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RESEARCH ARTICLE

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The effect of occlusal loading on secondary tooth eruption: An experimental study using a rat model

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Abstract

This study aimed to assess the impact of occlusal loading on secondary tooth eruption and to determine the extent to which altering the occlusal loading influences the magnitude of secondary eruption through an experimental rat model. The present sample consisted of 48 male Wistar rats. At the onset of the experiment, 24 rats were 4 weeks old (young rats) and 24 rats were 26 weeks old (adult). Within each age group, the rats were further divided into two equal subgroups (12 rats each), receiving either a soft- or hard-food diet for the 3-month duration of the experiment. The primary outcome was the tooth position changes relative to stable references in the coronal plane by evaluating the distance between the mandibular first molars and the inferior alveolar canal. Microcomputed tomography scans were taken from all rats at three standardized intervals over the 3-month study period. Descriptive statistics were calculated by age and diet over time, and the evolution of the outcomes were plotted by age and diet over time. Longitudinal data analysis via generalized estimating equations was performed to examine the effect of age, diet and time on the primary outcomes. Secondary tooth eruption was observed in all age groups (young and adult) regardless of diet consistency (soft or hard food). In young rats, the secondary eruption was greater in the animals fed a soft diet than those fed a hard diet. In adult rats, minimal difference in secondary tooth eruption were found between different diet consistencies. Occlusal loading influences secondary tooth eruption in teeth with an established occlusal contact. The quantity of eruption in growing rats is higher when occlusal loading is less, providing a certain amount of secondary tooth eruption occurs. This difference, however, is not evident in adult rats, at least during the given 3-month time frame.

KEYWORDS

continuous eruption, functional capacity, masticatory muscles, molars

1 | INTRODUCTION

Facial growth is an ongoing process throughout adulthood (Behrents, 1985; Lewis & Roche, 1988; Oesterle & Cronin, 2000). Similarly, the teeth continue to erupt even after reaching occlusion. As the jaws grow, the vertical distance between them increases, prompting the teeth to continue erupting, to maintain adequate occlusion. This study focuses on the detailed analysis of this process, which is defined as postocclusal or secondary tooth eruption. Secondary tooth eruption is crucial in sustaining balanced contacts between teeth. The extent of tooth eruption in both the posterior and anterior regions of the mandible and maxilla seems to correlate with the mandibular growth pattern (Poelmans et al., 2016).

For clinicians, secondary tooth eruption can present challenges, particularly in cases involving implant placement. As implants are osseointegrated, they lack the ability to move and consequently cannot adapt themselves to the secondary eruption of adjacent teeth. In instances of significant secondary tooth eruption, the implant and adjacent teeth may exhibit a vertical discrepancy, potentially compromising aesthetic outcomes. Infraposition, defined as a tooth being situated more gingivally than the occlusal plane, may be observed in such cases. Thilander et al. (1994) recommend placing implants after the completion of craniofacial growth and skeletal development. Detailed knowledge about the amount of secondary tooth eruption, and its timing, aids clinicians in strategically planning the placement of single-tooth implants in an age-appropriate manner (Fudalej et al., 2007). Nevertheless, Bernard et al. (2004) observed continuous eruption not only in young adults, but also in mature adults, emphasizing substantial interindividual variation in all age groups.

More studies have since been carried out attempting to investigate this question and their findings have been collated in a systematic review authored by Papageorgiou et al. (2018). This review revealed that, within the initial 5–15 years postimplantation, ~50% of implants experience infraposition. On average, this infraposition measures 0.58 mm. Moreover, a noteworthy 20% of the implants are at risk of substantial infraposition at some point. Notably, the study identified a considerable degree of heterogeneity in the occurrence of implant infraposition across the various studies analysed. In addition, a recent study investigated the long-term vertical discrepancy between single anterior maxillary implant-supported crowns and adjacent teeth, suggesting that the amount of vertical discrepancy may be unrelated to patient gender and age with great interindividual variation (Sauvin et al., 2022). Kiliaridis et al. (2019) found that the functional capacity of masticatory muscles, assessed by masseter muscle thickness, influences maxillary incisor secondary eruption, whereby individuals with stronger masticatory muscles experience less secondary eruption. Understanding potential factors influencing the magnitude of secondary tooth eruption would be extremely beneficial especially regarding patient selection and timely interventions.

The hypothesis of the present study proposes that modifying the occlusal loading of subjects after prolonged alteration of the consistency (hardness) of their food will influence the rate of secondary tooth eruption. The primary objective of this study was to quantify the magnitude of change in secondary eruption from alterations in occlusal loading resulting from dietary manipulations. The secondary objective was to assess whether adult individuals also undergo secondary tooth eruption, that might be modifiable with changes in diet consistency.

2 | MATERIALS AND METHODS

2.1 | Ethical approval

The current study received approval from the Ethics Committee for Animal Research under the reference number GE/15/20A. The reporting of this study adheres to the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) (Percie du Sert, 2020).

2.2 | Subjects

For this experimental prospective study, 48 male Wistar rats were included, 24 of which were 4 weeks old (classified as young growing rats) and 24 were 26 weeks old (classified as adult) at the onset of the experiment. In each age group, the rats were divided into two equal subgroups (12 rats each), differing only in diet consistency (receiving either soft or hard food), and were followed for a period of 3 months. The hard food consisted of normal rat pellets, while the soft food comprised ground rat pellets mixed with water, in a ratio of 100 g powdered pellets to 100 mL water. Before the experimental period, all young rats were fed through lactation and weaned by the 4-week mark, while all adult rats were fed with a standard hard food.

2.3 | Methods

All rats underwent scanning using in vivo microcomputed tomography (micro-CT) at three standardized intervals, employing the Quantum GX micro-CT imaging system from Perkin Elmer®. The specific parameters were as follows: field of view of 60 mm, 90 kV, 88 μA, scan mode set to 'high resolution' and a scanning time of 4 min.

The baseline scans were taken on the first day (Day 1, T0), intermediate scans at 2 months (Day 57, T1) and final scans at the end of the 3-month experimental period (Day 85, T2). Anaesthetic gas (isoflurane) was administered to induce sedation in the rats, with an initial concentration of 5% and 1 L/min during induction, and 3% and 1 L/min during the radiographic imaging phase.

Analysis of the micro-CT images was conducted using Osirix® image analysis software. Initially, the scans were reoriented in the three different CT planes. The objective was to evaluate the secondary eruption by a longitudinal series of measurements of the distance between the first mandibular molars and a stable structure as is the inferior alveolar canal (IAC) in the coronal plane (Figure 1). The measurements included the distance between the IAC and the vestibular and lingual cusps of the first mandibular molar (IAC-Cusp), and the distance between IAC and the groove of the same molar (IAC-Groove) (Figure 2). These measurements were taken on both the right and left sides of the mandible, resulting in a total of six measurements per CT scan per rat. In our analysis, the mean between the right and left sides was determined. The mean between the lingual and vestibular cusps was chosen to mitigate the risk of errors, considering that there are instances where a particular cusp may be more clearly visible. Additionally, the adoption of the mean helps minimize potential discrepancies arising from wear irregularities between cusps, thus enhancing the accuracy of our analysis. To assess whether the variation in secondary tooth eruption between rats fed a soft or hard diet is related to tooth wear, we selected the groove of the teeth as a reference point because this is unaffected by wear.

2.4 | Statistical analysis

Descriptive statistics were calculated by age and diet over time and the evolution of the outcomes were plotted by age and diet over time. Longitudinal data analysis was implemented via a series of population average generalized estimating equation (GEE) models using nonparametric bootstrap (500 iterations), robust SEs and an exchangeable correlation matrix, to examine the effect of age, diet and time, and their interactions on each of the two averaged outcomes across vestibular/lingual and right/left measurements. Predictive marginal plots were also drawn. All analyses were conducted using Stata 18.0 (Stata Corp.).

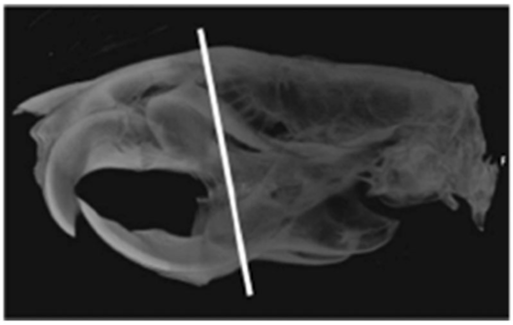

FIGURE 1 Microcomputed tomography coronal section of a male Wistar rat.

3 | RESULTS

Median and interquartile range since data not normally distributed are shown in Table 1. Spaghetti plots (Figure 3) of the observed measurements display the evolution of the outcomes per diet, age over time. For both outcomes, there is variability among animals which is more pronounced among young animals (Figures 3 and 4, red lines). The evolutionary patterns are similar across different diet groups and between young and adult individuals.

Secondary tooth eruption (as measured by the IAC-cusp and IAC-groove distances) was consistently observed in both the young and adult age groups, regardless of dietary consistency. For both outcomes, for young animals there is an upward trend. For adult animals the increase over time is smaller compared to young animals. The GEE multivariable regression model results are shown in Table 2. For both outcomes, the main effects for age, time and diet are highly significant, as well as the age*time interaction. The time*diet interaction was not significant and is not included in the model.

For both outcomes, higher distances are predicted for adult animals compared to young animals. Soft diet results in higher distances for both the IAC-cusp and IAC-groove distances compared

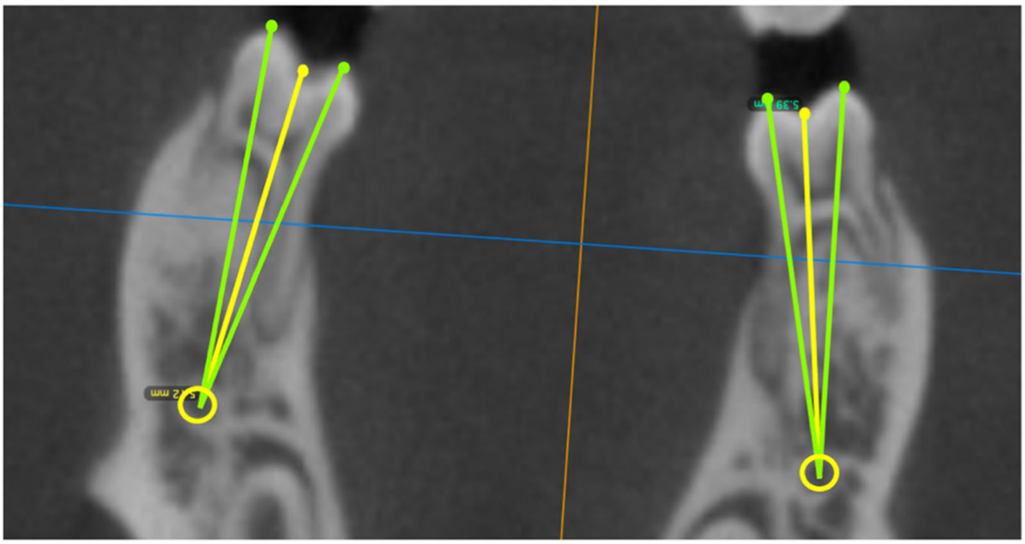

FIGURE 2 Measurements from the inferior alveolar canal (IAC) to the mandibular molar lingual and vestibular cusps (IAC-cusp; green lines) and the mandibular molar groove (IAC-Groove; yellow lines).

TABLE 1 Descriptive statistics (median and interquartile range) for distance from inferior mandibular canal to cusp (top) and groove (bottom) by age, diet and time.

Distance of inferior mandibular canal to cusp (median/interquartile range)						
	Soft diet			Hard diet		
	Day 0	Day 57	Day 85	Day 0	Day 57	Day 85
Young	3.95 (0.16)	5.41 (0.15)	5.53 (90.15)	3.93 (0.24)	5.28 (0.22)	5.40 (0.22)
Adult	5.89 (0.26)	6.06 (0.32)	6.03 (0.29)	5.85 (0.28)	6.15 (0.34)	6.15 (0.32)
Distance of inferior mandibular canal to groove (median/interquartile range)						
	Soft diet			Hard diet		
	Day 0	Day 57	Day 85	Day 0	Day 57	Day 85
Young	3.66 (0.15)	5.03 (0.15)	5.33 (0.05)	3.5 (0.30)	5.00 (0.34)	5.21 (0.32)
Adult	5.77 (0.31)	5.77 (0.30)	5.81 (0.30)	5.46 (0.27)	5.75 (0.37)	5.84 (0.27)

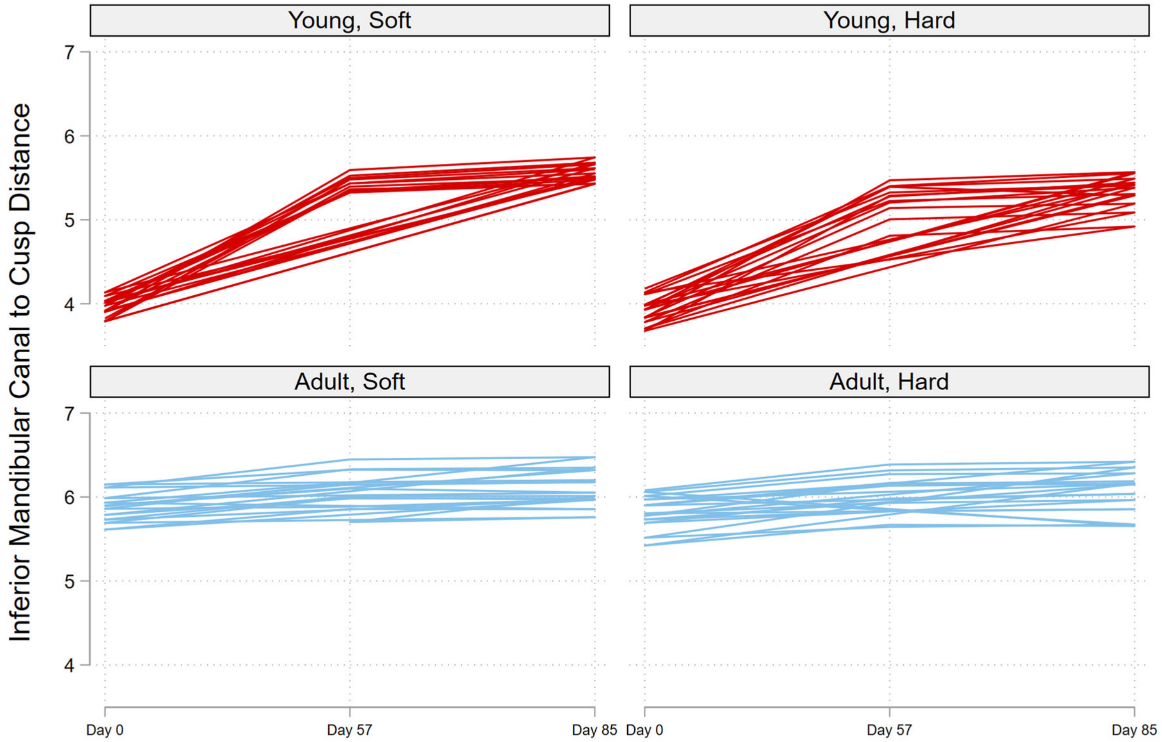

FIGURE 3 Observed evolution of inferior mandibular canal distance to cusp by age group, diet group over time.

to hard diet. The significant age × time interaction is graphically shown by the difference in the evolution patterns between young and adult animals over time. The evolution over time shows a sharp increase from Day 0 (T0) to Day 57 (T1) and then a decreases from Day 57 (T1) to Day 85 (T2) for the young animals. A similar but less pronounced pattern is observed for the adult animals (Figures 5 and 6). Figures 5 and 6 are the predicted marginal evolutions after fitting the GEE models and considering the included predictors (diet, age, time and age × time interactions). The vertical at the three timepoints are the 95% confidence intervals bars.

4 | DISCUSSION

The present study has shown that occlusal loading influences secondary tooth eruption, whereby this eruption is higher with less occlusal loading (dictated by diet consistency). This effect of the diet however (soft vs. hard food, with an indirect effect on low vs. high occlusal loading) is seen only in young growing rats possibly due to their bigger amount of secondary eruption than that witnessed in adult animals. Moreover, the eruption differences between young rats fed a soft versus a hard diet are likely due to the change in occlusal loading and not explained by an increase in enamel wear of

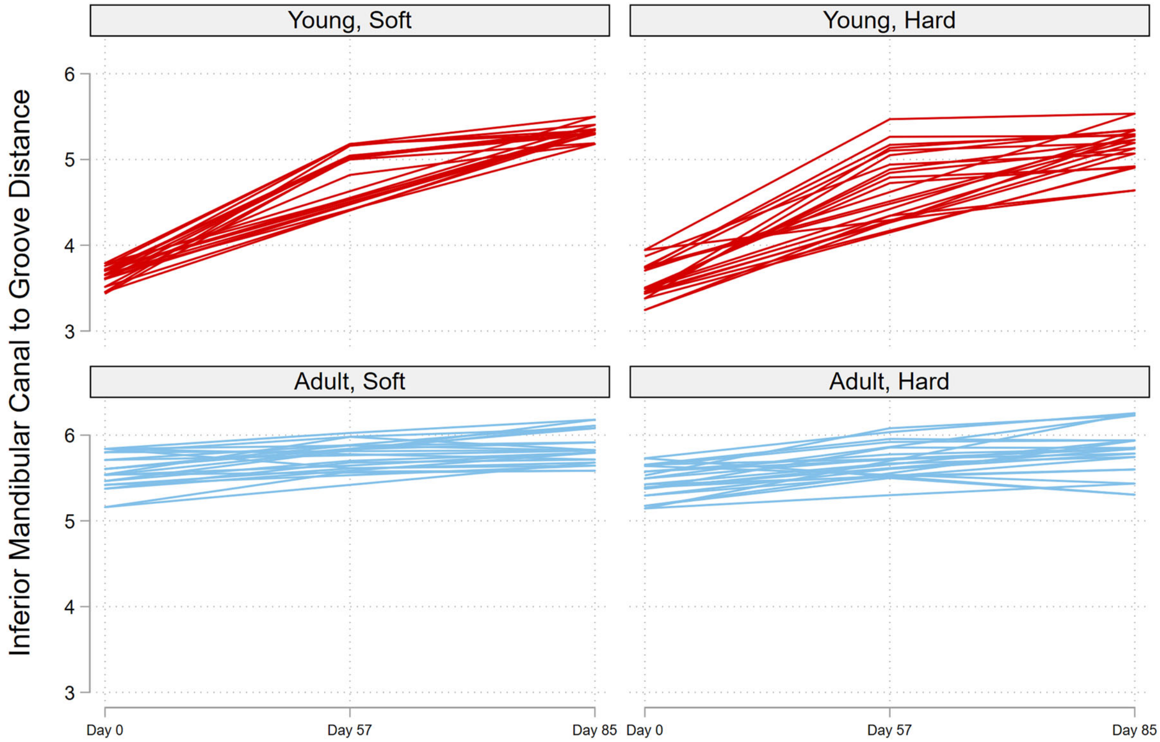

FIGURE 4 Observed evolution of inferior mandibular canal distance to groove by age group, diet group over time.

TABLE 2 GEE multivariable regression model estimates for the effect of age, time, age × time interaction and diet on the of inferior mandibular canal distance to cusp.

Covariate	IAC-Cusp Coefficient (95% CI)	*p*	IAC-Groove Coefficient (95% CI)	*p*
Age				
Young*	Reference		Reference	
Adult	1.90 (1.83, 1.96)	<.001	1.90 (1.83, 1.98)	<.001
Diet				
Soft*	Reference		Reference	
Hard	−0.08 (−0.12, −0.04)	<.001	−0.08 (−0.12, −0.04)	<.001
Time				
Day 0*	Reference		Reference	
Day 57	1.40 (1.32, 1.47)	<.001	1.41 (1.34, 1.49)	<.001
Day 85	1.51 (1.43, 1.59)	<.001	1.63 (1.55, 1.71)	<.001
Age × time interaction				
Adult#Day 57	−1.16 (−1.25, −1.08)	<.001	−1.16 (−1.26, −1.06)	<.001
Adult#Day 85	−1.26 (−1.35, −1.18)	<.001	−1.32 (−1.42, −1.22)	<.001

Abbreviations: 95% CI, 95% confidence interval; GEE, Generalized estimating equation; IAC, inferior alveolar canal; IAC-Cusp, IAC and the vestibular and lingual cusps of the first mandibular molar; IAC-Groove, distance between IAC and the groove of the same molar.

the teeth due to the hard food. In adult rats on other hand, occlusal loading does not affect the secondary eruption rate, at least not within the 3-month experimental period.

The low rate of secondary eruption in adult rats may explain the smaller differences observed. Another additional possible reason could be the well-trained masticatory muscles of the adult animals before the initiation of the experiment. It is likely that a longer duration may be required before a significant decline in the functional capacity of the elevator muscles could occur. Consequently, during the relatively short duration of our study (3 months), the weakening

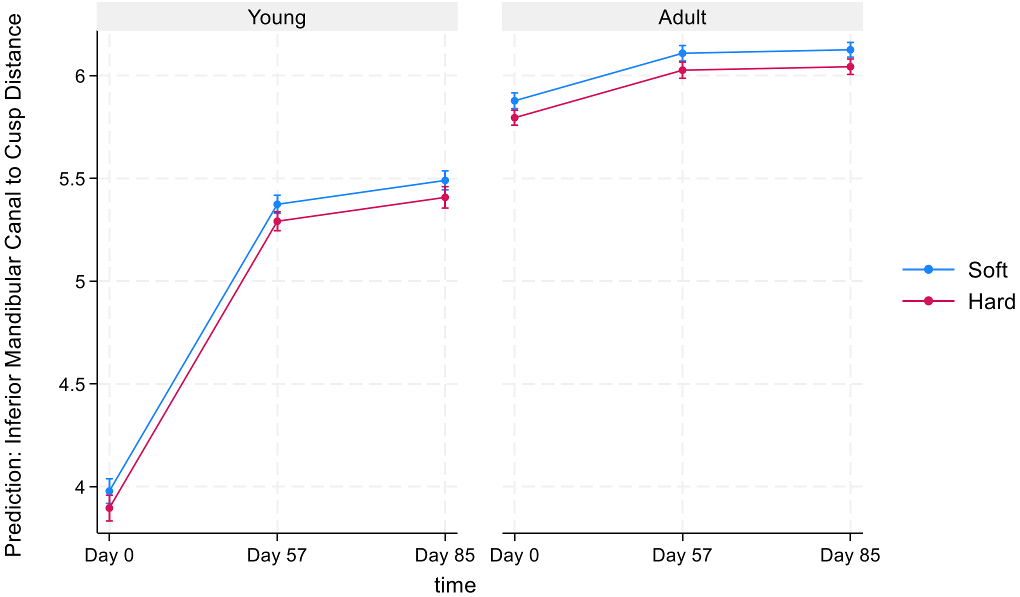

FIGURE 5 Predicted evolution of inferior mandibular canal distance to cusp by age group, diet group over time.

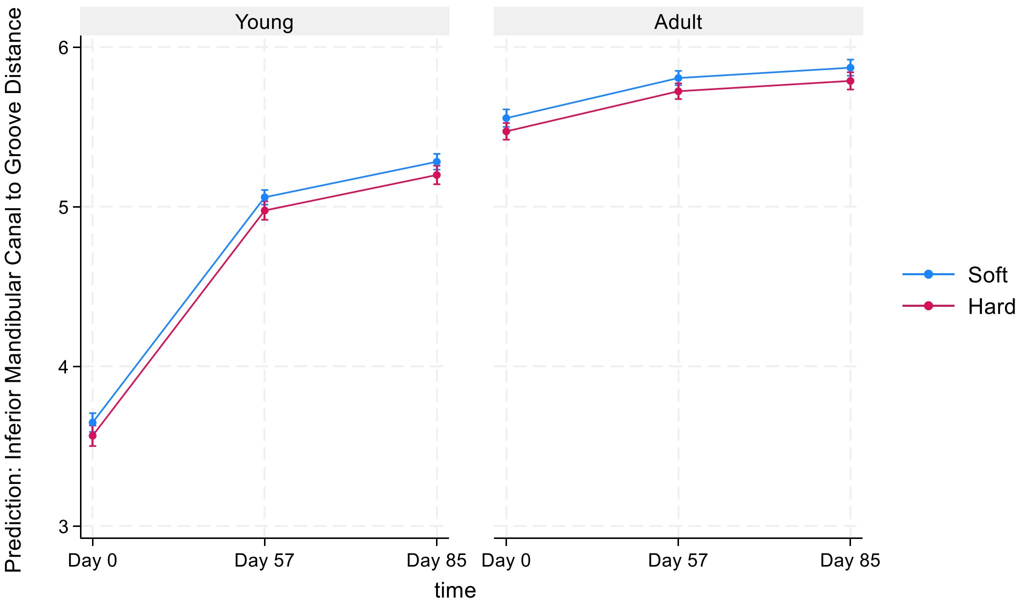

FIGURE 6 Predicted evolution of inferior mandibular canal distance to groove by age group, diet group over time.

of elevator muscles is only partial and may not have exerted a noticeable impact on the rather limited continuous eruption. Other studies have also shown that masticatory functional changes only lead to marginal changes, for example in mandibular morphology in adult rats (Ödman et al., 2008). This may mean that in adult animals, changes may either require more time to become evident, or it may be the case that changes in loading through modifications in diet consistency may not be sufficient to influence the smaller amount of secondary (continuous) eruption.

When comparing the present results to previous investigations, they align well with what has been shown regarding continuous tooth eruption in adults (Bernard et al., 2004; Poelmans et al., 2016; Sauvin et al., 2022; Thilander et al., 1994). The role of masticatory muscle function in influencing secondary tooth eruption is consistent with existing studies demonstrating that masticatory function modifies growth and eruption patterns. For instance, a study by Kiliaridis (1995) concluded that increased masticatory muscle function leads to an anterior growth rotation pattern of the mandible and well-developed angular, coronoid, and condylar processes. The present findings substantiate our hypothesis that the extent of secondary tooth eruption varies with respect to the occlusal loading exerted.

Knowledge of secondary tooth eruption is crucial for dental practitioners. It is important to understand or even predict the amount of secondary tooth eruption that will take place within a given time frame, to more appropriately plan the placement of single-tooth implants. Further studies are needed to gain better insight into which individuals are more prone to a larger amount of secondary tooth eruption. This information is equally vital for orthodontics. Secondary tooth eruption may influence the stability of orthodontic treatment in both the vertical and sagittal dimensions. Remmers et al. (2008) found that the stability of anterior openbite correction is rather poor, with 44% of patients presenting an openbite 5 years following the end of treatment. In a case of myotonic dystrophy (Antonarakis et al., 2019) a severe openbite treated by orthodontics and orthognathic surgery was found to be inherently unstable with severe long-term vertical relapse occurring, despite favourable posttreatment outcomes, and this was attributed to the significant continuous eruption of the posterior teeth related to the weakened masticatory musculature. The importance of considering dentofacial growth in long-term stability has repeatedly been advocated (Nanda & Nanda, 1992).

Identifying patients with a propensity for significant secondary tooth eruption is thus a prerequisite for adequate treatment planning. In the case of maxillary lateral incisor agenesis, for example, in high-risk patients, space closure may be a preferable option. Avoiding tooth replacement using an implant in these cases prevents the appearance of vertical discrepancy between the implant and the adjacent teeth. Moreover, in cases where relapse risk is high, a longer retention phase and postponing treatment until a later age may be advisable.

In the present study, we have evaluated the influence of occlusal loading on the continuous eruption of the mandibular molars using rats as an experimental model. Although molar eruption in rat may have many similarities to molar eruption in humans (Fujita et al., 2009, 2010), the translation and applicability of our findings to humans should be interpreted with caution. Additionally, the study exclusively involved male rats, and exploring potential sex differences could help provide further valuable insights. Lastly, only mandibular molar secondary tooth eruption was investigated in the present study and whether different amounts of secondary tooth eruption occur for the maxillary and mandibular teeth, or anterior and posterior teeth is as of yet not clearly understood.

Clinical long-term studies from several decades ago, albeit retrospective, illustrate secondary tooth eruption as is exemplified in the long-term growth studies of Behrents (1985) on adult individuals, as well as other studies on younger individuals (Iseri & Solow, 1996 and Thilander, 2009). Little is known, however, about the differential secondary tooth eruption of the mandibular versus maxillary teeth, or the anterior versus posterior teeth. A recent clinical study investigating secondary tooth eruption over a 20-year period found more secondary eruption in adolescents than adults, almost two times the amount of eruption in the maxillary molars than the mandibular molars, and more eruption in the mandibular incisors than molars (Poelmans et al., 2016). Undeniable, one commonality between all these studies is the large interindividual variation. An in-depth exploration into what factors may influence this secondary tooth eruption would be of great benefit. Interestingly, a clinical study has found that the functional capacity of masticatory muscles, as appraised through masseter muscle thickness measurements, influences secondary tooth eruption of the maxillary incisors. Individuals with stronger masticatory muscles demonstrate less secondary eruption (Kiliaridis et al., 2019). More data in this regard however are needed.

5 | CONCLUSIONS

The present study shows that occlusal loading influences secondary tooth eruption in teeth with an established occlusal contact. The quantity of eruption in growing rats is higher when occlusal loading is less. This difference however is not evident in adult rats, at least during the given 3-month time frame. Considering these results, evaluating the masticatory muscle functional capacity may prove advantageous in estimating the extent of secondary tooth eruption in our patients.

AUTHOR CONTRIBUTIONS

Conceptualization: Ourania Stergiopulos, Stavros Kiliaridis. *Data Curation*: Ourania Stergiopulos. *Formal analysis*: Nikolaos Pandis. *Investigation*: Ourania Stergiopulos, Aikaterini Lagou. *Writing—original draft preparation*: Ourania Stergiopulos. *Writing—review and editing*: Gregory S. Antonarakis, Stavros Kiliaridis. *Funding support*: Stavros Kiliaridis.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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PEER REVIEW

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