

# Does the degree of advancement during functional appliance therapy matter?

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**SUMMARY** The aim of this study was to assess the effect of varied degrees of mandibular advancement on condylar growth. Three hundred and thirty five 35-day-old female Sprague–Dawley rats were randomly divided into 10 experimental groups ( $n = 10$ ) and five control groups ( $n = 5$ ) for analysis of new bone formation and 10 experimental groups ( $n = 14$ ) and five control groups ( $n = 14$ ) for molecular analysis. The experimental animals were fitted with bite-jumping appliance to advance the mandible 2 and 4 mm. The rats were sacrificed on days 3, 7, 14, 21, and 30. A computer-assisted image analysing system was used to assess the quantity of new condylar bone formation. Molecular analysis utilizing real-time reverse transcription–polymerase chain reaction was used to assess the different levels of mRNA expression of different growth markers in the condyle.

One-way analysis of variance (ANOVA), with a Bonferroni multiple comparison test, showed significantly more newly formed bone in the 4 mm group compared with the 2 mm and control groups on days 21 and 30 ( $P < 0.05$ ). Most of the examined growth markers demonstrated a significant increase during the 4 mm advancement ( $P < 0.05$ ). Indian hedgehog (Ihh) mRNA showed a 7- and 5-fold change, parathyroid hormone-related peptide (PTHrP) a 5.2- and 3-fold change and type II collagen a 9.6- and 3.7-fold change in the 4 and 2 mm advancement groups, respectively.

Varied degrees of mandibular advancement result in different quantities of new bone formation and levels of expression of growth members: Ihh, PTHrP, and type II collagen.

## Introduction

The effects of functional appliance therapy on the correction of Class II malocclusions remain controversial (Franchi *et al.*, 1999; Du *et al.*, 2002; Hägg *et al.*, 2002; Johnston, 2005). Is it possible that the different outcomes of published reports are the results of different treatment protocols?

What is unclear is the following: Does the degree of mandibular advancement matter? Does the duration of treatment matter? Does the mode of advancement matter?

If the answer to any or all of these questions is ‘yes’, then we are a step closer to understanding the reasons behind this controversy. In other words, it is possible to hypothesize why some reports indicate a positive response while others a negative or no response to mandibular advancement.

Reviewing the literature, it is clear that the degree of advancement differs among studies; for example, Panerz (1982) and Ruf and Panerz (2006) advanced the mandible to an edge-to-edge position, while McNamara and Hugu (1981) and Tulloch *et al.* (1997) reported an advancement of 4–6 mm of protrusion and reactivation of the functional appliance when necessary; others advanced in small sequential steps (Falck and Fränkel, 1989). Generally, the degree of advancement differs in many investigations of functional appliance therapy (Weiland and Bantleon, 1995; Toth and McNamara, 1999; Bendeus *et al.*, 2002; Du *et al.*, 2002; Hägg *et al.*, 2002; Burkhardt *et al.*, 2003).

This highlights an important issue: the assessment of the effect of a small advancement versus a larger advancement on condylar growth. Obviously, there is a need to identify the markers for each stage of condylar growth and measure the levels of expression of these markers as a result of a small advancement and compare it with their levels of expression during a larger advancement. In doing so, the question of ‘condylar displacement versus condylar growth’ would have been addressed. Therefore, the aim of this study was to examine the effects of varied degrees of advancement on all the known stages of condylar growth: mesenchymal cell replication, differentiation to cartilage-making cells, cartilage growth, and finally bone formation leading to condylar growth. By assessing the molecular markers of each of these stages, condylar growth would have been assessed rather than condylar displacement.

The study of the effect of mandibular advancement on the growth of the mandibular condyle and glenoid fossa at histological and molecular levels has led to an understanding of the process involved in condylar growth and the factors synchronized to achieve it (Rabie and Hägg, 2002; Rabie *et al.*, 2002, 2003a,b,d,e, 2004; Shum *et al.*, 2004; Tang *et al.*, 2004; Tang and Rabie, 2005; Van Lam and Rabie, 2005). Indian hedgehog (Ihh), a transcription factor that is involved in late limb development by regulating chondrocyte proliferation and differentiation (McMahon, 2000), has been found to be the mechanotransduction

mediator in the condylar cartilage. It uses mechanical signals, resulting from forward mandibular positioning, to stimulate cellular proliferation in the condyle (Tang *et al.*, 2004). Hence, measuring the level of expression of Ihh is an indicator of cellular proliferation during mandibular advancement.

Parathyroid hormone-related peptide (PTHrP) retards further maturation of chondroblasts, and thereby allows further replication of chondrogenic cells; earlier increased expression of PTHrP in condylar cartilage on mandibular advancement was found by Rabie *et al.* (2003e). Measuring its level of expression under varied mandibular advancement could shed some light on how different advancements could affect condylar growth.

Type II collagen, a major component of the cartilage matrix of the condyles (Suda *et al.*, 1999) and the chondrocyte-specific marker (Rabie and Hägg, 2002), has been found to be increased on forward positioning of the mandible (Rabie *et al.*, 2003a). Cartilage is the template onto which bone will form; the more cartilage the more the potential of bone being accommodated on the condyle. Therefore, measuring type II collagen in response to varied mandibular advancements would help to understand how these different advancements affect the growth of the condyle.

The purpose of this study was to examine condylar tissue response to varied degrees of advancement by bite-jumping appliances, by examining the following:

1. The amount of new bone formed.
2. Quantitative analysis of three components of the extracellular matrix of the condylar tissue and factors regulating condylar growth, namely, Ihh, PTHrP, and type II collagen.

## Material and methods

### *Assessment of the amount of new bone formation in the mandibular condyle*

The animals in the current study were used according to The University of Hong Kong Committee on the Use of Live Animals in Teaching and Research guidelines ([http://www.hku.hk/facmed/04research\\_animal.htm](http://www.hku.hk/facmed/04research_animal.htm)). One hundred and twenty five rats were randomly divided into 100 experimental animals and 25 control animals.

Fifty rats were randomly selected to wear the 4 mm bite-jumping appliance and 50 the 2 mm jumping appliance, whereas for the 25 control animals no appliance was fitted. Each group of rats were sacrificed on days 3, 7, 14, 21, and 30 (Rabie *et al.*, 2001).

**Bite-jumping appliance.** Bite-jumping appliances were constructed according to the method used by Xiong *et al.* (2004). Briefly, the appliances were made of polymethylmethacrylate with identical inclined planes which were cemented to the upper central incisors of the experimental group. A lower crown of the same material

with an anterior inclined plane was also cemented to the lower incisors. The appliances were worn 24 hours per day producing a continuous forward and downward positioning of the mandible.

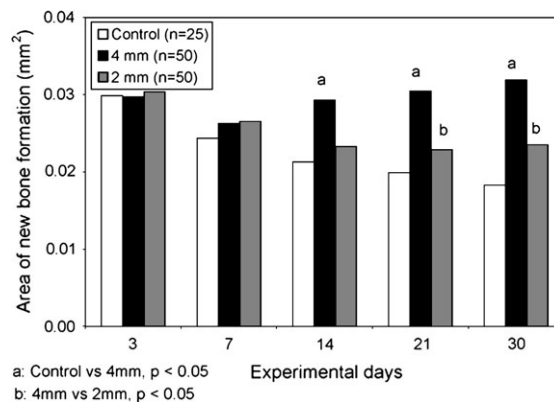
**Tissue preparation.** Tissue preparation was undertaken following the method reported by Rabie *et al.* (2001, 2003f). Briefly, immediately after the rats were sacrificed, the heads were fixed in 10 per cent paraformaldehyde and then carefully dissected along the middle sagittal plane, and the temporomandibular joints (TMJ) were harvested and decalcified with 20 per cent ethylenediaminetetraacetic acid. The specimens were then embedded in paraffin. Serial sections, 7 µm thick, were cut through the TMJ at the sagittal plane and mounted on glass slides. The sections were then stained with periodic acid and Schiff's reagent; the newly formed bone takes on a distinctive magenta colour.

**Quantitative analysis.** The amount of new bone was measured via a true colour RGB (red–green–blue) computer-assisted image analysing system (Leica Q5501W; Leica Microsystems Imaging Solutions Ltd, Cambridge, UK) with Leica Qwin Pro (version 2.2) software, following the method of Rabie *et al.* (2001). The measurements were carried out under a ×360 magnification light microscope (Leitz Orthoplan, Wetzlar, Germany).

**Statistical analysis.** The data were processed with the Statistical Package for the Social Sciences (version 13.01; SPSS Inc., Chicago, Illinois, USA). One-way ANOVA with a Bonferroni multiple comparison test was used to compare the mean differences in the amount of new bone formation at each time point. Ten randomly selected sections were used for method error analysis. The measurements were carried out on two occasions 1 month apart and were compared using the formula,

$$\pm \sqrt{\sum d^2 / 2n}$$

where  $d$  is the difference between the two registrations of a pair and  $n$  is the number of double registrations. A paired



**Figure 1** Comparison of the total amount of new bone formation in the condyle between the 4 mm group versus the 2 mm and control groups at days 3, 7, 14, 21, and 30.

**Table 1** Analysis of variance to test statistically significant differences in the amount of new bone formation between the 4 and 2 mm groups versus the control groups at each time point, at the  $P < 0.05$  level.

		Sum of squares	df	Mean square	<i>F</i>	Significance
Day 3	Between groups	0.0000	2	0.0000	0.03	0.975
	Within groups	0.0010	22	0.0000		
	Total	0.0010	24			
Day 7	Between groups	0.0000	2	0.0000	0.23	0.801
	Within groups	0.0010	22	0.0000		
	Total	0.0010	24			
Day 14	Between groups	0.0000	2	0.0000	4.97	0.017
	Within groups	0.0010	22	0.0000		
	Total	0.0010	24			
Day 21	Between groups	0.0000	2	0.0000	8.01	0.002
	Within groups	0.0010	22	0.0000		
	Total	0.0010	24			
Day 30	Between groups	0.0010	2	0.0000	22.88	<0.001
	Within groups	0.0000	22	0.0000		
	Total	0.0010	24			

**Table 2** Bonferroni test for multiple comparison of mean differences in the amount of new bone formation in the condyle between the 4 and 2 mm groups versus the control groups at each time point.

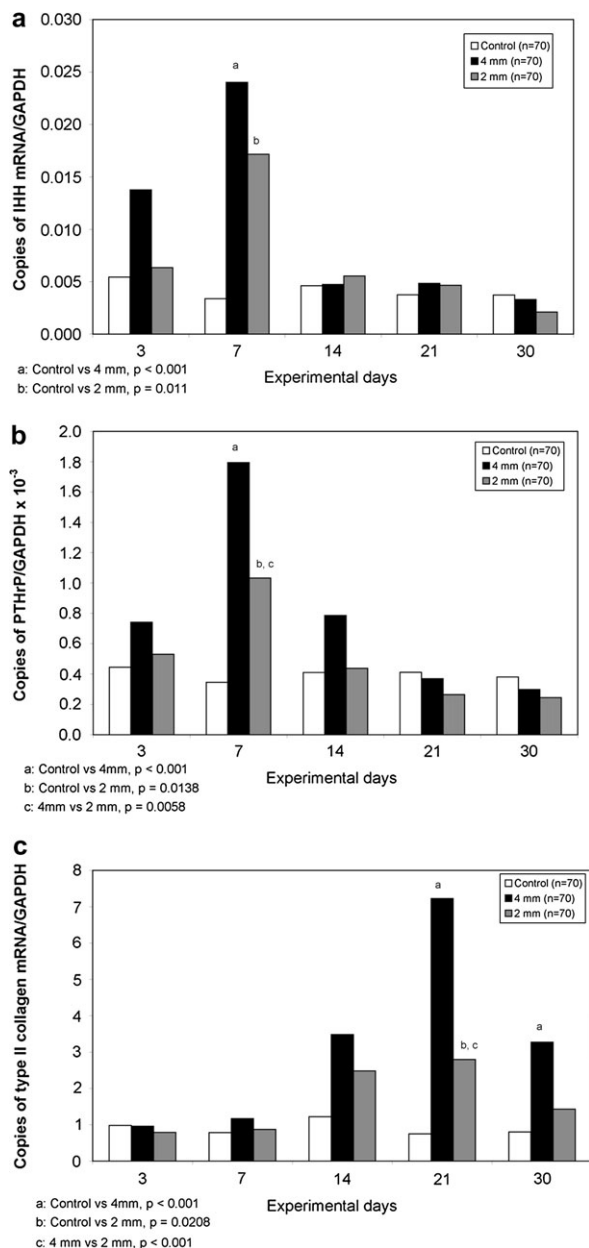
Dependent variable			Mean difference	Standard error	Significance	95% confidence interval	
						Lower bound	Upper bound
Day 3	Control	4 mm	0.0002	0.0034	1.0000	-0.0087	0.0091
	Control	2 mm	-0.0004	0.0034	1.0000	-0.0093	0.0085
	4 mm	Control	-0.0002	0.0034	1.0000	-0.0091	0.0087
	4 mm	2 mm	-0.0006	0.0028	1.0000	-0.0079	0.0066
	2 mm	Control	0.0004	0.0034	1.0000	-0.0085	0.0093
Day 7	2 mm	4 mm	0.0006	0.0028	1.0000	-0.0066	0.0079
	Control	4 mm	-0.0018	0.0032	1.0000	-0.0101	0.0065
	Control	2 mm	-0.0021	0.0032	1.0000	-0.0103	0.0062
	4 mm	Control	0.0018	0.0032	1.0000	-0.0065	0.0101
	4 mm	2 mm	-0.0003	0.0026	1.0000	-0.0070	0.0065
Day 14	2 mm	Control	0.0021	0.0032	1.0000	-0.0062	0.0103
	2 mm	4 mm	0.0003	0.0026	1.0000	-0.0065	0.0070
	Control	4 mm	-0.0094*	0.0032	0.0238	-0.0177	-0.0011
	Control	2 mm	-0.0034	0.0032	0.9056	-0.0117	0.0049
	4 mm	Control	0.0094*	0.0032	0.0238	0.0011	0.0177
Day 21	4 mm	2 mm	0.0060	0.0026	0.0979	-0.0008	0.0128
	2 mm	Control	0.0034	0.0032	0.9056	-0.0049	0.0117
	2 mm	4 mm	-0.0060	0.0026	0.0979	-0.0128	0.0008
	Control	4 mm	-0.0106*	0.0030	0.0053	-0.0183	-0.0029
	Control	2 mm	-0.0030	0.0030	0.9813	-0.0107	0.0047
Day 30	4 mm	Control	0.0106*	0.0030	0.0053	0.0029	0.0183
	4 mm	2 mm	0.0076*	0.0024	0.0147	0.0013	0.0139
	2 mm	Control	0.0030	0.0030	0.9813	-0.0047	0.0107
	2 mm	4 mm	-0.0076*	0.0024	0.0147	-0.0139	-0.0013
	Control	4 mm	-0.0136*	0.0021	0.0000	-0.0191	-0.0080
Day 30	Control	2 mm	-0.0051	0.0021	0.0772	-0.0107	0.0004
	4 mm	Control	0.0136*	0.0021	0.0000	0.0080	0.0191
	4 mm	2 mm	0.0084*	0.0018	0.0003	0.0039	0.0130
	2 mm	Control	0.0051	0.0021	0.0772	-0.0004	0.0107
	2 mm	4 mm	-0.0084*	0.0018	0.0003	-0.0130	-0.0039

\*The mean difference is significant at the 0.05 level.

*t*-test was performed to compare the two registrations. Analysis indicated no significant difference in repeated measurement [mean -0.00062, standard deviation (SD) 0.00156,  $P = 0.222$ , method error (mm<sup>2</sup>) 0.00113].

#### Molecular analysis of type II collagen, *Ihh*, and *PTHrP*

Two hundred and ten 35-day-old female Sprague-Dawley rats were randomly divided into 140 experimental animals



**Figure 2** Expression of (a) Indian hedgehog (Ihh), (b) parathyroid hormone-related peptide (PTHrP), and (c) type II collagen in the 4 mm group versus the 2 mm and control groups at days 3, 7, 14, 21, and 30.

and 70 control animals. Seventy experimental rats had an advancement of 4 mm, 70 a 2 mm advancement, while the remaining 70 rats served as the control group.

**Experimental design.** Fourteen rats from each group of animals were sacrificed on days 3, 7, 14, 21, and 30, respectively. Total RNA extraction, real-time reverse transcription–polymerase chain reaction, and quantification of mRNA were performed according to the method used in previous studies (Ng *et al.*, 2006a,b).

**Statistical analysis.** The data were input and processed using the SPSS version 13.0 for windows. One-way ANOVA with a Bonferroni multiple comparison test was used to

compare the mean differences in mRNA expression between the control and experimental groups at each time point.

## Results

### Bone formation

The 4 mm advancement group demonstrated significantly more bone compared with the 2 mm group on days 21 and 30 ( $P < 0.05$ ), the total amount of newly formed condylar bone was significantly increased on days 14, 21, and 30, when compared with the control group, with the highest amount being reached on day 30 ( $P < 0.05$ ).

Although the total amount of newly formed bone was more in the 2 mm group at all time points, when compared with the control group, the difference was not statistically significant (Figure 1 and Tables 1 and 2).

### Ihh mRNA expression

Ihh mRNA expression showed a decrease with age in the control group. There was a significant increase in Ihh mRNA expression, with the peak identified on day 7 for both the 4 and 2 mm advancement groups, showing a 7- and 5-fold change, respectively, compared with the control group ( $P < 0.05$ ). This then decreased to the control level from day 14 of appliance wear. The difference in expression level between the 4 and 2 mm groups was not statistically significant (Figure 2a and Tables 3 and 4).

### PTHrP mRNA expression

In the 4 mm group, there was a substantial increase in PTHrP mRNA expression on day 7, with the difference being statistically significant when compared with the control group, with a mean 5.2-fold change ( $P < 0.001$ ).

For the 2 mm group, there was a significant increase in PTHrP expression at day 7, with 3.0-fold increase compared with the control group, followed by a return to baseline level ( $P < 0.05$ ).

When the data from the 4 mm group were compared with the 2 mm group at day 7, the level of PTHrP was statistically more in the 4 mm group, with a 1.8-fold increase ( $P < 0.01$ ; Figure 2b and Tables 5 and 6).

### Type II collagen mRNA expression

In the 4 mm advancement group, there was an increase in type II collagen mRNA on experimental days 21 and 30; the increase was significant when compared with the 2 mm and control groups ( $P < 0.05$ ). The maximum expression level was reached on experimental day 21, showing a 9.6-fold increase compared with the control group.

In the 2 mm advancement group, there was a significant increase in type II collagen mRNA on experimental day 21, showing 3.7-fold change compared with the control group ( $P < 0.05$ ).

**Table 3** Analysis of variance to test statistically significant differences in Indian hedgehog mRNA expression between the 4 and 2 mm groups versus the control groups at each time point, at the  $P < 0.05$  level.

		Sum of squares	df	Mean square	<i>F</i>	Significance
Day 3	Between groups	0.0006	2	0.0003	2.64	0.084
	Within groups	0.0043	39	0.0001		
	Total	0.0049	41			
Day 7	Between groups	0.0031	2	0.0016	10.15	<0.001
	Within groups	0.0060	39	0.0002		
	Total	0.0092	41			
Day 14	Between groups	0.0000	2	0.0000	0.92	0.406
	Within groups	0.0001	39	0.0000		
	Total	0.0002	41			
Day 21	Between groups	0.0000	2	0.0000	1.58	0.218
	Within groups	0.0001	39	0.0000		
	Total	0.0001	41			
Day 30	Between groups	0.0000	2	0.0000	1.99	0.150
	Within groups	0.0002	39	0.0000		
	Total	0.0002	41			

**Table 4** Bonferroni test for multiple comparison of mean differences in Indian hedgehog mRNA expression between the 4 and 2 mm groups versus the control groups at each time point.

Dependent variable			Mean difference	Standard error	Significance	95% confidence interval	
						Lower bound	Upper bound
Day 3	Control	4 mm	-0.0084	0.0040	0.1261	-0.0183	0.0016
	Control	2 mm	-0.0010	0.0040	1.0000	-0.0110	0.0090
	4 mm	Control	0.0084	0.0040	0.1261	-0.0016	0.0183
	4 mm	2 mm	0.0074	0.0040	0.2135	-0.0026	0.0174
	2 mm	Control	0.0010	0.0040	1.0000	-0.0090	0.0110
	2 mm	4 mm	-0.0074	0.0040	0.2135	-0.0174	0.0026
Day 7	Control	4 mm	-0.0206*	0.0047	0.0003	-0.0324	-0.0089
	Control	2 mm	-0.0145*	0.0047	0.0111	-0.0263	-0.0028
	4 mm	Control	0.0206*	0.0047	0.0003	0.0089	0.0324
	4 mm	2 mm	0.0061	0.0047	0.6066	-0.0057	0.0179
	2 mm	Control	0.0145*	0.0047	0.0111	0.0028	0.0263
	2 mm	4 mm	-0.0061	0.0047	0.6066	-0.0179	0.0057
Day 14	Control	4 mm	0.0000	0.0007	1.0000	-0.0019	0.0018
	Control	2 mm	-0.0009	0.0007	0.7058	-0.0027	0.0009
	4 mm	Control	0.0000	0.0007	1.0000	-0.0018	0.0019
	4 mm	2 mm	-0.0008	0.0007	0.7754	-0.0027	0.0010
	2 mm	Control	0.0009	0.0007	0.7058	-0.0009	0.0027
	2 mm	4 mm	0.0008	0.0007	0.7754	-0.0010	0.0027
Day 21	Control	4 mm	-0.0010	0.0006	0.3459	-0.0026	0.0006
	Control	2 mm	-0.0009	0.0006	0.4560	-0.0025	0.0006
	4 mm	Control	0.0010	0.0006	0.3459	-0.0006	0.0026
	4 mm	2 mm	0.0001	0.0006	1.0000	-0.0015	0.0016
	2 mm	Control	0.0009	0.0006	0.4560	-0.0006	0.0025
	2 mm	4 mm	-0.0001	0.0006	1.0000	-0.0016	0.0015
Day 30	Control	4 mm	0.0004	0.0008	1.0000	-0.0017	0.0024
	Control	2 mm	0.0016	0.0008	0.1932	-0.0005	0.0036
	4 mm	Control	-0.0004	0.0008	1.0000	-0.0024	0.0017
	4 mm	2 mm	0.0012	0.0008	0.4443	-0.0008	0.0033
	2 mm	Control	-0.0016	0.0008	0.1932	-0.0036	0.0005
	2 mm	4 mm	-0.0012	0.0008	0.4443	-0.0033	0.0008

\*The mean difference is significant at the 0.05 level.

When the data from the 4 mm group were compared with the 2 mm group on experimental day 21, the level of expression of mRNA of type II collagen was significantly increased in the 4 mm group, a 2.6-fold increase ( $P < 0.001$ ; and Tables 7 and 8).

## Discussion

The findings demonstrate that varied degrees of advancements produce different amounts of newly formed bone in the condyles.



**Table 5** Analysis of variance to test statistically significant differences in parathyroid hormone-related peptide mRNA expression between the 4 and 2 mm groups versus the control groups at each time point, at the  $P < 0.05$  level.

		Sum of squares	df	Mean square	<i>F</i>	Significance
Day 3	Between groups	0.66	2	0.33	2.69	0.080
	Within groups	4.77	39	0.12		
	Total	5.43	41			
Day 7	Between groups	14.70	2	7.35	20.08	<0.001
	Within groups	14.28	39	0.37		
	Total	28.98	41			
Day 14	Between groups	1.24	2	0.62	3.17	0.053
	Within groups	7.60	39	0.20		
	Total	8.84	41			
Day 21	Between groups	0.16	2	0.08	2.31	0.113
	Within groups	1.35	39	0.04		
	Total	1.51	41			
Day 30	Between groups	0.13	2	0.07	1.67	0.201
	Within groups	1.52	39	0.04		
	Total	1.65	41			

**Table 6** Bonferroni test for multiple comparison of mean differences in parathyroid hormone-related peptide mRNA expression between the 4 and 2 mm groups versus the control groups at each time point.

Dependent variable			Mean difference	Standard error	Significance	95% confidence interval	
						Lower bound	Upper bound
Day 3	Control	4 mm	-0.2976	0.1322	0.0902	-0.6282	0.0331
	Control	2 mm	-0.0849	0.1322	1.0000	-0.4155	0.2458
	4 mm	Control	0.2976	0.1322	0.0902	-0.0331	0.6282
	4 mm	2 mm	0.2127	0.1322	0.3467	-0.1179	0.5433
	2 mm	Control	0.0849	0.1322	1.0000	-0.2458	0.4155
	2 mm	4 mm	-0.2127	0.1322	0.3467	-0.5433	0.1179
Day 7	Control	4 mm	-1.4486*	0.2287	0.0000	-2.0206	-0.8765
	Control	2 mm	-0.6878*	0.2287	0.0138	-1.2598	-0.1157
	4 mm	Control	1.4486*	0.2287	0.0000	0.8765	2.0206
	4 mm	2 mm	0.7608*	0.2287	0.0058	0.1887	1.3328
	2 mm	Control	0.6878*	0.2287	0.0138	0.1157	1.2598
	2 mm	4 mm	-0.7608*	0.2287	0.0058	-1.3328	-0.1887
Day 14	Control	4 mm	-0.3771	0.1669	0.0886	-0.7946	0.0404
	Control	2 mm	-0.0281	0.1669	1.0000	-0.4456	0.3894
	4 mm	Control	0.3771	0.1669	0.0886	-0.0404	0.7946
	4 mm	2 mm	0.3490	0.1669	0.1292	-0.0685	0.7665
	2 mm	Control	0.0281	0.1669	1.0000	-0.3894	0.4456
	2 mm	4 mm	-0.3490	0.1669	0.1292	-0.7665	0.0685
Day 21	Control	4 mm	0.0414	0.0702	1.0000	-0.1343	0.2170
	Control	2 mm	0.1464	0.0702	0.1311	-0.0293	0.3220
	4 mm	Control	-0.0414	0.0702	1.0000	-0.2170	0.1343
	4 mm	2 mm	0.1050	0.0702	0.4284	-0.0706	0.2806
	2 mm	Control	-0.1464	0.0702	0.1311	-0.3220	0.0293
	2 mm	4 mm	-0.1050	0.0702	0.4284	-0.2806	0.0706
Day 30	Control	4 mm	0.0820	0.0747	0.8366	-0.1048	0.2688
	Control	2 mm	0.1356	0.0747	0.2314	-0.0512	0.3224
	4 mm	Control	-0.0820	0.0747	0.8366	-0.2688	0.1048
	4 mm	2 mm	0.0536	0.0747	1.0000	-0.1332	0.2404
	2 mm	Control	-0.1356	0.0747	0.2314	-0.3224	0.0512
	2 mm	4 mm	-0.0536	0.0747	1.0000	-0.2404	0.1332

\*The mean difference is significant at the 0.05 level.

The 4 mm group showed a significant increase in the total amount of new bone formation in the condyle when compared with the 2 mm and control groups (Figure 1). This clearly shows that the total amount of new bone

formation differs between groups treated with various degrees of advancement.

A direct correlation between mechanical stress and bone adaptation was demonstrated by Wolff (1892), who

**Table 7** Analysis of variance to test statistically significant differences in type II collagen mRNA expression between the 4 and 2 mm groups versus the control groups at each time point, at the  $P < 0.05$  level.

		Sum of squares	df	Mean square	<i>F</i>	Significance
Day 3	Between groups	0.31	2	0.16	2.01	0.148
	Within groups	3.05	39	0.08		
	Total	3.36	41			
Day 7	Between groups	1.11	2	0.55	2.78	0.074
	Within groups	7.77	39	0.20		
	Total	8.88	41			
Day 14	Between groups	35.98	2	17.99	2.50	0.095
	Within groups	280.17	39	7.18		
	Total	316.15	41			
Day 21	Between groups	306.95	2	153.47	42.55	<0.001
	Within groups	140.69	39	3.61		
	Total	447.63	41			
Day 30	Between groups	46.18	2	23.09	13.9	<0.001
	Within groups	64.77	39	1.66		
	Total	110.94	41			

**Table 8** Bonferroni test for multiple comparison of mean differences in type II collagen mRNA expression between the 4 and 2 mm groups versus the control groups at each time point.

Dependent variable			Mean difference	Standard error	Significance	95% confidence interval	
						Lower bound	Upper bound
Day 3	Control	4 mm	0.0237	0.1057	1.0000	-0.2406	0.2880
	Control	2 mm	0.1940	0.1057	0.2219	-0.0703	0.4583
	4 mm	Control	-0.0237	0.1057	1.0000	-0.2880	0.2406
	4 mm	2 mm	0.1703	0.1057	0.3453	-0.0940	0.4346
	2 mm	Control	-0.1940	0.1057	0.2219	-0.4583	0.0703
	2 mm	4 mm	-0.1703	0.1057	0.3453	-0.4346	0.0940
Day 7	Control	4 mm	-0.3791	0.1687	0.0911	-0.8011	0.0429
	Control	2 mm	-0.0849	0.1687	1.0000	-0.5069	0.3371
	4 mm	Control	0.3791	0.1687	0.0911	-0.0429	0.8011
	4 mm	2 mm	0.2942	0.1687	0.2670	-0.1278	0.7162
	2 mm	Control	0.0849	0.1687	1.0000	-0.3371	0.5069
	2 mm	4 mm	-0.2942	0.1687	0.2670	-0.7162	0.1278
Day 14	Control	4 mm	-2.2619	1.0130	0.0941	-4.7962	0.2724
	Control	2 mm	-1.2633	1.0130	0.6595	-3.7976	1.2710
	4 mm	Control	2.2619	1.0130	0.0941	-0.2724	4.7962
	4 mm	2 mm	0.9986	1.0130	0.9911	-1.5357	3.5329
	2 mm	Control	1.2633	1.0130	0.6595	-1.2710	3.7976
	2 mm	4 mm	-0.9986	1.0130	0.9911	-3.5329	1.5357
Day 21	Control	4 mm	-6.4774*	0.7179	0.0000	-8.2732	-4.6815
	Control	2 mm	-2.0471*	0.7179	0.0208	-3.8429	-0.2512
	4 mm	Control	6.4774*	0.7179	0.0000	4.6815	8.2732
	4 mm	2 mm	4.4303*	0.7179	0.0000	2.6344	6.2261
	2 mm	Control	2.0471*	0.7179	0.0208	0.2512	3.8429
	2 mm	4 mm	-4.4303*	0.7179	0.0000	-6.2261	-2.6344
Day 30	Control	4 mm	-2.4705*	0.4871	0.0000	-3.6890	-1.2520
	Control	2 mm	-0.6271	0.4871	0.6166	-1.8455	0.5914
	4 mm	Control	2.4705*	0.4871	0.0000	1.2520	3.6890
	4 mm	2 mm	1.8434*	0.4871	0.0016	0.6250	3.0619
	2 mm	Control	0.6271	0.4871	0.6166	-0.5914	1.8455
	2 mm	4 mm	-1.8434*	0.4871	0.0016	-3.0619	-0.6250

\*The mean difference is significant at the 0.05 level.

hypothesized that bone increased its density and strength in the area that was exposed to stress, while those areas that were not stimulated became weaker and lost bone density.

Bone formation has been stimulated by bending in regions with the highest bending strains in the rat tibia (Raab-Cullen *et al.*, 1994). Similar forces applied only in the form of pressure loading do not stimulate tibial

formation either at the contact site or between loading pads (Raab-Cullen *et al.*, 1994). These results suggest that externally applied forces as a result of increased bending strains influence bone formation, mass, and strength. Mandibular advancements could produce such bending strains on the mandibular condyle and ramus. Rabie *et al.* (2001) reported that mandibular advancement creates deformation of the mesenchymal cells in the proliferative layers of the condylar cartilage; this cellular deformation creates a strain alignment that leads the cells to be orientated in the direction of the pull. Obviously, varied degrees of advancement could produce levels of tensile strains or mechanical strains leading to different amounts of bone formation.

Interestingly, the 2 mm group produced more bone when compared with the controls, but the difference was insignificant; this points to a possibility of having to surpass a threshold to solicit a response.

Turner *et al.* (1994) reported that bone formation occurred where bending strains were above a loading threshold of 40 N or approximately 1050  $\mu$ m strain. Such levels increased both the bone-forming surface and the mineral apposition rate and subsequently increased the bone formation rate as much as 6-fold. No evidence of increased bone formation was seen for applied strains below 1050. This could explain the fact that 4 mm advancement produced significantly more bone when compared with the controls, but 2 mm advancement resulted in an insignificant difference in the amount of bone produced compared with the controls.

The results of the current study point to a similar response of condylar tissues to mechanical loading resulting from mandibular advancement. The larger advancement, which could have subjected condylar tissue to more mechanical strain, resulted in more bone than the smaller advancement. However, this is still an observation and does not explain the mechanism behind such a response.

Tang *et al.* (2004) reported the presence of Ihh, a mechanotransduction mediator that reads and understands mechanical forces created as a result of mandibular advancement and converts them into condylar growth. In the present study, both the 4 and 2 mm groups expressed more Ihh than the untreated group (Figure 2a). Ihh was found to adopt the PTHrP pathway in the condyle (Ng *et al.*, 2006a). The Ihh in the larger advancement group (4 mm) led to more expression of PTHrP than in the 2 mm advancement group and the untreated controls (Figure 2b). How does this influence the ultimate growth of the condyle? PTHrP delays cartilage cell maturation (Rabie *et al.*, 2003e) and thereby allows more mesenchymal cell proliferation. The larger the pool of mesenchymal cells, the more the potential for cells to differentiate into chondroblasts (Rabie *et al.*, 2003d). The more the chondrogenic cells, the more the possibility of forming cartilage in the condyle. That is why, in this study, the

amount of type II collagen, the major component of condylar cartilage, was measured.

The 4 mm group produced more type II collagen than the 2 mm and control groups (Figure 2c). Cartilage is the template onto which bone forms. The larger the cartilage template, the more bone it can accommodate. Therefore, it is clear from the data presented above that the more significant the advancement the greater the formation of mesenchymal cells, cartilage, and bone. This is an indication of more growth as seen by the significant increase in the expression of the factors that regulate the stages of condylar growth.

It is also important to relate the results of the present investigation to earlier research published in the literature. The markers discussed above, Ihh, type II collagen, and PTHrP, as well as the amount of bone formed in response to stepwise advancement were found to be significantly more than their levels of expression as a result of one single advancement (Rabie *et al.*, 2003c; Ng *et al.*, 2006a,b). However, in the current study, the same quantitative approach was used to examine the tissue response to varied degrees of advancement: 2 and 4 mm, rather than the mode of advancement, that is, stepwise versus single advancement.

## Conclusion

Varied degrees of advancements produce different amounts of bone in the mandibular condyle. The mechanism behind such an effect on condylar growth was delineated. The variation in the degree of advancements produce unequal levels of mechanical strain which trigger different levels of cellular responses in the form of mechanotransduction mediators (Ihh), regulator of cell maturity (PTHrP), amount of cartilage (type II collagen), and ultimately bone. Thus, pointing out the presence of a minimum threshold of strain that should be surpassed to solicit a response could lead to more growth of the condyle in the form of new bone formation. This threshold should be identified clinically as it could influence the tissue response to mandibular advancement.

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