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Title page

Title: Diurnal variation in orthodontic pain: clinical implications and pharmacological management

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Abstract

Pain is a bio-physiological phenomenon characterized by a circadian rhythm. A better understanding of diurnal variability in orthodontic pain perception would not only enhance our knowledge about how orthodontic pain intensity fluctuates over the 24-h day, but it also has great potential to improve the clinical management of orthodontic pain. Since the administration timing of pharmacological interventions has a direct influence on their effectiveness, a sound knowledge of the timing of peak pain intensity would allow clinicians to better coordinate the administration timing of analgesics. The objective of this study was to explore and quantify the diurnal variation in orthodontic pain over a period of seven days following initial arch wires placement. A multilevel linear spline model was used for secondary data analysis. Data were obtained from an earlier published high quality randomized controlled trial involving 85 participants (42 males and 43 females; mean age 14.1 years and SD 2.0). Results showed significant diurnal variability in pain intensity during the first two days of force application for both sexes. However, females showed significantly greater diurnal variation in the pain than males. Clinical and research implications of observed diurnal variability in orthodontic pain perception are discussed.

Introduction

The International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage".[1] This well accepted definition of pain also describes the characteristics of orthodontic pain. Orthodontic pain is characterized by sensory and emotional components,[2] is a subjective phenomenon displaying substantial interindividual variability,[3-6] and is caused by potential periapical tissue injury[7, 8] in response to orthodontic force application.

Pain engages multiple neural circuits in the brain, involving the brainstem, the thalamus, and the cortex. These higher-level cognitive and emotional responses to pain exert their influence over pain perception either by a direct modulation of neural pain pathways or through a wide range of neurotransmitters, including natural endogenous pain relieving opioids such as β-endorphin.[9] This "top-down" feedback on sensory processing plays an important role in pain perception, essentially providing a "gate" for the transmission of nociceptive information to the brain.[9] Furthermore, tissue injury acts as a stress factor provoking a defensive biological response in the form of a nervous-endocrine-immune super system that responds as a whole to the tissue injury.[9]

Pain is essentially a bio-physiologically driven phenomenon and follows a circadian rhythm (daily fluctuations in pain level). The circadian rhythm is related with the existence of 24-h daily variations in plasma and brain concentrations of pain regulators such as β-endorphin and the Interleukins.[10-12] The existing pain literature shows that the rhythmic influences on pain increases with an increase in the pain intensity.[13] In other words, more intense the pain, greater the change in a person's sensitivity to the pain across the day. Pollmann[14] systematically studied the daily variations of dental pain and reported that the pain threshold follows a circadian rhythm, reaching its maximum in the afternoon (least pain intensity) and minimum

at night (highest pain intensity). Similarly, Jones and Chan[15] reported diurnal variation in orthodontic pain with higher pain intensity during the evenings and nights. However, in the 25 years which have followed this early work, the diurnal variability in orthodontic pain perception has received scant attention.

A better understanding of diurnal variability in orthodontic pain perception has important clinical and research implications. A better understanding would not only enhance our knowledge about how orthodontic pain intensity fluctuates during the 24-h day, but it also has great potential to improve the clinical management of orthodontic pain. The traditional approach of analgesic administration at regular intervals does not take into account the time-dependent variations in pain intensity and the pharmacokinetics of analgesics. In a recent network meta-analyses, we found that it is important to take into account administration timing as well as the pharmacokinetics (such as plasma half-life) of analgesics while evaluating their effectiveness[16, 17], as the administrative timing has a direct influence on the effectiveness profile.[17]

This paper explores diurnal variation in orthodontic pain perception utilizing intensive longitudinal data (ILD) obtained from an earlier published high quality randomized controlled trial (RCT). High quality RCTs are considered the 'gold standard' for evidence synthesis.[18] The ILD, which involves many repeated measurements per subject, is ideally suited for investigating circadian variation of orthodontic pain.

Methodology

Data from an RCT designed to investigate the effect of two different initial aligning arch wires on pain perception (N = 85, 42 males and 43 females; mean age 14.1 years and SD 2.0 years) [19] were used to measure the circadian variation of orthodontic pain. Participants were randomly assigned to multistranded stainless steel or superalistic arch wire groups using a

stratified (on age, sex and initial crowding) randomization technique. Two age groups were considered, 11–14 and 14–17 years. Pre-adjusted Edgewise Appliances (PEA) with 0.022 x 0.028-inch slot twin brackets (Roth prescription, Gemini Metal Brackets; 3M Unitek Corporation, Monrovia, CA, USA) were bonded directly to the mandibular dentition using light-cure composite resin (Transbond XT; 3M Unitek Corporation), and either 0.0175-inch multistranded stainless steel (Six-stranded, UnitekTM Coaxial Wire; 3M Unitek Corporation) or 0.016-inch superelastic nickel–titanium (austenitic active, preformed ovoid, superelastic arch wire; 3M Unitek) was used as an initial aligning arch wire. Only the mandibular arch was bonded until the completion of the study. The follow-up period was 14 days.

The outcome, pain, was assessed by using the 100-mm Visual Analogue Scale (VAS) at baseline (before arch wire placement) and 32 pre-specified follow-up points. On day 0, the follow-up points were: 1-h; 2-h; 4-h; 6-h; 12-h. On day 1 through 14, pain was not assessed at *a priori* specified time (hours) of the day but rather participants recorded pain in the morning, the afternoon and the bedtime. Participants were requested to mark a line across the VAS (0-100) corresponding to perceived pain at each time point. For all participants, the bonding procedure and initial arch wire placement were carried out between 10.00 am and 11.00 am, though on different days, to ensure that the follow-up time points for pain assessment were the same across all individuals. The trial was meticulously planned to achieve high methodological quality and to minimize confounding by using a blocked stratified randomization for three potential confounders: age, sex and the amount of initial crowding.[19] These trial characteristics make it ideally suitable for current research work. Further methodological details can be found in the original study[19].

The data analyzed in this paper comprised of 2040 observations (mean initial crowding 6.57 mm and SD 1.37) across 24-time points over one week's duration (day 0 morning till day 7 bedtime). As the time points in original study were defined as morning, afternoon and

bedtime except for day 0 (day of arch wire placement), we chose three-time points on day 0 (which were recorded in hours) and re-coded these as morning, afternoon and bedtime as follow: 1-h as day 0 (morning), 4-h as day 0 (afternoon) and 12-h as day 0 (bedtime). Thus, the day 0 (morning) marks the first pain assessment included in this study, which occurred 1-h after the initial arch wire placement, day 0 (afternoon) corresponds to second pain assessment which occurred 4-h after the initial arch wire placement, and day 0 (bedtime) defines the third pain assessment which occurred 12-h after the initial arch wire placement. From day 1 to day 7, we used the same time points as defined in the original study (morning, afternoon and bedtime). Thus, we analyzed data for total 24 timepoints (See Figure 1). We focused on analyzing the pain data for the first seven days because beyond the first week, daily pain data was not available from the original study.

Statistical analysis

A multilevel linear spline model was used for data analysis which offers a great flexibility in modelling nonlinear pain trajectories and appropriately accounts for the subject-specific variability in pain scores. The model was fitted using iterative generalized least squares (IGLS) using the MLwiN (version 2.36) multilevel modelling software[20] calling it from within Stata (version 14) [21] via the user-written 'runmlwin' command.[22] The IGLS estimation method is equivalent to maximum likelihood (ML) method of estimation.

The pain trend over one week's time period followed a curvilinear pattern (initial increase in pain which reaches at peak intensity and then starts declining). We fitted linear splines to capture this complex pattern whilst still providing easily interpretable parameter estimates.[23] Introductions to linear spline multilevel models including discussion of knots selection, interpretation of parameters can be found in the literature.[24]

A range of multilevel linear spline models with different numbers and timings of knots were explored. All models included subject-level (level 2) random effects (intercept and

random coefficients on the linear splines) to appropriately account for the individual-specific variability in pain perception.[23] Likelihood ratio tests were used compare the fit of nested models. Akaike's information criterion (AIC) and the Bayesian information criterion (BIC) were used for fit evaluation of non-nested models.[23] Predicted random effects and residuals were examined to test the random effects and residual normality and homogeneity of variance asssumptions.[23]

An acceptable model fit was achieved by selecting eight knots at the following time points: day 0 afternoon, day 0 bedtime, day 1 morning, day 1 afternoon, day 1 bedtime, day 2 morning, day 3 bedtime, and day 5 bedtime which resulted in a total of 9 linear splines segments. Table 1 presents a description of each linear spline segment. Model fit involved all potential covariates available from the original study (age, sex, initial crowding and the aligning arch wires). As part of the model building exercise, non-significant covariates were dropped from the final model. Although empirical evidence suggests that age-sex interactions have a significant influence on orthodontic pain perception and should be included in the analysis,[4] the limited sample size available in current study did not allow us to estimate such effects (model failed to converge).

Results

Results from final multilevel linear spline model are displayed in Table 1. Only fixed effect estimates (population mean estimates) are displayed as these were the focus of the current study. The model predicted gender-specific mean trajectories showing differences in the pain perception between male and female subjects are presented in Figure 1. The intercept shows that compared to male subjects (coded as 0) who reported a mean average pain of 3.19 mm on day 0 morning, females (coded as 1) experienced significantly greater pain (estimate 2.58, $p < 0.001$, 95% CI 1.40 3.77). However, the rate of increase in pain from day 0 morning to day 0 afternoon (Spline 1) for females was significantly lower (estimate -2.39, $p = 0.018$,

95% CI -4.37 -0.40) as compared to males. The steepest rise in pain, for both male and female subjects, was from day 0 afternoon to day 0 bedtime (Spline 2) where female reported significantly higher rate of increase in pain (estimate 7.19, $p < 0.001$, 95% CI 3.93 10.45) as compared to males. While male subjects showed a plateau around day 0 bedtime to day 1 morning (Spline 3) with no significant change in pain (estimate 0.06, $p = 0.934$, 95% CI -1.35 1.47), the pain for female subjects continued to rise at a significant rate during this period (estimate 4.06, $p < 0.001$, 95% CI 2.08 6.04). From day 1 morning to day 1 afternoon (Spline 4), males reported significant decrease in pain (estimate -1.60, $p = 0.026$, 95% CI -3.00 -0.19) and the rate of decrease in pain for females compared to males was even higher (estimate -2.91, $p = 0.004$, 95% CI -4.88 -0.95). Compared to male subjects who experienced no significant change in pain from day 1 afternoon to day 1 bedtime (Spline 5) and from day 1 bedtime to day 2 morning (Spline 6), females reported a significant rise in pain (estimate 4.35, $p < 0.001$, 95% CI 2.41 6.30) from day 1 afternoon to day 1 bedtime followed by significant decrease in pain (estimate -5.96, $p > 0.001$, 95% CI -8.20 -3.72) from day 1 bedtime to day 2 morning, suggesting a much greater daily variability in pain perception as compared to their male counterparts. From day 2 morning to day 3 bedtime (Spline 7), day 3 bedtime to day 5 bedtime (Spline 8) and day 5 bedtime to day 7 bedtime (Spline 9), both male and female subjects reported a consistent decrease in pain and were not significantly different from each other except from day 2 morning to day 3 bedtime (Spline 7) where rate of decrease in females was significantly higher as compared to males (estimate -0.79, p 0.037, 95% CI -1.53 -0.05).

In the final model, for which the results are presented above, data was combined across ages, initial crowding and the aligning arch wires as initial model fit revealed that the age (estimate 0.05, $p = 0.47$, 95% CI -0.09 - 0.19), initial crowding (estimate -0.03, $p = 0.63$, 95% CI -0.17 0.10) and the type of aligning arch wires (estimate 0.06, $p = 0.64$, 95% CI -0.21 0.34) had no significant effects on orthodontic pain perception while the effect of sex was significant

(estimate 1.04, p = 0.000, 95% CI 0.76 1.32). The likelihood ratio test confirmed no worsening of model fit (Chi square = 0.77, degrees of freedom = 3, p = 0.855) after omitting these covariates from the final model.

Discussion

The present study explored diurnal variation in orthodontic pain over one week's time after initial aligning arch wire placement. A multilevel linear spline model was used which takes into account subject variability in pain perception around the population mean pain trajectory. Results confirm previous findings that pain starts almost immediately after orthodontic force application[19, 25] and follows a curvilinear pattern reaching at its peak intensity after 24 hours and then starts declining after 2-3 days, and gradually tails off after approximately a week.[3, 4, 15, 19 3, 4, 26] The study's findings also support the fact that sex has a significant influence on the orthodontic pain perception as females experienced greater pain at all time points compared to male subjects.[3-6]

The most interesting finding of this study was a significant diurnal variation in pain perception especially within the first two days of orthodontic force application. This is the first study which empirically tested this phenomenon in the context of orthodontic pain and supports the findings of earlier studies which examined this phenomenon under different pain conditions, including dental pain.[11, 13-15, 27] The current study's findings show that both male and female subjects experienced diurnal variation around the peak pain intensity level (day 1), and this variability was significantly higher for females. These findings also support the hypothesis that the rhythmic modulation of pain sensitivity increases with an increase in pain intensity.[13] Results also lend support for the facts that dental pain threshold reaches its peak in the afternoon[14] and individuals experience lesser pain during the afternoon as compared to the night and morning.[15]

Various factors can contribute to the observed diurnal variation in pain perception. Orthodontic tooth movement is essentially a bio-physiologically driven phenomenon involving biological mediators. The Interleukin-1beta (IL-1beta) is the first mediator to regulate bone remodeling in response to orthodontic force and plays an important role in orthodontic pain perception by inducing the release of pain producing pro-inflammatory mediators.[28] A recent study[25] demonstrated that the IL-1beta concentration increases after 1 h of orthodontic force application and peaks after 24 h of force application.[29] Evidence shows that the plasma and brain concentrations of these pain regulator mediators such as Interleukins follow a circadian rhythm and thus are primarily responsible for the diurnal pain variation in pain perception.[10-12] Emerging evidence suggests that orthodontic pain can be significantly influenced by routine daily activities such as physical activity.[26] Thus, it can be hypothesized that day time engagement in routine physical activities at schools (as most orthodontic patients are school going children) may contribute to lower orthodontic pain perception in the afternoon, as observed in this current study.

The diurnal variability in orthodontic pain perception should be thoroughly considered while designing orthodontic trials as it has a direct influence on a study's outcome. It is a common error not to carefully consider and report the timing of orthodontic force application and erroneously conclude that peak pain intensity occurs on day 1 morning and equating it to 24-h after force application. That would hold true only if force was applied in the morning and not in the evening. We have highlighted this issue in our earlier studies.[4, 19, 26]

Clinical implications and pharmacological management

Pain initiates extensive complex neural and extra neural physiological processes affecting overall health, functional capability, and sense of well-being.[30, 31] Pain can also lead to anxiety and suffering. All these effects of pain can profoundly influence individuals'

perceptions, experiences and interpretations of pain. Therefore, orthodontic pain management is of vital importance. The field of pain research is expanding at an unprecedented pace resulting in improved understanding and evolution of new concepts of pain, thereby providing new directions for pain research and pain management. Pain has no 'specialty' boundaries defined by field of research, therefore, from the patient's perspective, orthodontic pain should be considered as significant as any other pain.

An understating of diurnal variation in orthodontic pain perception has important implications in clinical practice, as it has a direct role in ensuring effective pain management by using pharmacological interventions, and even placebo treatments. The most effective analgesia following administration of pharmacological interventions to control pain can only be achieved if the maximum blood level of analgesics occurs at the same time as the peak in pain intensity.[32] In view of this, administration of analgesics should be based on anticipated peaks in the pain intensity rather than wait for pain to occur and increase with time.[32]

Since the administration timing and plasma half-life period of analgesics routinely used for orthodontic pain management have a direct influence on their effectiveness,[16, 17] the traditional approach of analgesic administration at regular intervals, which does not take into account the time-dependent variations in the orthodontic pain intensity, is suboptimal. A better understanding of daily variability in pain perception as well as knowledge of the plasma half-life of analgesics can play a major role in enhancing their effectiveness while minimizing their side effects. Based on such knowledge, a clinician may build his/her own analgesic protocol using multimodal analgesic therapy. An important component of multimodal analgesic therapy is the pre-emptive analgesic followed by prescription of an adjunct analgesic in the form of post-operative analgesics which may involve prescription of long acting analgesics.

As pain starts within the first hour of orthodontic force application and the steepest rise in pain occurs from afternoon to bedtime, clinicians can prescribe a pre-emptive analgesic to manage immediate pain followed by a long acting analgesic (such as Etoricoxib, Pirocixam, Naproxen or Lumiracoxib) that would reduce the build-up of inflammatory response initiated by the orthodontic force, thereby minimizing the steepest rise in the pain by preventing the 'wind-up phenomenon'.[33] Thereafter, analgesic with short to medium plasma half-life periods such as ibuprofen, aspirin or acetaminophen can be used for daily pain management. A knowledge of the fact that pain is relatively more severe during morning and evening as compared to the afternoon suggests that patients can be advised to take analgesics accordingly and need not be prescribed routine analgesics as 6-8 hourly. This judicious use of analgesics would not only enhance their effectiveness but also minimize the side effects. Thus, it is advisable to make patients aware of the diurnal variation they are likely to experience in the intensity of their pain, and to encourage them to take analgesics at the times of day when highest pain is anticipated, rather than wait for peaks in their pain.[32] It also seems prudent to encourage patients to interact with other people and engage in some form of activity during the day.[32]

Interestingly, the placebo effect, which can produce up to 40% of the pain threshold elevation produced by a normal analgesic, also shows significant circadian variations.[34] In a dental study involving healthy individuals, it was reported that the pain threshold changes significantly depending on the administration timing of the placebo.[34] When a placebo is labelled as an analgesic, there is a greater and quicker increase of the pain threshold during the day-time than during the night hours when the pain threshold ascends less or may even drop down.[34] These findings open up new possibilities for future research which would provide evidence for rationale use of analgesics and placebo for pain management based on our understanding of diurnal variability in orthodontic pain perception.

Study limitations

Our study has several limitations relating to the data. As the study involved a secondary data analysis, we could not examine the influence of all covariates which could have potentially affected the daily variability in the orthodontic pain intensity. For example, our study did not consider the psychological variables which may account for the reported sex-related differences in pain perception because the original study did not provide information on such factors. Further, due to the limited sample size, we could not explore the influence of age-sex interaction effects on the diurnal variation in orthodontic pain perception. As we studied the sex-related differences in the rate of change in pain intensity, rather than focusing on the individual timepoint, the statistically significant results may not necessarily be clinically significant. We believe that more research is needed in this direction to better understand the wide array of clinical issues related to the diurnal variation in pain. Lastly, as the original study defined the pain assessment time points as morning, afternoon and evening from day 1 onwards, it was not possible for us to examine hourly fluctuation in the pain intensity. Future studies may wish to use even more flexible measurement schedules to explore this aspect of diurnal variability in orthodontic pain perception.

Conclusion

The findings suggest a significant diurnal variation in orthodontic pain perception, especially during the first two days of orthodontic force application. In this study, where orthodontic force was applied in the morning, the steepest rise in pain occurred between the afternoon and bedtime on the same day. On the following day, pain was significantly less during the afternoon as compared to the morning and bedtime. Both male and female subjects displayed diurnal variability; however, females showed significantly higher pain intensity at all time points and a greater diurnal variability in pain perception as compared to males.

Figure captions

Figure 1 Model fitted mean pain trajectories for male and female subjects with 95% confidence intervals.

References

1. International Association for the Study of Pain. IASP Taxonomy. Available from: https://www.iasp-pain.org/Taxonomy.
2. Sandhu SS. Validating the factor structure and testing measurement invariance of modified Short-Form McGill Pain Questionnaire (Ortho-SF-MPQ) for orthodontic pain assessment. *J. Orthod.* 2017:1-10.
3. Sandhu SS, Leckie G. Orthodontic pain trajectories in adolescents: Exploring the between- and within-subject variability in pain perception. *Am. J. Orthod. Dentofacial Orthop.* 2016;149:491-500.
4. Sandhu SS, Sandhu J. Orthodontic pain: an interaction between age and sex in early and middle adolescence. *Angle Orthod.* 2013;83:966-972.
5. Scheurer PA, Firestone AR, Burgin WB. Perception of pain as a result of orthodontic treatment with fixed appliances. *Eur. J. Orthod.* 1996;18:349-357.
6. Bergius M, Berggren U, Kiliaridis S. Experience of pain during an orthodontic procedure. *Eur. J. Oral Sci.* 2002;110:92-98.
7. Storey E. The nature of tooth movement. *Am. J. Orthod.* 1973;63:292-314.
8. Furstman L, Bernick S. Clinical considerations of the periodontium. *Am. J. Orthod.* 1972;61:138-155.
9. McMahon S, Koltzenburg M, Tracey I, Turk DC. *Wall & Melzack's Textbook of Pain, 6th Edition*: Elsevier Health Sciences; 2013.
10. Nilsonne G, Lekander M, Åkerstedt T, Axelsson J, Ingre M. Diurnal Variation of Circulating Interleukin-6 in Humans: A Meta-Analysis. *PLoS One.* 2016;11:e0165799.

11. Labrecque G, Karzazi M, Vanier M-C. Biological Rhythms in Pain and Analgesia. In: Redfern PH, Lemmer B, eds. *Physiology and Pharmacology of Biological Rhythms*. Berlin, Heidelberg: Springer Berlin Heidelberg; 1997:619-649.

12. Labrecque G, Vanier M-C. Rhythms, pain and pain management. In: Redfern P, Redfern PH, eds. *Chronotherapeutics*: Pharmaceutical Press, London (UK); 2003:211-234.

13. Koch HJ, Raschka C. Diurnal Variation of Pain Perception in Young Volunteers Using the Tourniquet Pain Model. *Chronobiol. Int.* 2004;21:171-173.

14. Pöllmann L. The duality of pain, demonstrated by the circadian variation in the tooth sensitivity. Vol 111981:S45-S45.

15. Jones M, Chan C. The pain and discomfort experienced during orthodontic treatment: a randomized controlled clinical trial of two initial aligning arch wires. *Am. J. Orthod. Dentofacial Orthop.* 1992;102:373-381.

16. Sandhu SS, Cheema MS, Khehra HS. Comparative effectiveness of pharmacologic and nonpharmacologic interventions for orthodontic pain relief at peak pain intensity: A Bayesian network meta-analysis. *Am. J. Orthod. Dentofacial Orthop.* 2016;150:13-32.

17. Sandhu SS, Piepho HP, Khehra HS. Comparing the effectiveness profile of pharmacological interventions used for orthodontic pain relief: an arm-based multilevel network meta-analysis of longitudinal data. *Eur. J. Orthod.* 2017.

18. Sandhu SS, Sandhu J, Kaur H. Reporting quality of randomized controlled trials in orthodontics—what affects it and did it improve over the last 10 years? *The European Journal of Orthodontics.* 2015;37:356-366.

19. Sandhu SS, Sandhu J. A randomized clinical trial investigating pain associated with superelastic nickel–titanium and multistranded stainless steel archwires during the

initial leveling and aligning phase of orthodontic treatment. *J. Orthod.* 2013;40:276-285.

20. Rasbash J, Steele F, Browne W, Goldstein H. A User's Guide to MLwiN, Version 2.36. Centre for Multilevel Modelling, University of Bristol2016.

21. StataCorp. *Stata Statistical Software: Release 14*. College Station, TX: StataCorp LP; 2015.

22. Leckie G, Charlton C. runmlwin: A Program to Run the MLwiN Multilevel Modeling Software from within Stata. *Journal of Statistical Software.* 2013;52:1-40.

23. Rabe-Hesketh S, Skrondal A. *Multilevel and Longitudinal Modeling Using Stata, Third Edition (Volumes I and II)*: Stata Press Publication; 2012.

24. Howe LD, Tilling K, Matijasevich A, et al. Linear spline multilevel models for summarising childhood growth trajectories: A guide to their application using examples from five birth cohorts. *Stat. Methods Med. Res.* 2016;25:1854-1874.

25. Luppanapornlarp S, Kajii TS, Surarit R, Iida J. Interleukin-1beta levels, pain intensity, and tooth movement using two different magnitudes of continuous orthodontic force. *Eur. J. Orthod.* 2010;32:596-601.

26. Sandhu SS, Sandhu J. Effect of physical activity level on orthodontic pain perception and analgesic consumption in adolescents. *Am. J. Orthod. Dentofacial Orthop.* 2015;148:618-627.

27. Pollmann L, Harris PH. Rhythmic changes in pain sensitivity in teeth. *Int. J. Chronobiol.* 1978;5:459-464.

28. Giannopoulou C, Dudic A, Kiliaridis S. Pain discomfort and crevicular fluid changes induced by orthodontic elastic separators in children. *The journal of pain : official journal of the American Pain Society.* 2006;7:367-376.

29. Lee KJ, Park YC, Yu HS, Choi SH, Yoo YJ. Effects of continuous and interrupted orthodontic force on interleukin-1beta and prostaglandin E2 production in gingival crevicular fluid. *Am. J. Orthod. Dentofacial Orthop.* 2004;125:168-177.

30. Chapman CR, Tuckett RP, Song CW. Pain and stress in a systems perspective: reciprocal neural, endocrine, and immune interactions. *J. Pain.* 2008;9:122-145.

31. Apkarian AV, Bushnell MC, Treede RD, Zubieta JK. Human brain mechanisms of pain perception and regulation in health and disease. *Eur. J. Pain.* 2005;9:463-484.

32. Glynn CJ, Lloyd JW. The diurnal variation in perception of pain. *Proc. R. Soc. Med.* 1976;69:369-372.

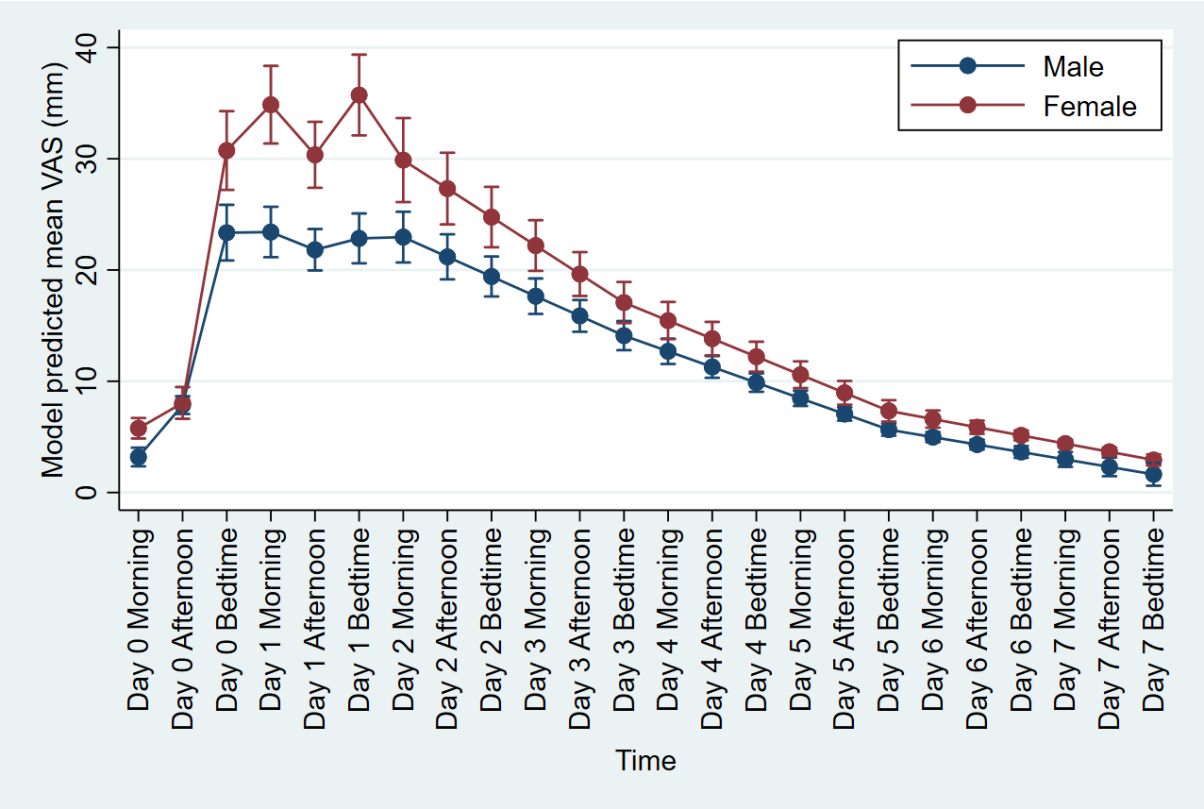
33. Sinatra RS, Jahr JS, Watkins-Pitchford JM. *The essence of analgesia and analgesics*: Cambridge University Press; 2010.

34. Pöllmann L. Circadian variation of potency of placebo as analgesic. *Funct. Neurol.* 1987;2:99-103.

Table 1 Diurnal variability in mean average pain trajectories estimated using Multilevel linear spline model (Significant parameters are highlighted).

Parameter*	Estimate	Standard Error	p value	95% Confidence Interval	
				lower	upper
Intercept	**3.19**	**0.43**	**0.00**	**2.35**	**4.03**
Female	**2.58**	**0.60**	**0.00**	**1.40**	**3.77**
Spline 1: day 0 morning to day 0 afternoon	**4.67**	**0.72**	**0.00**	**3.25**	**6.08**
Spline 2: day 0 afternoon to day 0 bedtime	**15.5**	**1.18**	**0.00**	**13.18**	**17.82**
Spline 3: day 0 bedtime to day 1 morning	0.06	0.72	0.93	-1.35	1.47
Spline 4: day 1 morning to day 1 afternoon	**-1.60**	**0.71**	**0.03**	**-3.00**	**-0.19**
Spline 5: day 1 afternoon to day 1 bedtime	1.02	0.71	0.15	-0.36	2.41
Spline 6: day 1 bedtime to day 2 morning	0.11	0.81	0.89	-1.48	1.71
Spline 7: day 2 morning to day 3 bedtime	**-1.77**	**0.27**	**0.00**	**-2.30**	**-1.24**
Spline 8: day 3 bedtime to day 5 bedtime	**-1.41**	**0.11**	**0.00**	**-1.63**	**-1.19**
Spline 9: day 5 bedtime to day 7 bedtime	**-0.67**	**0.09**	**0.00**	**-0.84**	**-0.50**
Female * Spline 1: day 0 morning to day 0 afternoon	**-2.39**	**1.01**	**0.02**	**-4.37**	**-0.40**
Female * Spline 2: day 0 afternoon to day 0 bedtime	**7.19**	**1.66**	**0.00**	**3.93**	**10.45**
Female * Spline 3: day 0 bedtime to day 1 morning	**4.06**	**1.01**	**0.00**	**2.08**	**6.04**
Female * Spline 4: day 1 morning to day 1 afternoon	**-2.91**	**1.00**	**0.00**	**-4.88**	**-0.95**
Female * Spline 5: day 1 afternoon to day 1 bedtime	**4.35**	**0.99**	**0.00**	**2.41**	**6.30**
Female * Spline 6: day 1 bedtime to day 2 morning	**-5.96**	**1.14**	**0.00**	**-8.20**	**-3.72**
Female * Spline 7: day 2 morning to day 3 bedtime	**-0.79**	**0.38**	**0.04**	**-1.53**	**-0.05**
Female * Spline 8: day 3 bedtime to day 5 bedtime	-0.21	0.16	0.18	-0.53	0.10
Female * Spline 9: day 5 bedtime to day 7 bedtime	-0.07	0.12	0.59	-0.31	0.17

* Each spline (Spline 1, Spline 2 and so on) captures a rate of change in pain score for male subjects as a function of time (male subjects coded as 0).
Each Female * Spline interaction effect represents difference in the rate of change in pain score between male and female subjects (male subjects coded as 0; female subjects coded as 1).


Model predicted mean VAS (mm)
0
10
20
30
40
Male
Female
Day 0 Morning
Day 0 Afternoon
Day 0 Bedtime
Day 1 Morning
Day 1 Afternoon
Day 1 Bedtime
Day 2 Morning
Day 2 Afternoon
Day 2 Bedtime
Day 3 Morning
Day 3 Afternoon
Day 3 Bedtime
Day 4 Morning
Day 4 Afternoon
Day 4 Bedtime
Day 5 Morning
Day 5 Afternoon
Day 5 Bedtime
Day 6 Morning
Day 6 Afternoon
Day 6 Bedtime
Day 7 Morning
Day 7 Afternoon
Day 7 Bedtime
Time