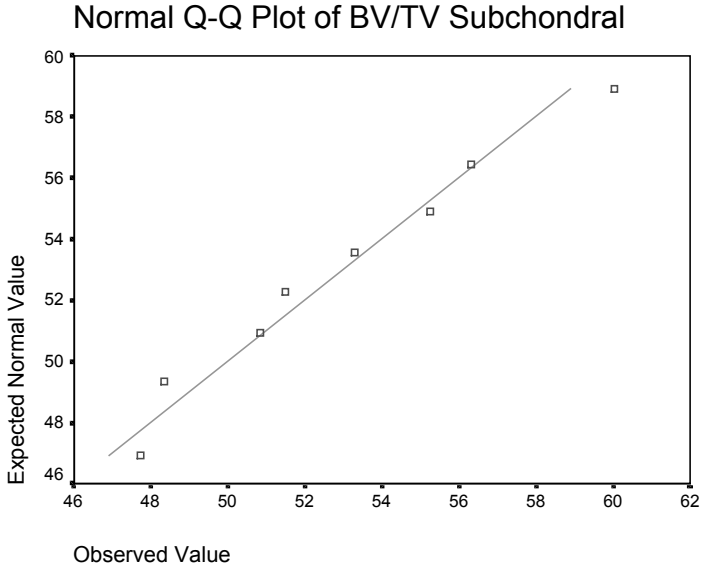


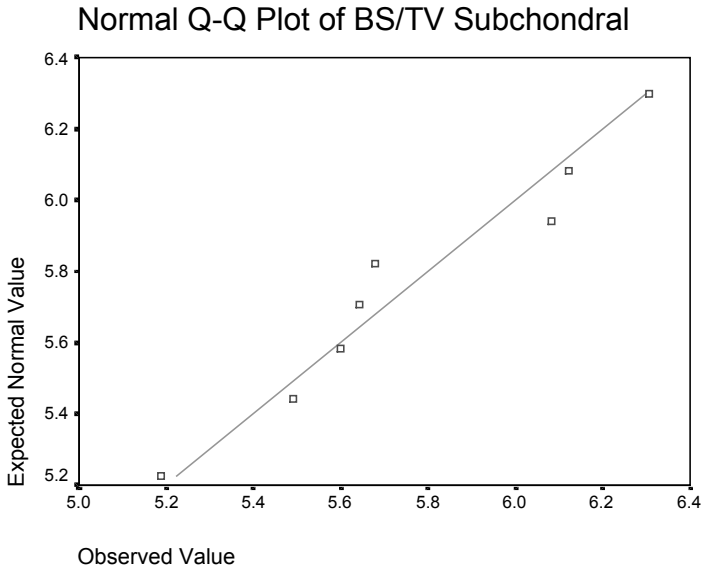

Normal Q-Q Plot of Tb.Th. Central
Expected Normal Value
.25
.24
.23
.22
.21
.20
.19
.19
.20
.21
.22
.23
.24
.25
.26
Observed Value

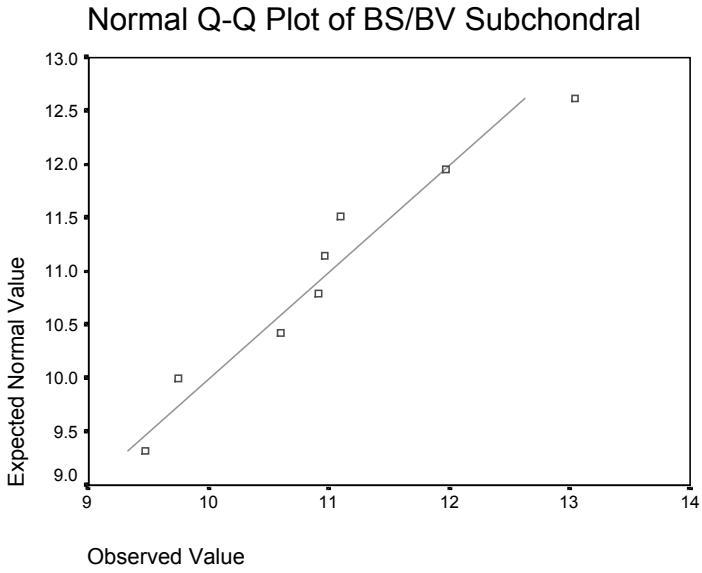

Normal Q-Q Plot of Tb.Sp. Central
Expected Normal Value
.4
.3
.2
.2
.3
.4
.5
Observed Value

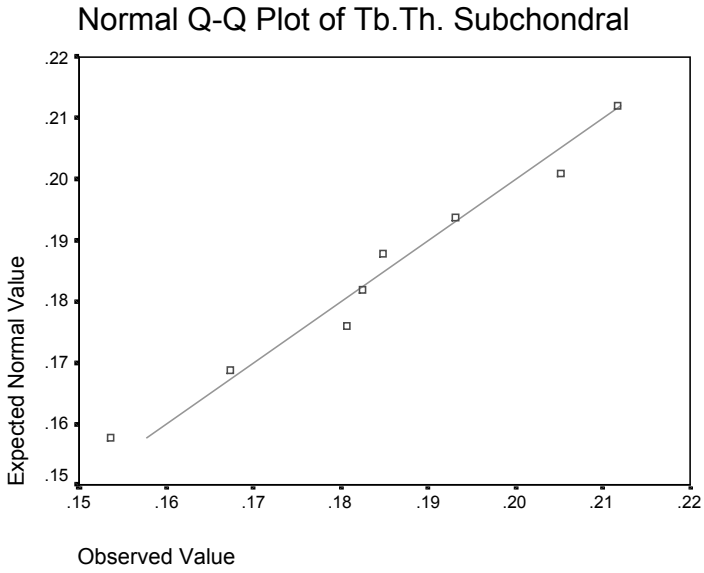

Normal Q-Q Plot of Tb.N. Central
Expected Normal Value
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.6
1.7
1.8
1.9
2.0
2.1
2.2
Observed Value

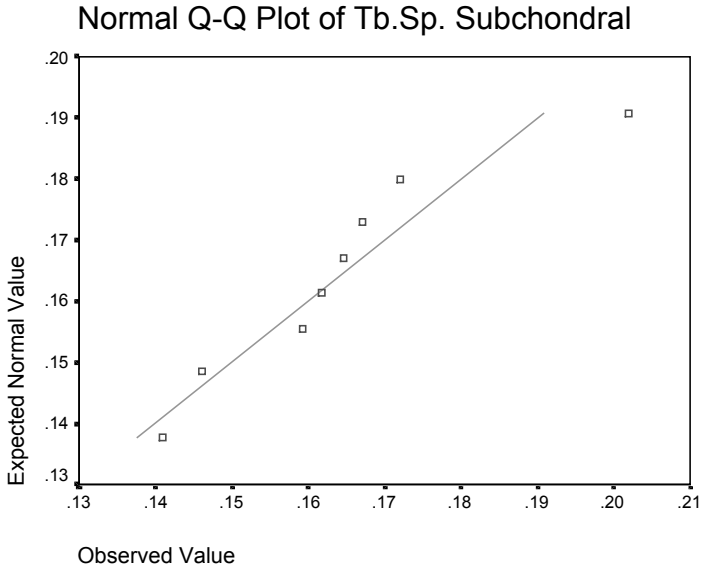
Trabecular Bone Structural Indices: Subchondral Region

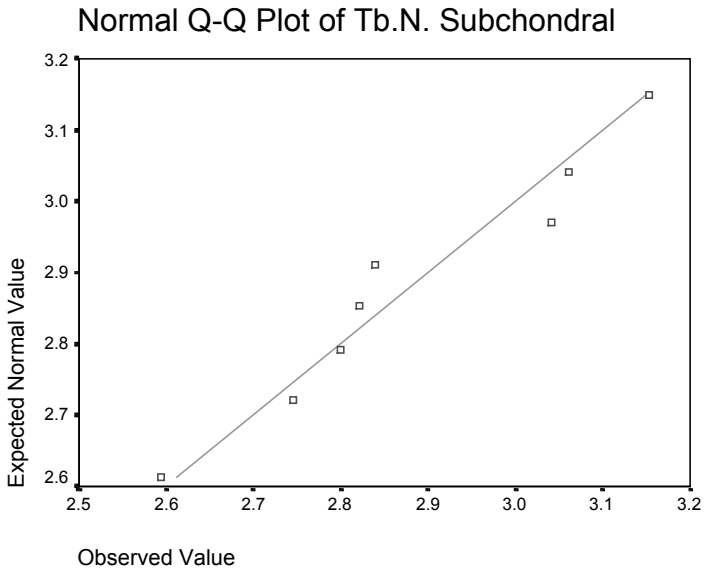




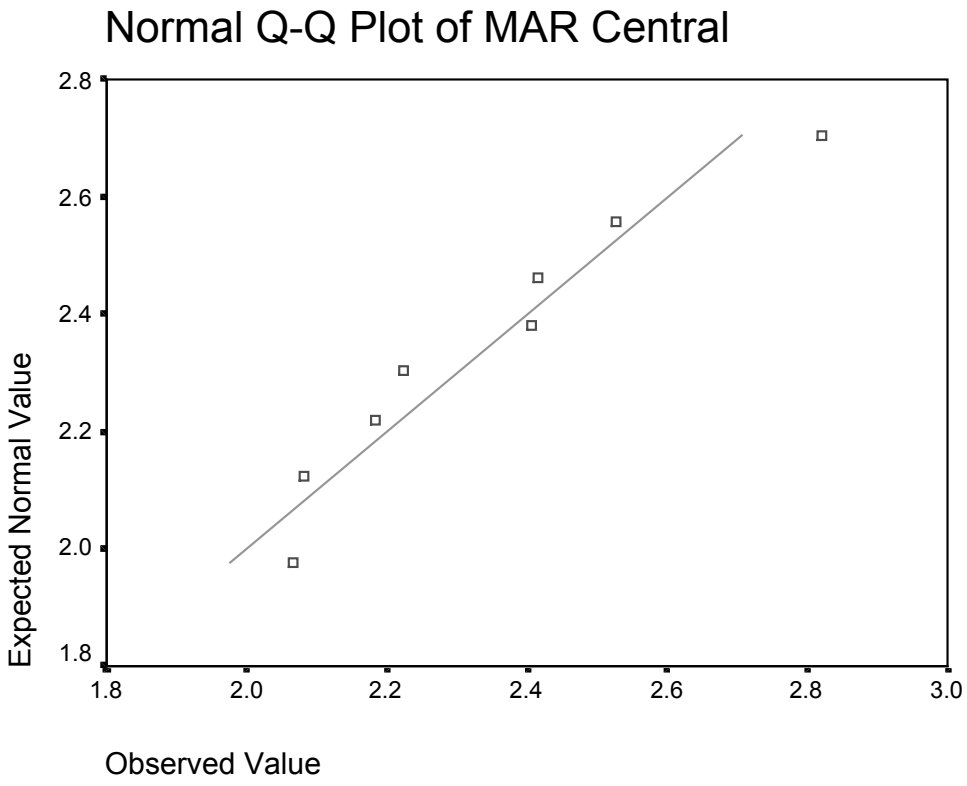


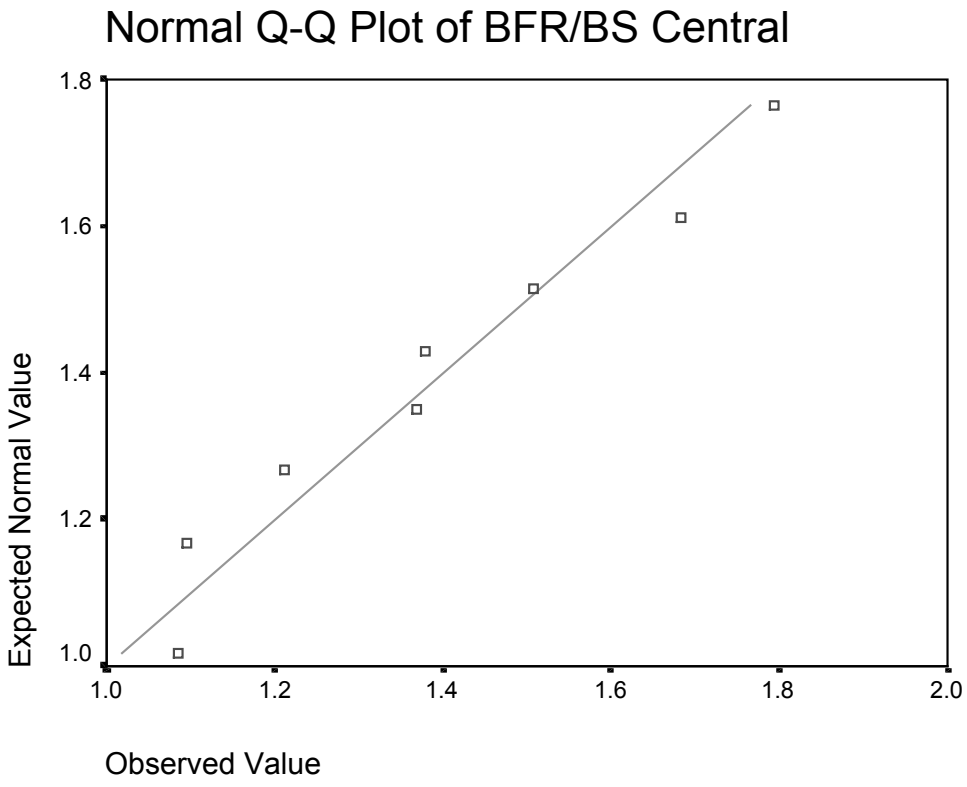

Normal Q-Q Plot of Tb.Th. Subchondral
Expected Normal Value
.22
.21
.20
.19
.18
.17
.16
.15
.15
.16
.17
.18
.19
.20
.21
.22
Observed Value

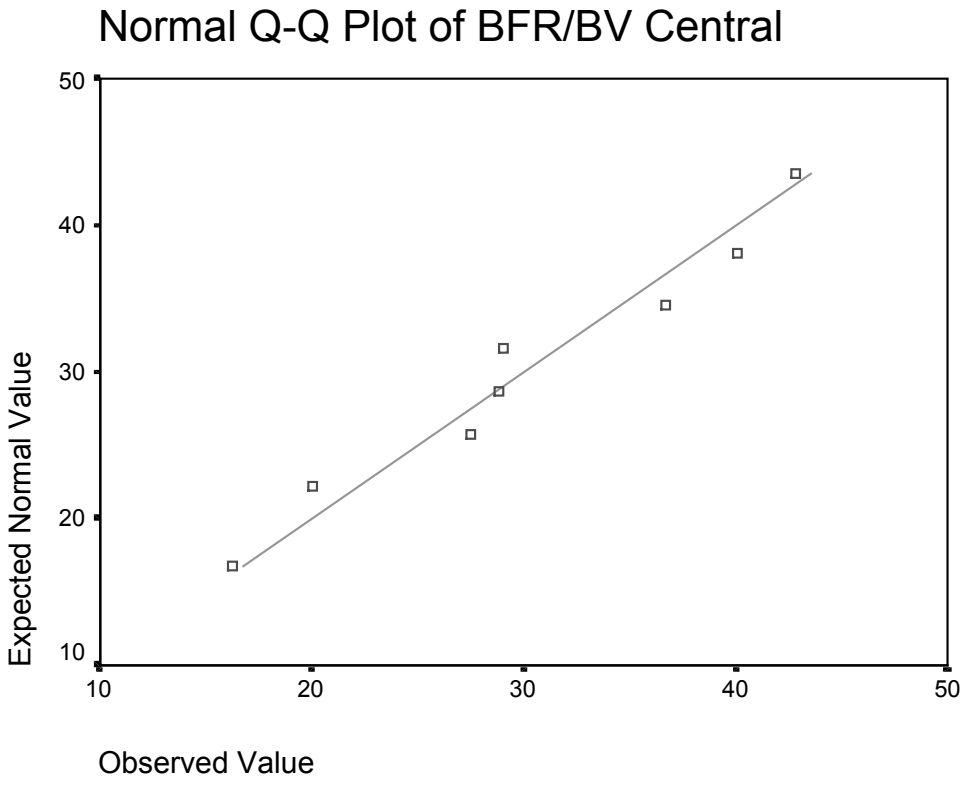

Normal Q-Q Plot of Tb.Sp. Subchondral
Expected Normal Value
.20
.19
.18
.17
.16
.15
.14
.13
.13
.14
.15
.16
.17
.18
.19
.20
.21
Observed Value

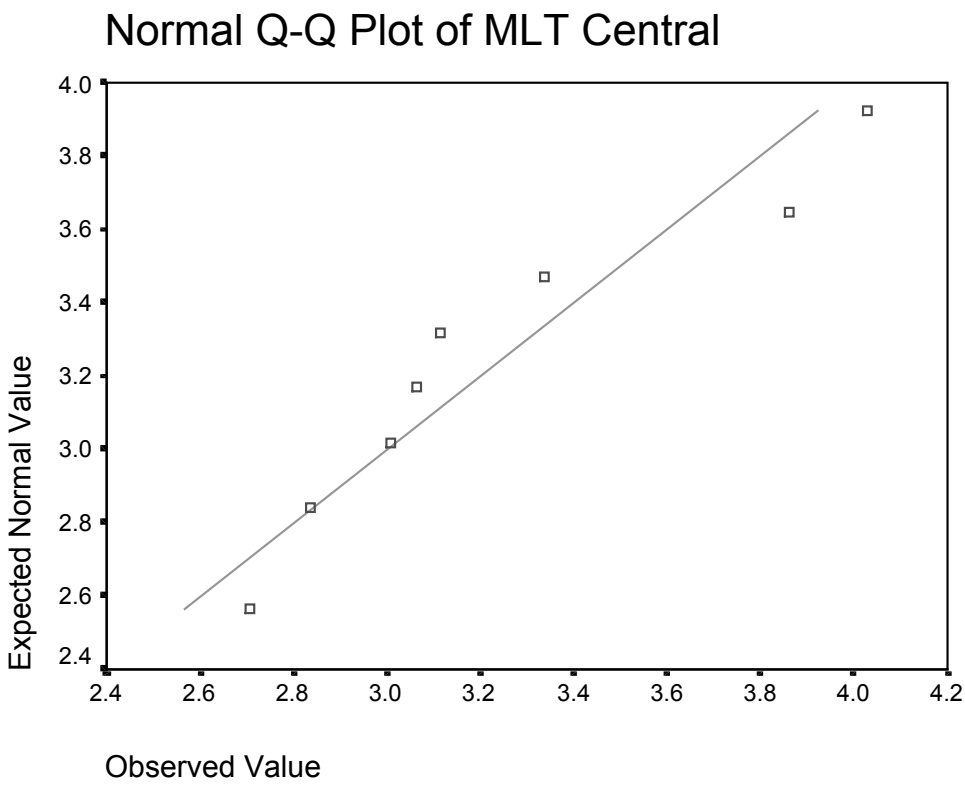

Normal Q-Q Plot of Tb.N. Subchondral
Expected Normal Value
3.2
3.1
3.0
2.9
2.8
2.7
2.6
2.5
2.6
2.7
2.8
2.9
3.0
3.1
3.2
Observed Value

Trabecular Bone Dynamic Indices: Central Region

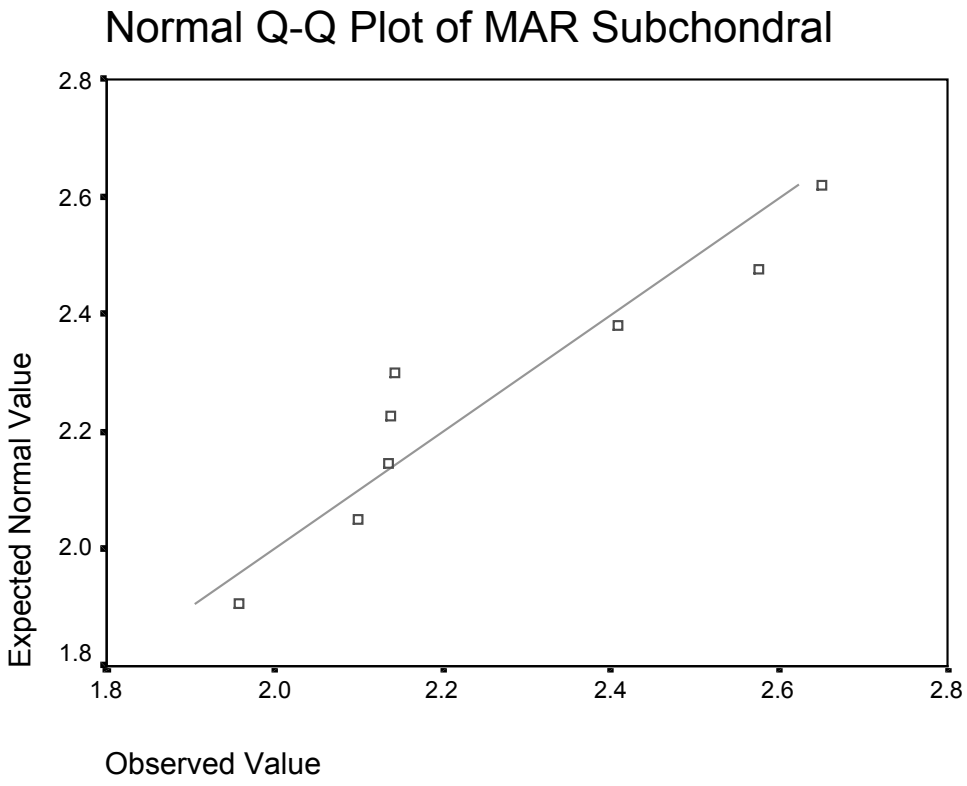


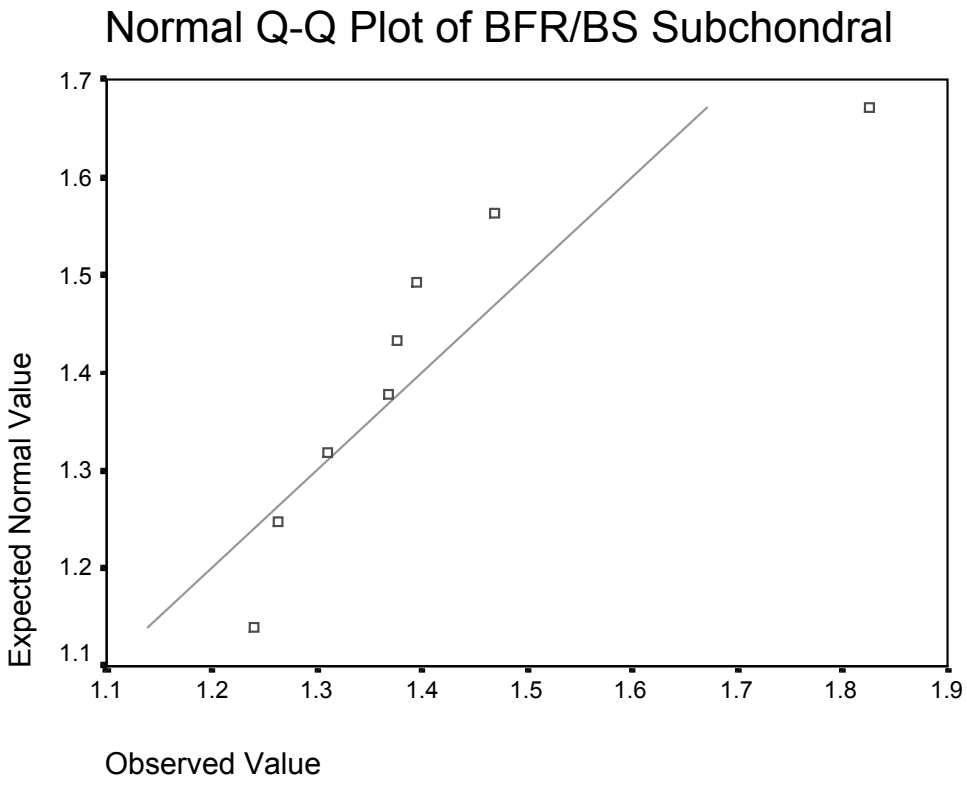


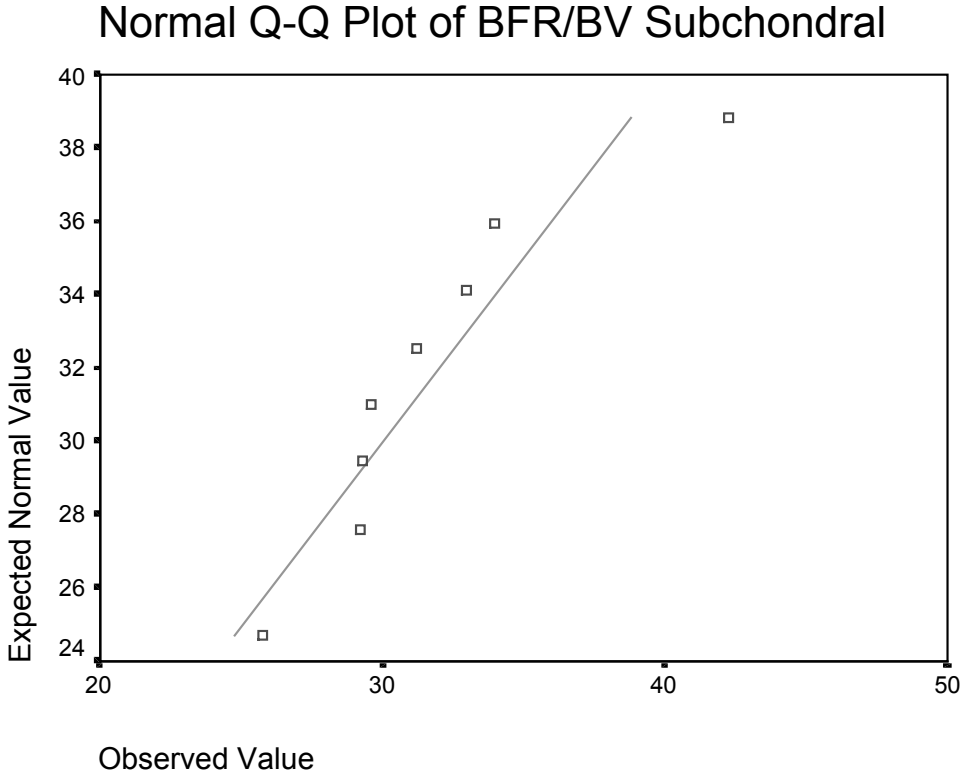

Normal Q-Q Plot of BFR/BV Central
Expected Normal Value
Observed Value
10
20
30
40
50

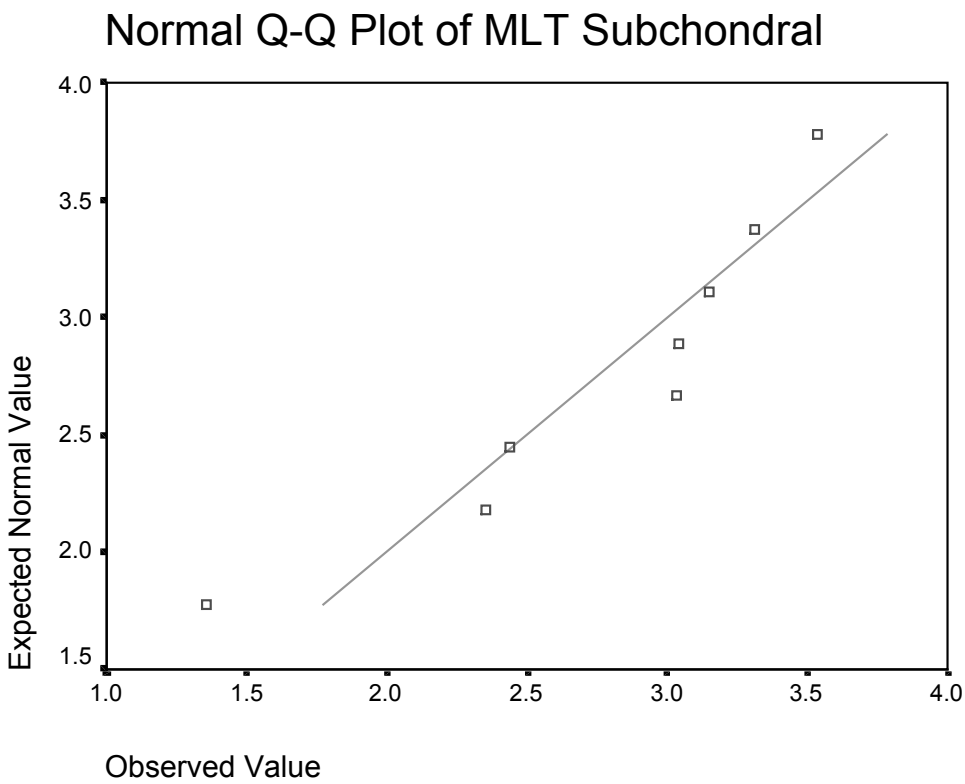

Normal Q-Q Plot of MLT Central
Expected Normal Value
Observed Value
2.4
2.6
2.8
3.0
3.2
3.4
3.6
3.8
4.0
4.2

Trabecular Bone Dynamic Indices: Subchondral Region



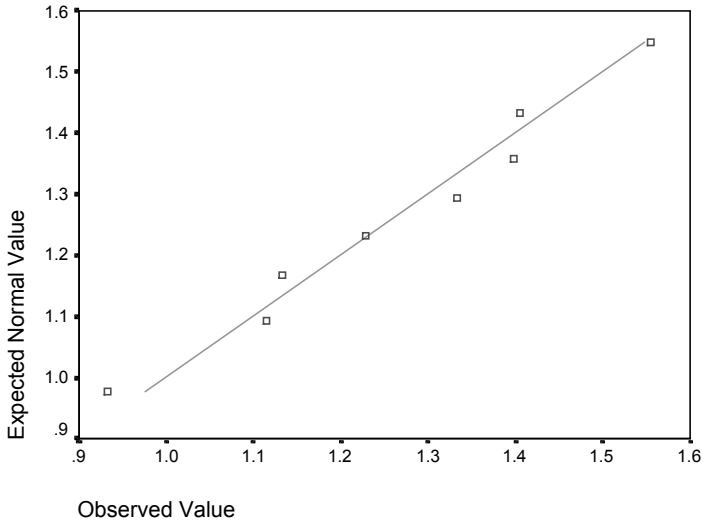



Normal Q-Q Plot of BFR/BV Subchondral
Expected Normal Value
40
38
36
34
32
30
28
26
24
20
30
40
50
Observed Value

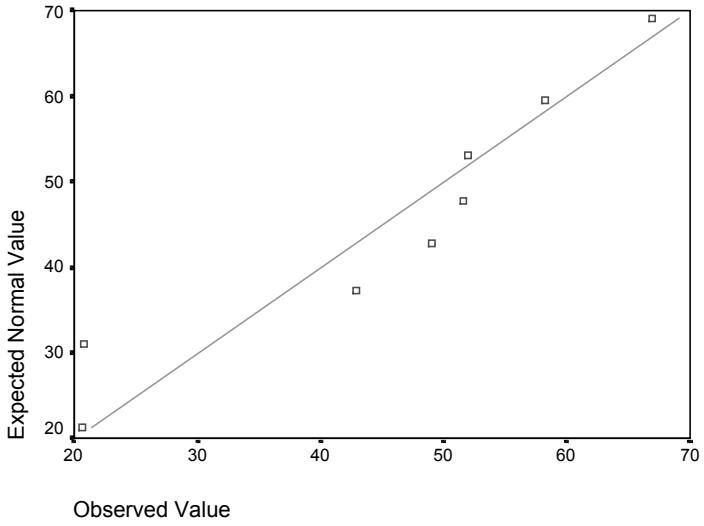

Normal Q-Q Plot of MLT Subchondral
Expected Normal Value
4.0
3.5
3.0
2.5
2.0
1.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Observed Value

Trabecular Bone Forming and Resorbing Indices: Central Region

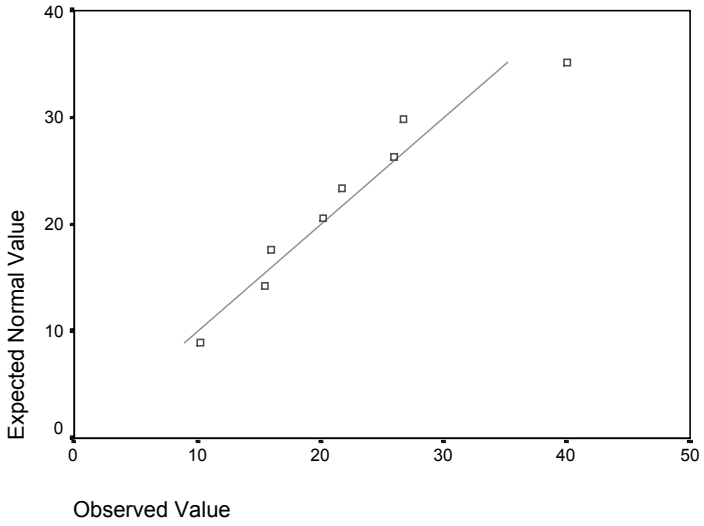
Normal Q-Q Plot of OSW Central

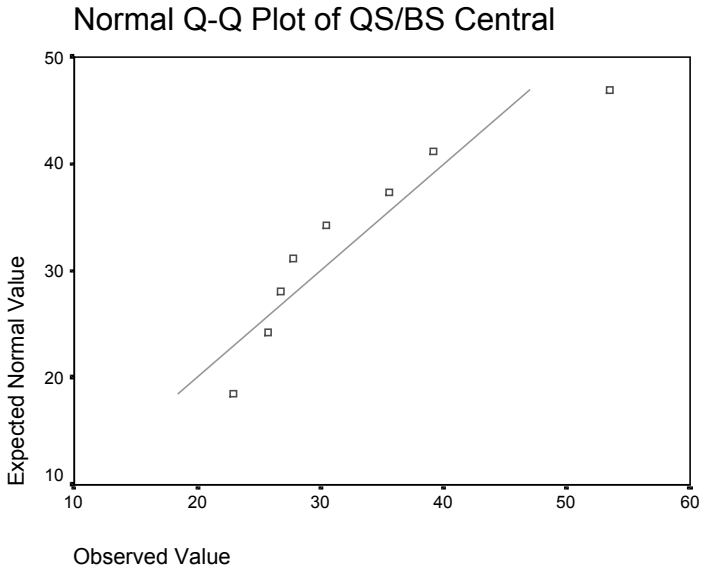


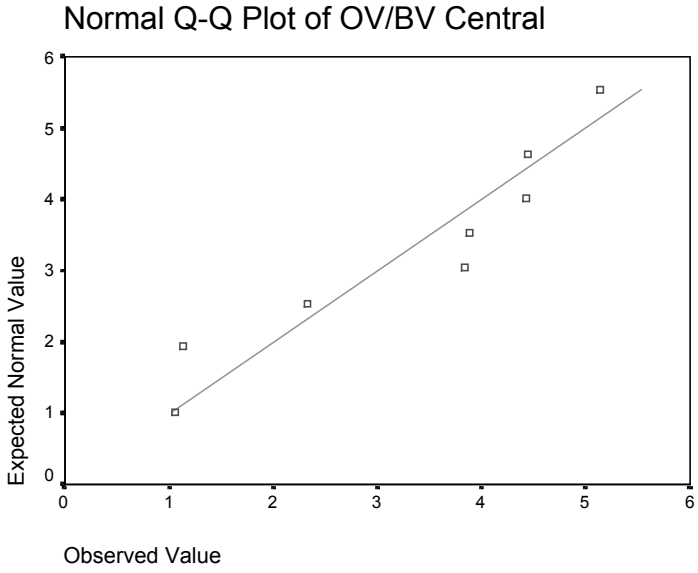
Normal Q-Q Plot of OS/BS Central

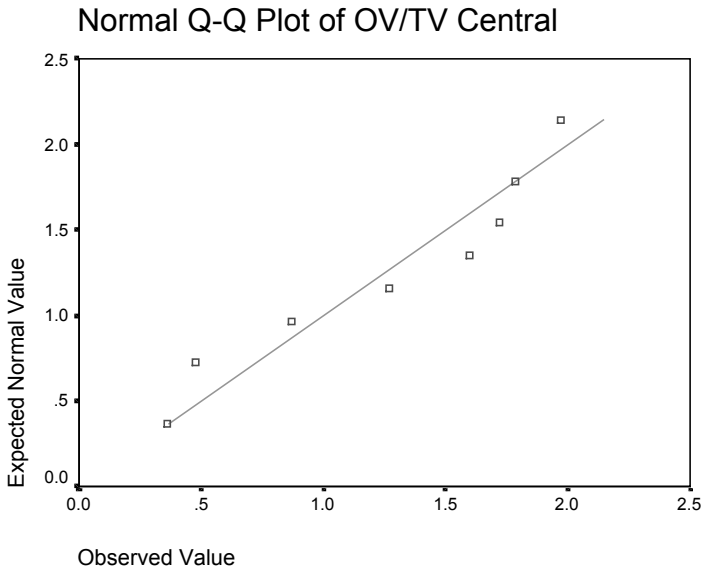


Normal Q-Q Plot of ES/BS Central



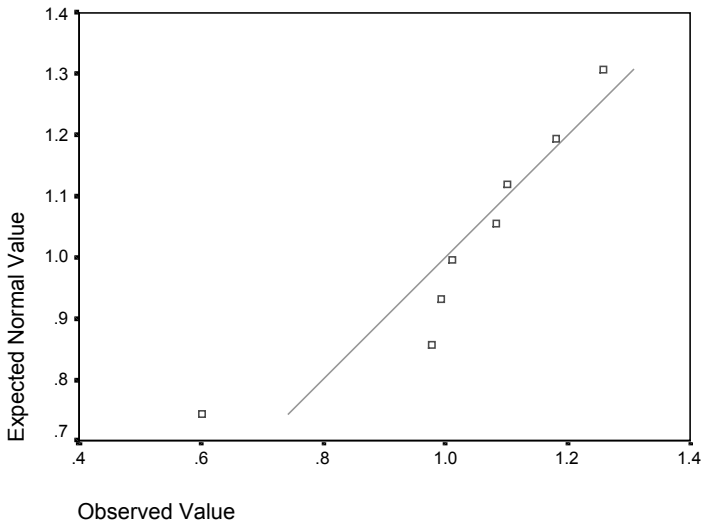

Normal Q-Q Plot of QS/BS Central
Expected Normal Value
Observed Value
50
40
30
20
10
10
20
30
40
50
60


Normal Q-Q Plot of OV/BV Central
Expected Normal Value
Observed Value
6
5
4
3
2
1
0
0
1
2
3
4
5
6

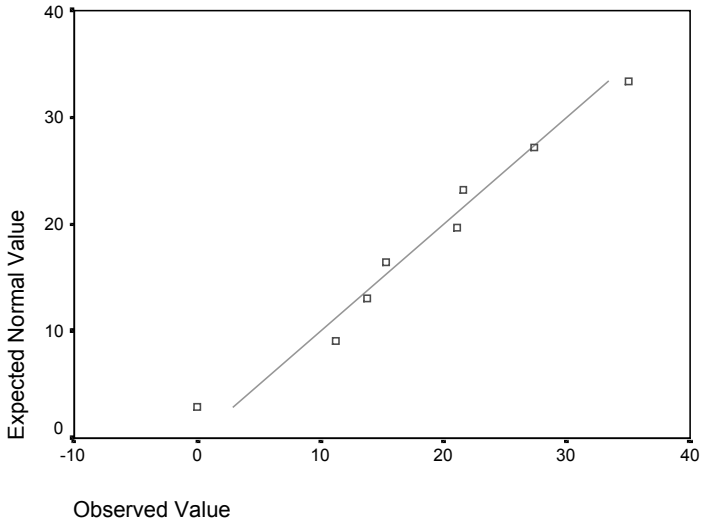

Normal Q-Q Plot of OV/TV Central
Expected Normal Value
Observed Value
2.5
2.0
1.5
1.0
.5
0.0
0.0
.5
1.0
1.5
2.0
2.5

Trabecular Bone Forming and Resorbing Indices: Subchondral Region

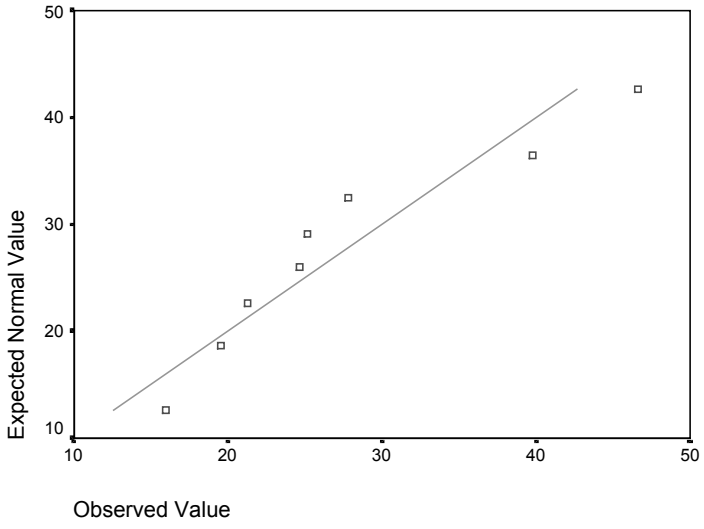
Normal Q-Q Plot of OSW Subchondral

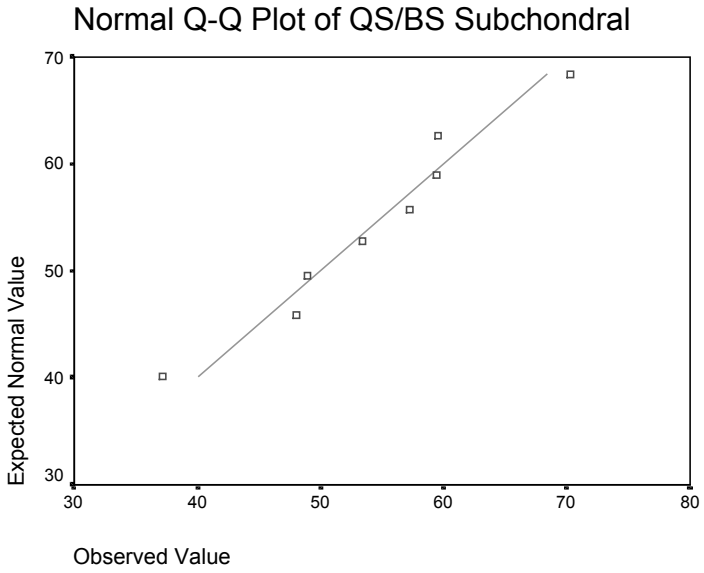


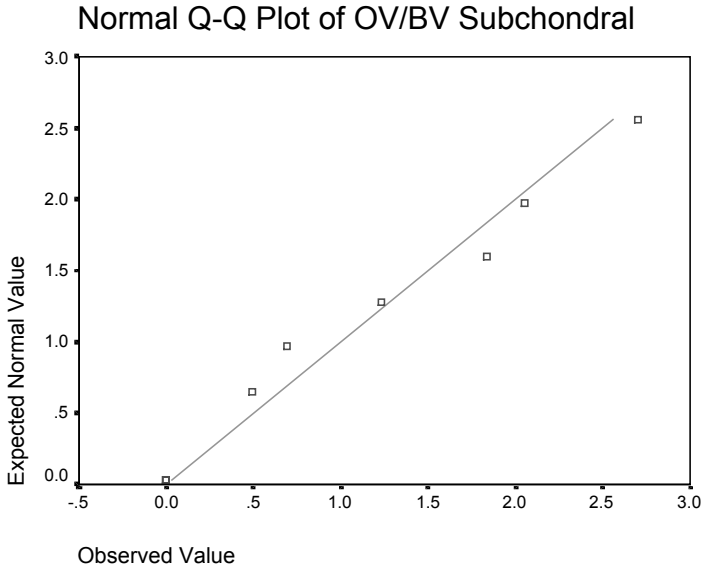
Normal Q-Q Plot of OS/BS Subchondral

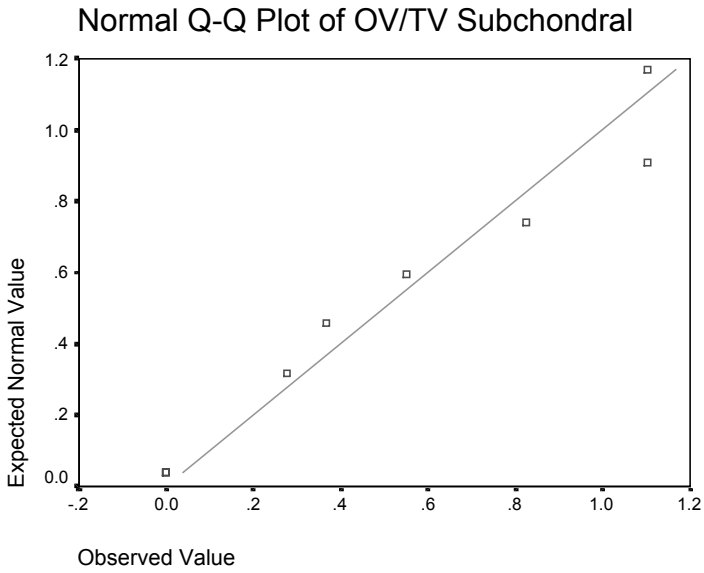


Normal Q-Q Plot of ES/BS Subchondral

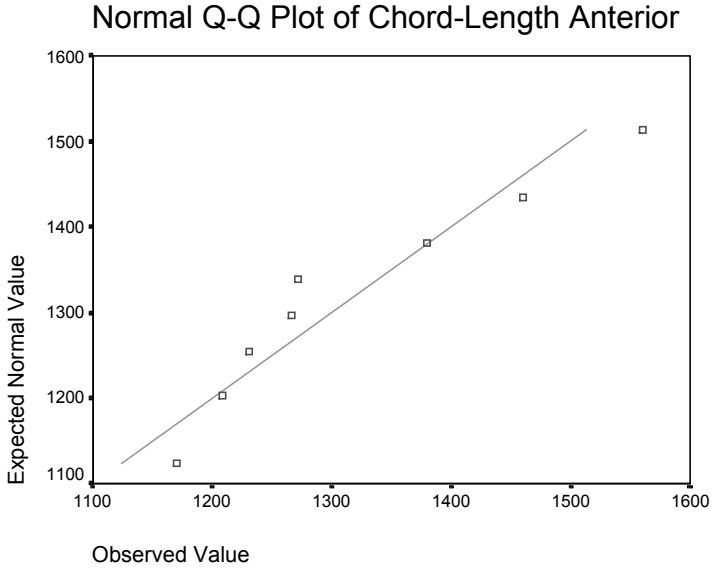


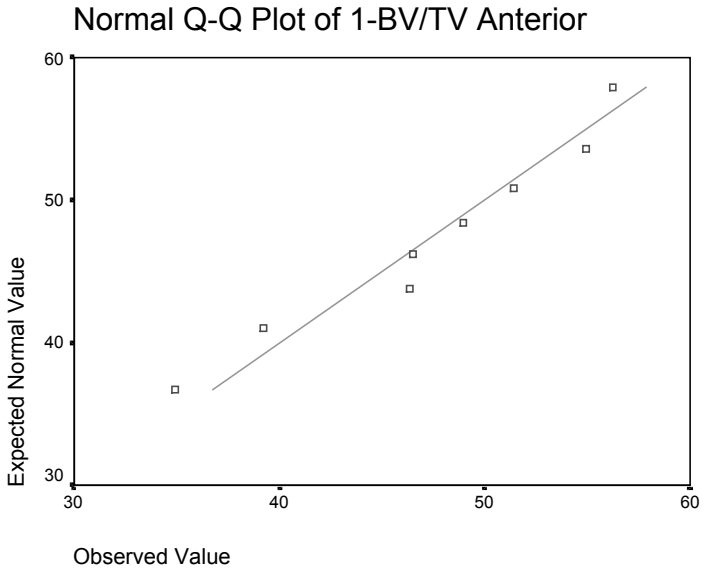

Normal Q-Q Plot of QS/BS Subchondral
Expected Normal Value
70
60
50
40
30
30
40
50
60
70
80
Observed Value

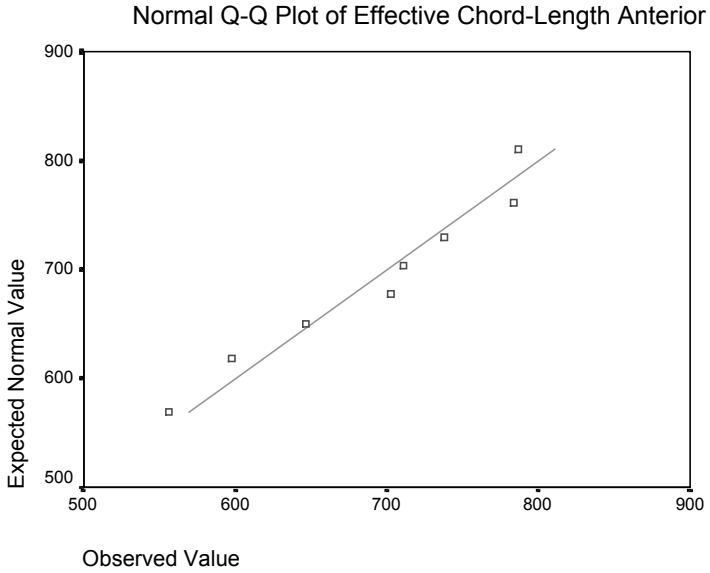

Normal Q-Q Plot of OV/BV Subchondral
Expected Normal Value
3.0
2.5
2.0
1.5
1.0
.5
0.0
-.5
0.0
.5
1.0
1.5
2.0
2.5
3.0
Observed Value


Normal Q-Q Plot of OV/TV Subchondral
Expected Normal Value
1.2
1.0
.8
.6
.4
.2
0.0
-.2
0.0
.2
.4
.6
.8
1.0
1.2
Observed Value

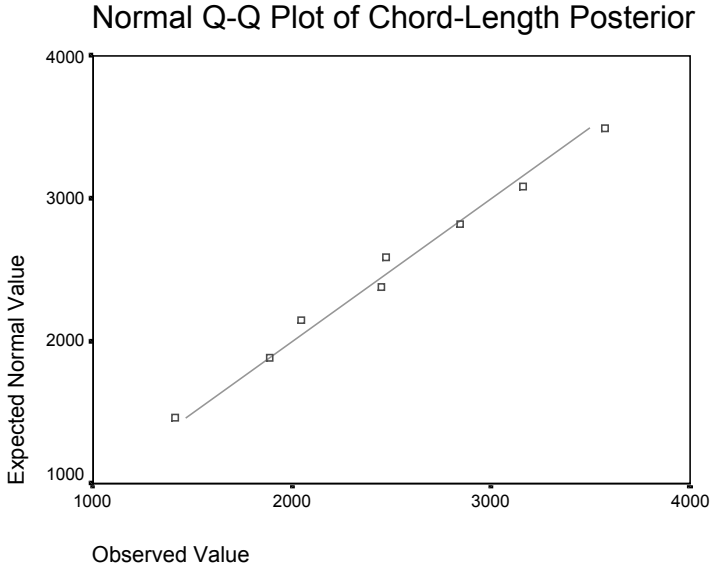
Cortical Bone Structural Indices: Anterior Region

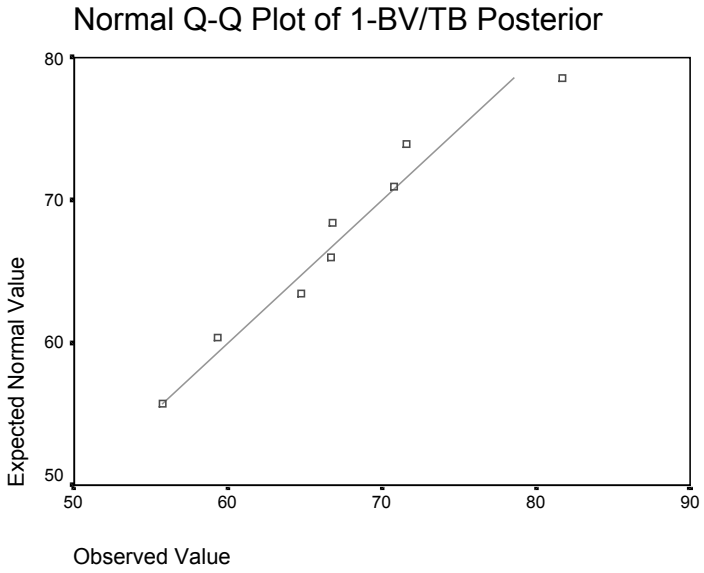


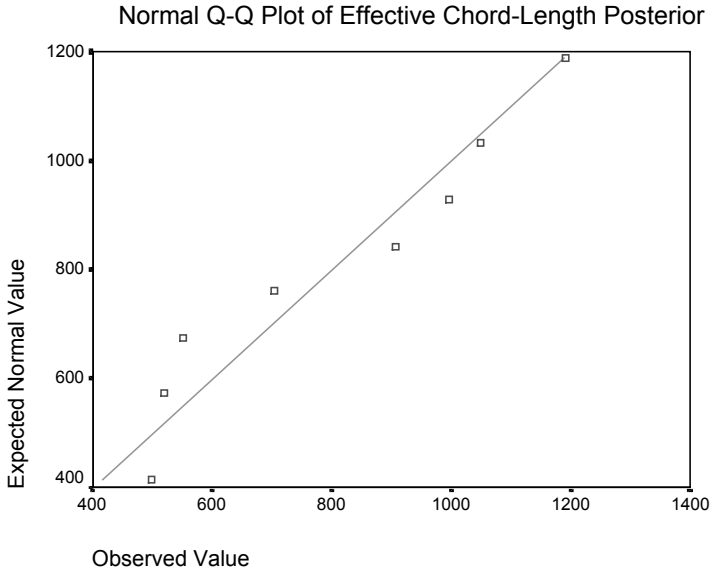




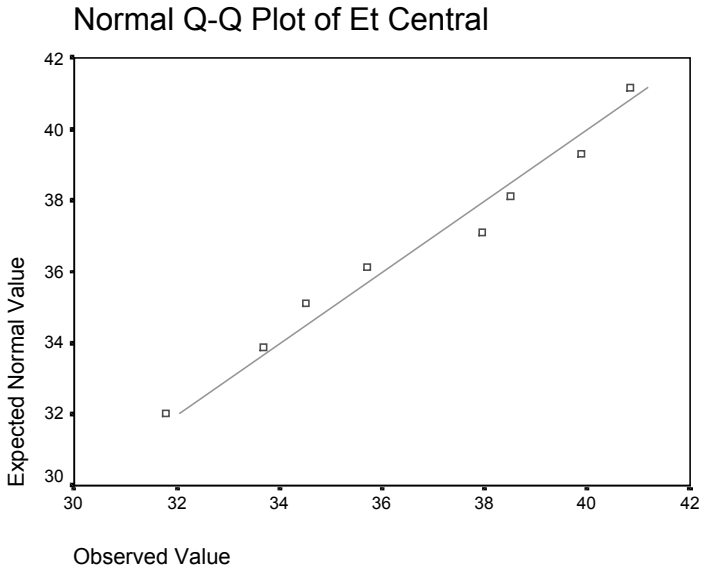
Cortical Bone Structural Indices: Posterior Region

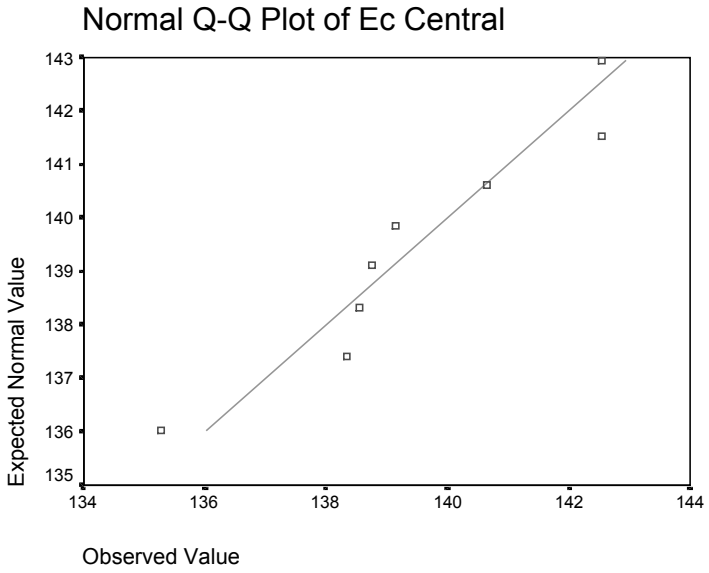


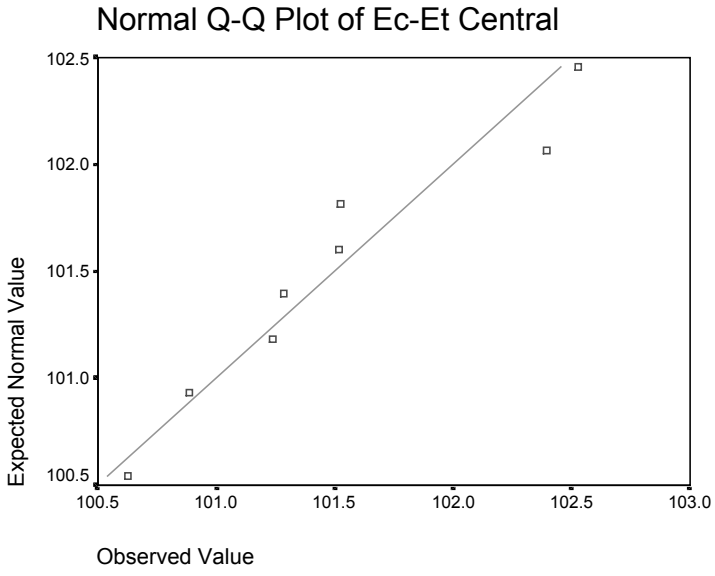


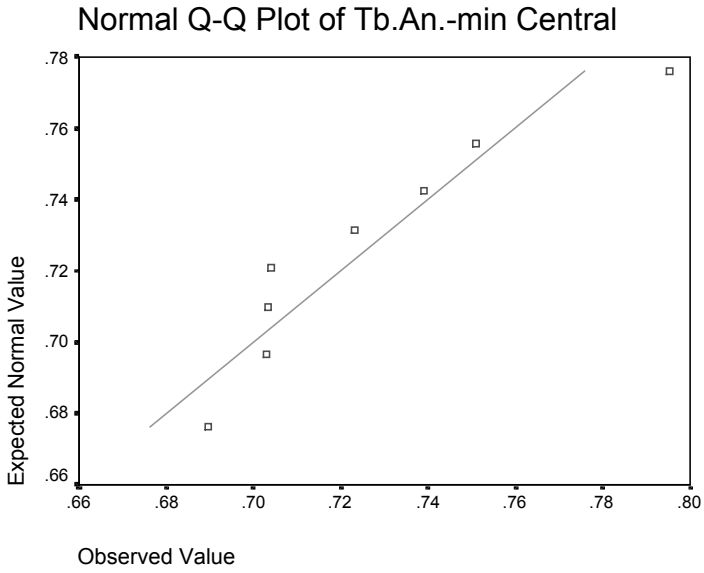


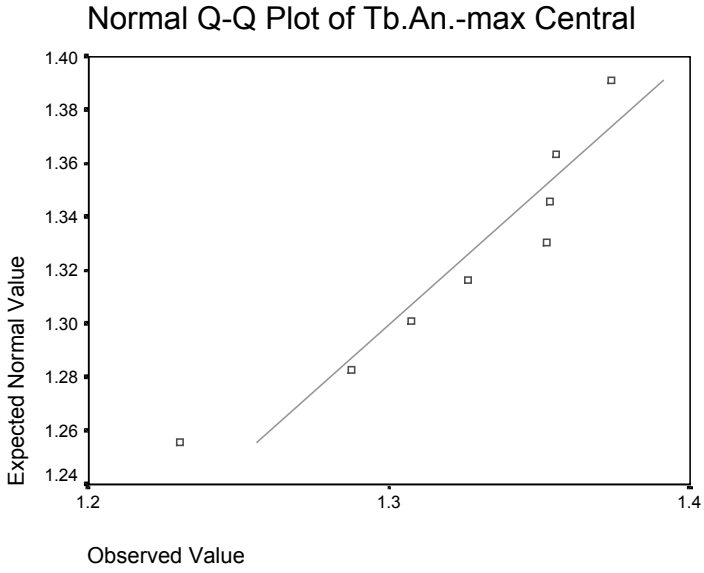
Trabecular Bone Anisotropy: Central Region



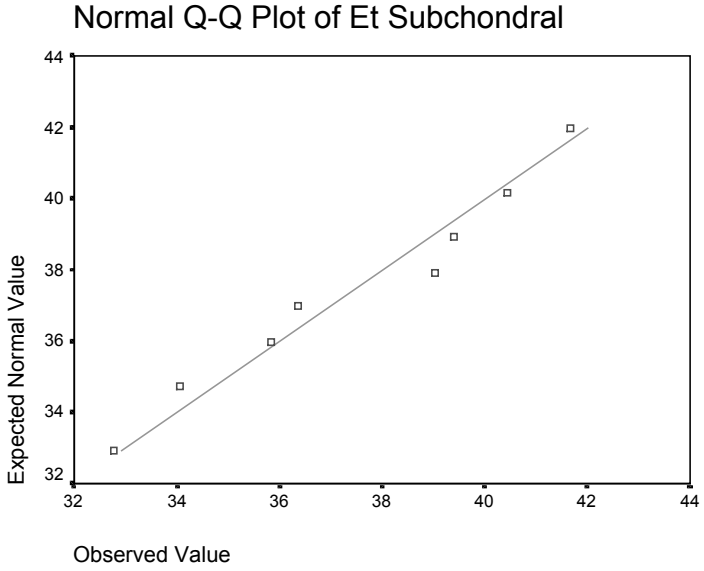


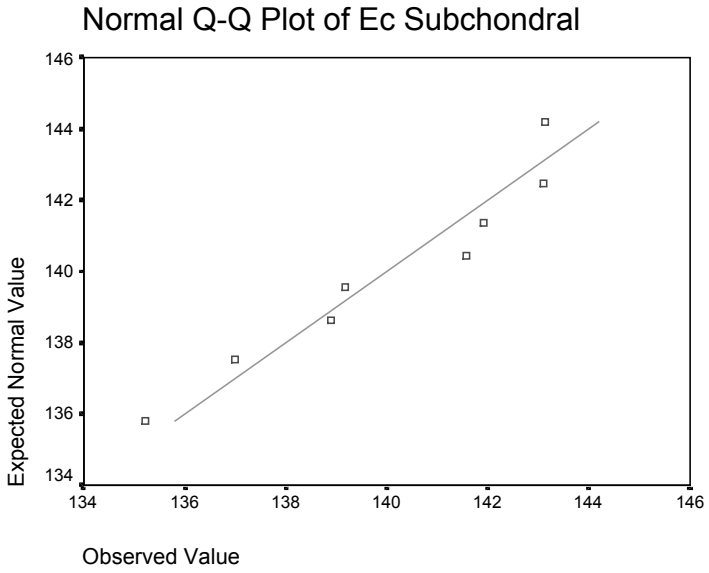


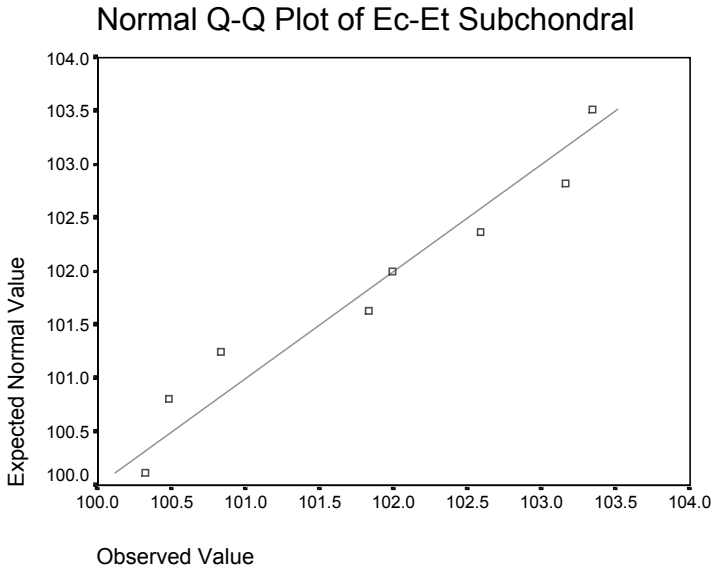

Normal Q-Q Plot of Tb.An.-min Central
Expected Normal Value
.78
.76
.74
.72
.70
.68
.66
.66
.68
.70
.72
.74
.76
.78
.80
Observed Value

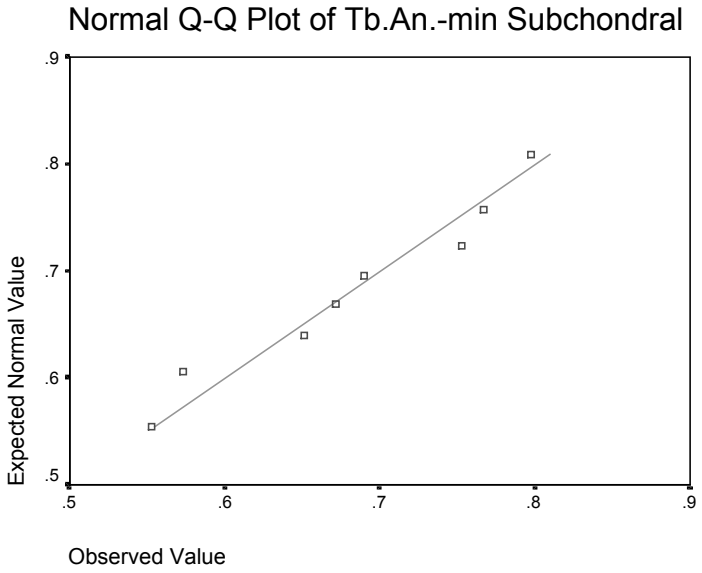

Normal Q-Q Plot of Tb.An.-max Central
Expected Normal Value
1.40
1.38
1.36
1.34
1.32
1.30
1.28
1.26
1.24
1.2
1.3
1.4
Observed Value

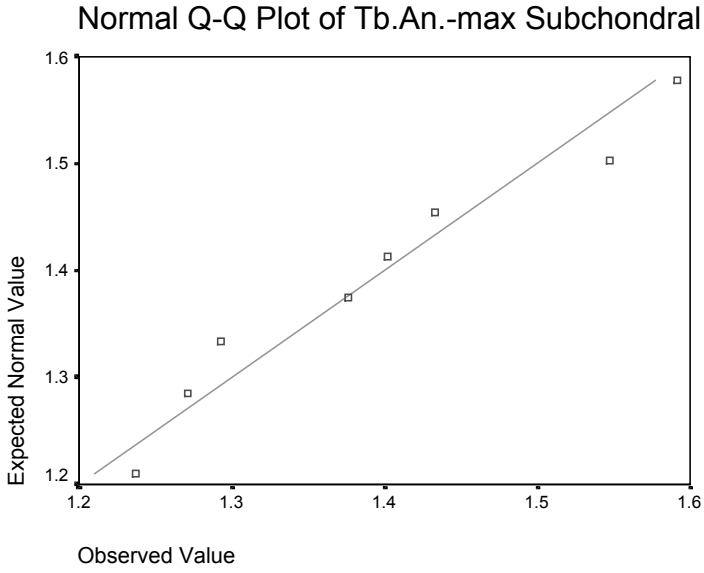
Trabecular Bone Anisotropy: Subchondral Region








Normal Q-Q Plot of Tb.An.-min Subchondral
Expected Normal Value
.9
.8
.7
.6
.5
.5
.6
.7
.8
.9
Observed Value


Normal Q-Q Plot of Tb.An.-max Subchondral
Expected Normal Value
1.6
1.5
1.4
1.3
1.2
1.2
1.3
1.4
1.5
1.6
Observed Value