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Original Article

Induced mandibular condylar growth in a sheep model after functional appliance treatment

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This paper reports on changes in mandibular condylar growth in sheep fitted with experimental functional appliances. The paper also addresses the similarities of mandibular condylar growth in sheep and humans. Experimental functional appliances were developed, constructed and applied to four, four-month old, male sheep. Another four sheep served as matched controls. All animals received fluorochrome bone labels during the study and their weight gain also was recorded. Fifteen weeks later, all animals were sacrificed and each temporo-mandibular joint and left metacarpus were removed. Undecalcified mid-sagittal sections of the joint and metacarpus were prepared for fluorescence microscopy. 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 (2.30 mm), which is also similar to previous reports in humans. These findings suggest the appropriateness of using sheep as a model for quantitative histological analysis when investigating the mechanisms of functional appliance treatment.
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Introduction

This study aims to evaluate mandibular condylar growth modified by an experimental functional appliance treatment.

Functional appliances have been reported to achieve correction of Class II discrepancy in human subjects through increasing the mandibular length[1–3] and rotating the mandible.[4,5] These changes have been suggested to result both from stimulation of mandibular condylar growth beyond that which would normally occur in growing children, and redirection of condylar growth from an upward and forward vector to a more posterior orientation.[6] While morphological changes in the mandibular condyle have been observed after functional appliance treatment,[7,8] the mechanism remains unclear. 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. Moreover, although proper control of the environmental factors is of great importance, complicated individual variations arise from patient compliance, growth and factors related to treatment planning.[6] Consequently, human studies usually have limitations of weak research design, small sample size, or incomplete collection of important information.[9]

Animal models have played an important role in investigating the mechanisms involved in functional appliance treatment. However, the limitations of using findings generated from animal studies to explain the human condition have been acknowledged. For example, the sagittal discrepancy does not usually exist in animals and the experiments actually created a Class III malocclusion rather than effecting the correction of Class II malocclusion.[10,11] In addition, there are considerable differences between the anatomy of the cranial base, upper face and mandible of man and that of other animals. Even the use of non-human primates does not overcome this limitation because man is unique in having a chin with lower incisors and their alveolar process growing more or less vertically.[10]

Animal studies are even more critical when quantitative analysis is proposed. Such studies have shown significant growth modifications with various appliances, but the equivalent treatment and retention periods have been too long to allow appropriate comparison to comparable treatment in human beings.[12,13] Furthermore, growth rates in animals are usually greater than in human beings, so that the appearance of adaptive changes in the temporo-mandibular joint (TMJ) of animals must be viewed in this regard. Consequently, quantification of these changes for extrapolation to the human situation is questionable.[11] As a result, animal experimentation produces results that must always be cautiously interpreted.

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 a functional appliance treatment. It has been reported that sheep have similar TMJ anatomical features to humans,[14] and the adaptive responses in the TMJ to mandibular displacement have been found to be comparable to previous studies conducted on rodents and non-human primates.[15–19] Hence, we chose a sheep model to study simulated functional appliance treatment effects in the correction of Class II discrepancy, with particular reference to the growth and development of the temporo-mandibular joint.

Determining the distance between fluorochrome bone label bands is a well-accepted method of measuring long bone growth and is a method that has also been applied to assess the growth of the mandibular condyle in rats.[20] The present study employed fluorochrome bone markers to record mandibular condylar growth in a sheep model over a short active treatment and retention period, the timing of treatment being comparable to that in human beings. 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.

Materials and methods

Experimental functional appliance treatment and tissue sample collection

Eight, four-month old, castrated male Merino sheep from the same flock were purchased from the Veterinary Services Division of The Institute of Medical and Veterinary Science (IMVS) in Adelaide and randomly assigned to experimental or control groups with four in each group. During the experimental period, the animals were kept at the animal house of the IMVS and each fed with 4 kg per day of lucerne chaff during the experimental period. This experiment was approved by the Animal Ethical Committees of Adelaide University and the IMVS. During the study period, the animals functioned normally, maintaining weight within the normal range for their age. The quantity of feed was believed to be sufficient for each animal.

Full arch impressions using silicon impression material [*]were taken for all the animals under general anaesthesia. One pair of refractory models was duplicated for each experimental animal. In the sheep, the upper arch is significantly wider than the lower arch and the teeth cannot occlude on both sides simultaneously. Therefore, both the upper and lower refractory models were separated along the midline, and the occlusion of either the left or right side was established according to the pattern of tooth attrition. Completed refractory models were mounted in a semi-adjustable articulator. Protrusive occlusion was achieved by setting the upper member of the articulator horizontally backward for approximately 4 mm. The wax pattern of the appliance was then constructed at the protrusive position, with minimal occlusal interference. Cast appliances were fabricated using chrome cobalt dental alloy[†]. The appliance was cemented using glass ionomer cement[‡] about one week after the impressions were taken. Both the completed appliance and the posturing effect of the appliance are shown in Figure 1. The total treatment period was 15 weeks.

At the beginning of the study, calcein label[§] (5 mg/kg) was given intra-muscularly to all the animals. Prior to sacrifice, Tetracycline label[**] (25mg/kg) and alizarin red S label[††] (30mg/kg) were given intra-muscularly to all animals at 14 and 3 days, respectively.

After sacrifice, the TMJs were removed *en bloc* by sectioning parallel and perpendicular to the occlusal plane, then processed and embedded in methylmethacrylate[‡‡]. In young sheep, *histomorphometric* analysis of the metacarpus has been recognised to

* Kerr, USA
† Dentaurum, Germany
‡ GC Fuji IX, Japan
§ Sigma Aldrich, Germany
** Intervet Pty Ltd, Australia
†† Ajax Chemicals, Australia
‡‡ BDH Laboratory Supplies, UK

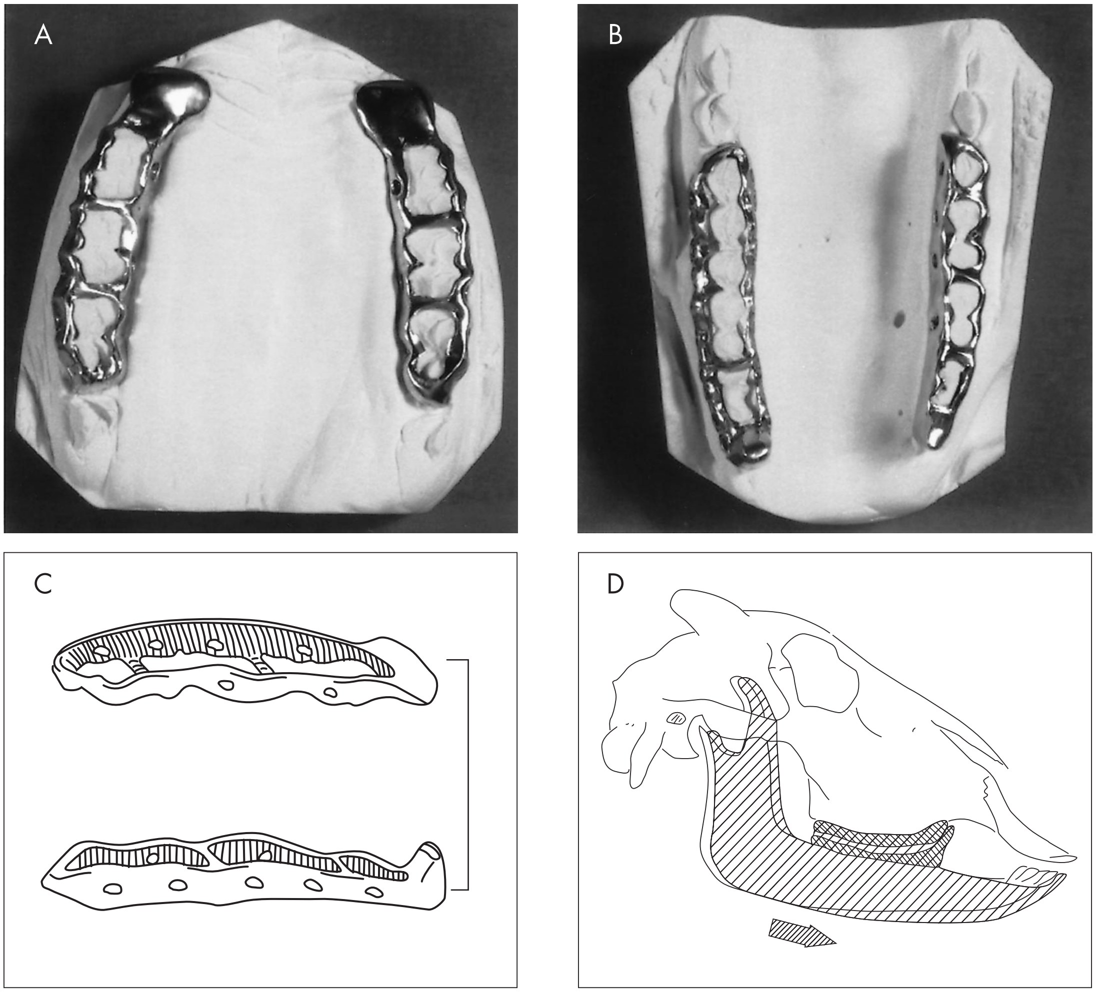


Figure 1. The functional appliance designed for mandibular displacement in sheep.
A: Occlusal view of upper components.
B: Occlusal view of lower components.
C: Schematic presentation showing the relationship of upper and lower components.
D: Schematic presentation showing displacement of the mandible.

be reliable in assessing bone growth.[21] Therefore, the left metacarpus also was collected. Unstained 10 micrometre thickness mid-sagittal sections from each TMJ and metacarpus in the standard region were cut on a microtome§§ for examination with fluorescent and light microscopy.

Bone histomorphometry

Mandibular condylar growth

The distance between the calcein and alizarin red fluorescent bands was measured along four directions (Figure 2), following the protocol designed for quantifying mandibular condylar dimension in rats.[22] A x6.3 microscope objective was selected to ensure that the fluorescent bands were clearly seen. All the measurements were performed by one author (B. M.) with a vernier calliper attached to a fluorescent microscope*** with an accuracy of 0.1 mm.

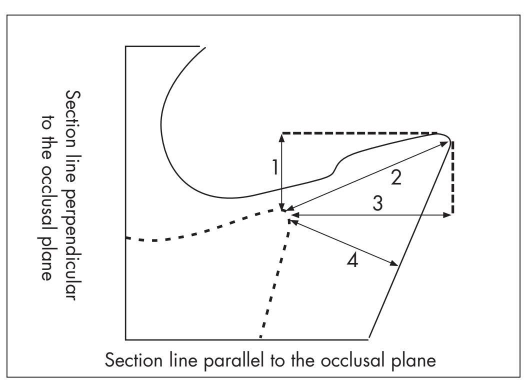


Figure 2. Measurements made from the mandibular condyles of sheep injected with fluorochrome bone labels. 1. Growth in height perpendicular to the occlusal plane. 2. Growth in a postero-superior direction measured parallel to the axis of the condylar process. This is the longest distance between the two fluorescent bands. 3. Growth in length parallel to the occlusal plane. 4. Growth in a postero-inferior direction measured as the shortest distance between the two fluorescent bands.

§§ Polycut, Germany

*** Zeiss, Germany

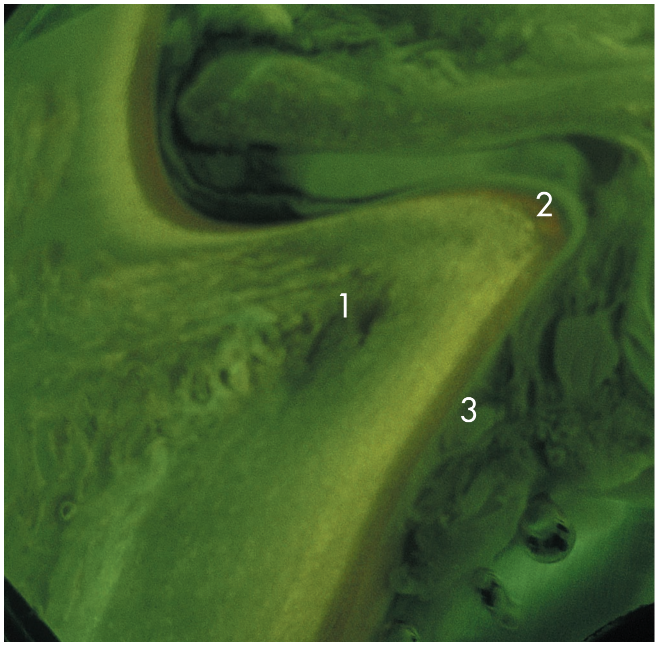
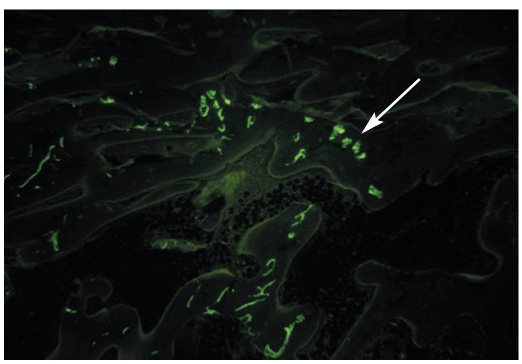


Figure 3. Fluorochrome bone labels calcein (green), tetracycline (yellow) and alizarin red (red) labels are clearly seen in embedded specimens from inner to outer parts of the mandibular condyle (upper left).
1: Calcein label (x 2.5);
2: Alizarin label in sub-chondral bone (x 6.3);
3: Alizarin label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



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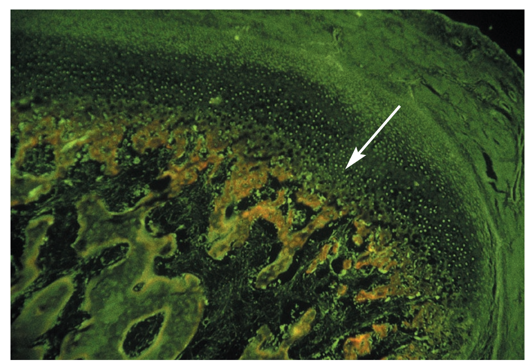

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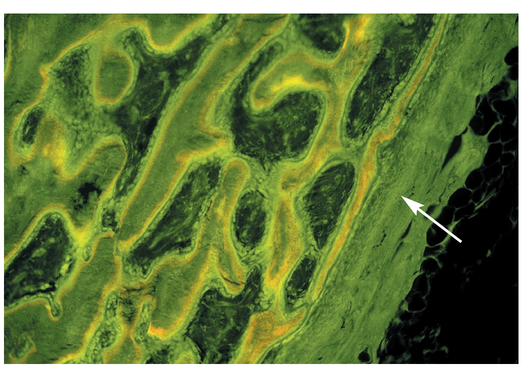

3.

Growth of metacarpus

The total growth of the metacarpus growth plate was determined by measuring the distance between the metacarpal fronts of the calcein and alizarin red fluorescent bands,[21] along the metacarpal trabeculae in the direction of the shaft bone, following the methods given by Hansson.[23]

Error evaluation

For evaluation of the measurement error, the condyle and metacarpus measurements were repeated after three months and the error calculated according to Dahlberg.[24]

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

where S is the measurement error, d is the difference between repeated measurements and n is the number of repeated measurements.

Table I. Mandibular condylar growth, metacarpus growth and weight gain in the experimental and control animals (variables are illustrated in Fig.2).

Condylar Growth		Left Side (mm) (N=4)		Right Side (mm) (N=4)	
Variable		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
Growth		Metacarpus Growth (mm) (N=4)		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

Data analysis

Means and standard deviations were calculated for mandibular condylar growth in four different directions (both left and right sides), metacarpus longitudinal growth and weight gain among the animals in both the control and experimental groups.

Growth data for the condyle were analysed using multivariant analysis of variance (MANOVA), with "control or treatment" and "left or right side" as fixed factors, and "metacarpus growth" and "weight gain" as covariants. Insignificant factors were removed from the analysis in a stepwise manner. Condylar growth in four directions was then adjusted by multivariant analysis of covariance (MANCOVA). When appropriate, analysis of covariance (ANCOVA) was performed, marginal means and mean differences were estimated. The SPSS software package††† was used for all analyses. A p-value < 0.05 indicated statistical significance.

Results

In the bone of the embedded TMJ and metacarpus samples, three fluorescent bands were clearly identified. The position of fluorescent bands in the TMJ is illustrated in Figure 3. The metacarpus growth in the control sheep during the study period varied from 4.8 to 7.5 mm. In the experimental sheep, the metacarpus growth varied from 5.0 to 7.1 mm.

The mandibular condylar growth in sheep was found to be largest in the postero-superior direction (variable 2), followed by growth in the posterior direction (variable 3) and postero-inferior 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. In the experimental sheep, the condylar growth varied from 8.5 to 13.3 mm.

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

From multivariant analysis, "left or right side" was found to have no significant effect on condylar growth (F = 2.35; P = 0.15). No combined effects were found between covariants and treatment

††† Version 9.0; Chicago, USA

Table II. Adjusted condylar growth (mm) using metacarpus growth and weight gain as covariates (variables are illustrated in Figure 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 (-0.55,1.88)	1.422	0.256	0.196
	Experimental	2.75	2.74				
2	Control	10.64	9.93	2.21 (1.37, 3.01)	32.624	0.001	0.999
	Experimental	11.43	12.14				
3	Control	10.15	9.44	2.30 (1.39, 3.21)	30.452	0.001	0.999
	Experimental	11.03	11.74				
4	Control	6.74	6.61	0.60 (-0.63, 1.98)	1.278	0.280	0.181
	Experimental	7.16	7.29				

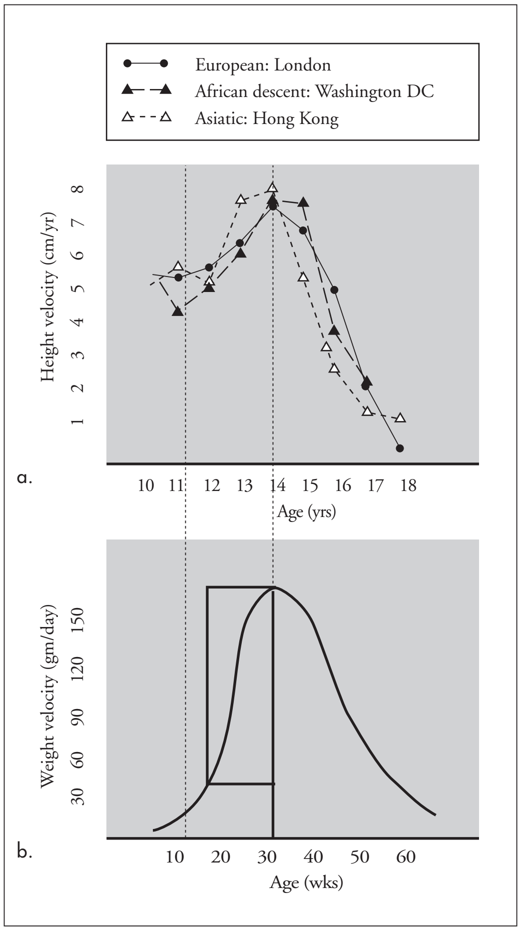

Figure 4: Comparison of the growth in sheep to that of humans; European boys (London), Asiatic boys (Hong Kong) and boys of African origin (Washington DC).
Between dotted lines (a): the adolescent growth spurt[25] and (b): the pubertal growth spurt in sheep.[26] The marked area covers a 15-week period in sheep that is comparable to 2 years of growth in humans.

(F = 3.21; P = 0.85 for treatment and weight gain, and F = 1.47; P = 0.31 for treatment and metacarpus growth, respectively). Data for both sides were pooled for further analysis. Metacarpus growth and weight gain were found to be a covariant 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 both the postero-superior and posterior directions 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 II. The induced growth was estimated to be largest in the posterior direction (variable 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 4a).[25] The ascending part of the spurt was found to cover a period of from two to three years. In sheep, weight increment is a convenient measurement of growth velocity (gm/day) and the pubertal growth spurt is evident (Figure 4b).[26] In the present study, a 15-week treatment period was chosen to cover the ascending portion of the pubertal growth spurt (Figure 4b, marked area). By comparing the pattern of the curves, growth during the treatment period in sheep is deemed to be comparable to two years of growth in adolescent humans.

It was particularly interesting to find that normal condylar growth and induced condylar growth following functional appliance intervention in sheep was comparable to growth in humans. In humans, the annual condylar growth rate during the pubertal maximum was reported by Bjork[27] as 5.5 mm/year, with a range of from 4.5 to 8 mm/year. A lower annual condylar growth rate during the similar growth period was reported by Buschang and co-workers[28] 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) 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 two years in humans, the condylar growth rate in the sheep model is 5.3 mm/year, which is comparable to that reported for humans.

With such an apparently comparable rate, 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 to the increased human condylar growth of from 1 to 3 mm suggested by Graber,[29] but slightly smaller than the reported 4 mm of condylar increment induced by the Herbst appliance.[30] This difference might be explained by the appliances used in the sheep model having a different mode of action; that is, the forces generated by chewing had an intermittent pattern, whereas Herbst appliances create more continuous forces.

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

The limitations of a sheep model are acknowledged. Sheep, like rodents and many other animal models, have a mandibular condylar growth vector that 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 (one possible mechanism of functional appliance treatment in humans)[6] cannot be fully simulated. In the present study, slight and less superior condylar growth (statistically insignificant) associated with additional posterior condylar growth suggested that redirection of condylar growth from a postero-superior vector to a more posterior orientation occurred. Whether or not the mechanism of the redirection of condylar growth in sheep is similar to that in humans remains equivocal.

Conclusion

Limited previous experimental and clinical animal and human studies have investigated the growth modification effects of functional appliance treatment upon the mandibular condyle. The present study determined that, in contrast to the rodent's, the sheep's mandibular condyle exhibits similar growth in length to human growth and within a comparable period. Moreover, the condylar growth induced by functional appliance intervention was similar. The results obtained provide new information regarding condylar growth and growth modification. As such the sheep model is a model for quantitative histological analysis in the study of biological responses. 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.

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