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Original Research Article

Functional Significance of the Curve of Spee: Electromyographic Analysis of Young Adults—A Preliminary Study

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ABSTRACT

Background: The curve of Spee is a fundamental anatomic feature of dental occlusion that influences mandibular movement, muscle coordination, and masticatory efficiency. In contemporary orthodontics, standardized leveling and flattening of this curve are frequently adopted, as originally proposed by Andrews. However, in prosthetic and restorative dentistry, the management of the curve of Spee often requires a more individualized approach. Given the high prevalence of fixed orthodontic treatment, understanding the functional consequences of altering this occlusal curvature is clinically relevant. **Objective:** This pilot study aimed to investigate the effects of leveling the antero-posterior occlusal curve on muscular activity and functional harmony of the stomatognathic system in young adults previously treated with fixed orthodontic appliances. **Materials and Methods:** Sixteen young adult subjects with a history of fixed orthodontic treatment were enrolled. Muscular activity was assessed by surface electromyography using a four-channel Kinelock electromyographic device wirelessly connected to a dedicated personal computer. Electromyographic recordings were used to evaluate masticatory muscle function in relation to the occlusal configuration and the characteristics of the curve of Spee. **Results:** Electromyographic analysis revealed variations in muscular activity associated with modifications of the curve of Spee. The findings suggest that standardized flattening of the occlusal curve may influence neuromuscular balance and masticatory function, highlighting interindividual differences in functional adaptation. **Conclusion:** Within the limitations of this pilot study, the curve of Spee appears to be a parameter requiring individualized consideration to optimize functional harmony of the stomatognathic apparatus. Standardized leveling procedures may have unavoidable functional consequences, supporting the need for personalized occlusal management in orthodontic, prosthetic, and restorative treatments.

Keywords—*Curve of spee, Electromyographic analysis, Young adults.*

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INTRODUCTION

The curve of Spee is an anatomical curve described by German anatomist Ferdinand Graf Von Spee in 1890, after whom it is named. It is a curve that is described as “an ideal line connecting the cusps, that is, the top of the natural teeth, starting from the canines and passing through the premolars to the molars of the same half of the mouth.”¹ Spee realized how this curved line could optimize tooth contact between elements on antagonistic arches. The curve of Spee is defined as a dental parameter determined by the mesiodistal inclinations of the latero-posterior elements of the mandibular dental arch.² As Osborn demonstrated, this arrangement allows a more efficient distribution of forces and masseter action.² Osborn also noted that the inclination of the masseter was parallel to the long axis of the lower first molars.²⁻¹⁰ The normal functions of the stomatognathic apparatus (chewing, swallowing, and phonation) result in contacts between antagonistic arches, and the consequent distribution of forces along the bone structure differs between patients because of several variable parameters, including the Spee curve. A similar sagittal arrangement of dental elements maximizes muscle efficiency during chewing.² These different muscle forces and the direction of the fibers could contribute to the mandibular shape and, consequently, could influence the inclination of the occlusal plane.¹¹ The curve of Spee is thus a dental parameter, which is probably affected and, in turn, influences both hard and soft tissues.¹² A similar curve in the sagittal plane is found in several mammalian taxa, including modern humans and fossil hominids. It went from a totally flat curve with a wide retromolar space, found in Neanderthals and Australopithecus, to the progressive change in the hominid craniofacial structure, which also included the appearance and variation of the curve in sapiens.¹³ The presence/absence of this feature may be related to the functional variation of the masticatory apparatus and also to broader dentognathic spatial dynamics. It can be argued that this morphological relationship is probably due to the developmental and functional integration between the maxillary and mandibular dental arches.¹³ Pathological situations, ethnicity, genetic components, and age may be part of the factors influencing the curve of Spee. This anatomical curve not only varies in relation to evolution

but also varies interpersonally and even within the same individual, depending on age. Studies show that it varies according to the subject’s stage of dentition. Marshall et al. noted how the occlusal plane is flat in full deciduous dentition.¹⁴ Similar results were reported by Ash et al.¹⁵, who suggested that deciduous dentition exhibits a curve of Spee that ranges from flat to slight, whereas dentition in adulthood exhibits a more pronounced Spee curve, which is stabilized and maintained. Thus, if in deciduous dentition the flattened curve is physiological, the same cannot be said for the curve in adulthood. Often, however, there is a tendency to alter this curve to reduce its depth or level it off, especially in the orthodontic school that follows Andrews, who believed that this curve should be reduced or eliminated to improve occlusal contact.⁴

While that is true in static structures, it must nevertheless be remembered that the mandible is an organ that performs its functions primarily in movement. When designing dental treatment that is aimed at maintaining or restoring ideal functional harmony, not only must the centric movements of the mandible be taken into account but also the eccentric and excursive components of mandibular movements. In fact, this curve favors the posterior disocclusion in protrusive movements.¹⁶ As proof of this, a clinical examination that can be carried out is to ask patients to perform a protrusive movement by bringing the upper incisal margins into a head-to-head position with the lower ones. Subsequently, the amount of posterior disocclusion should be noted, as well as the presence of any precontact or contact that obstructs the movement. Dental extrusions can pathologically alter this curve and lead to a similarly altered occlusal plane¹⁷, with the presence of posterior protrusive interference, which can cause abnormal activity—hyperactivity—in mandibular elevator muscles, particularly the masseter and temporalis muscles¹⁸, whose activity was examined in this study using electromyographic tracings. Excessive interference can also cause wear and tear, the fracture of restorations, and temporomandibular joint dysfunction. In confirmation of this, it has been observed that problems such as temporomandibular disorders are more prevalent in patients in whom the curve of Spee has been altered following orthodontic treatment.¹⁹ Such complications can be avoided by reconstructing a curve of Spee that is

in harmony with the subject's stomatognathic apparatus, and in particular with the structure of the mandibular condyle.¹⁸ The purpose of this study is to investigate the curve of Spee in relation to muscle activity, assessed by electromyographic analysis, in a group of 16 young adults with previous fixed orthodontic treatment.

MATERIALS AND METHODS

Digital imprints and electromyographic tracings of the anterior masseter and temporalis muscles were taken in a group of 16 subjects. The age of the subjects examined ranged from 20 to 25 years. All the subjects had Angle Class I occlusal relationships and had undergone previous fixed orthodontic treatment.

The study consists of three phases: 1. Measurement of the curve of Spee on digital imprints and assessment of the extent of occlusal contact, using the Cerec software, version 5.2.4 (Dental CAD/CAM software; Dentsply Sirona Inc., Charlotte, NC, USA, 2022); 2. Analysis of the electromyographic tracings obtained from the same subjects, study of the indices obtained from the electromyographic recordings, and evaluation of the fit bite system of the Kinelock electromyograph software (4T srl Cislago Italy); 3. Correlation of the data obtained.

Materials

Fingerprints were taken using the Primescan Connect intraoral scanner. For the evaluation of the occlusal contacts, the obtained digital surveys were studied using the Cerec 5.2.4 software. Data measurements of the maximum depth of the curve of Spee were made on digital models, with the Blender computer graphics computer-aided design (CAD) software (version 2023). In order to detect information about the muscular activity of the same subjects, the 4-channel Kinelock electromyographic device was used, wirelessly connected to a dedicated personal computer (PC). The readings were carried out using disposable bipolar Ag/AgCl electrodes. Before placing these electrodes, cotton swabs with an alcoholic solution were used to cleanse the skin. For the neutralization of occlusal contacts, cotton rolls with a diameter of 10 mm were used. Latex gloves were used to conduct the study. The data obtained from the present study were recorded using the Panasonic Lumix GH4 camera.

Methods

The fingerprints of upper and lower dental arches and of the position of maximum intercuspitation (bite) of each of the 16 examined subjects were considered. With the Cerec 5.2.4 software, it was possible to visualize the colorimetric evaluations of the extent of the occlusal contacts (generated by the articulation of the opposing dental arches in maximum intercuspitation). On these fingerprints, the curves of Spee were evaluated, both in the right and left side of the mandibular arch.

The maximum depth of the curve of Spee was measured in the mandibular arch as the greatest perpendicular distance between the tips of the buccal cusps of the mandibular teeth and a plane of measurement that went from the canines to the tip of the distal cusp of the most posterior ipsilateral tooth of the same dental arch. The maximum depth was studied by plotting several segments with the software, starting from the cusps of the latero-posterior elements perpendicular to the curve, and selecting the one having the greatest value corresponding to the point of maximum depth of the curve. The same subjects underwent electromyographic studies to assess activity of the masticatory muscles: both anterior temporalis and masseter.

The Kinelock electromyograph, equipped with eight channels, connected wirelessly to a dedicated PC, was used for the study. Bipolar Ag/AgCl electrodes, with 10-mm diameter and 1-mm inter-electrode distance, were used for all the subjects and muscle surfaces, using a gelled surface treated with conductive paste and a reference electrode placed at the center of the forehead on a silent area. Before placing the electrodes on the skin, it was necessary to clean it with a cotton swab soaked in methylated spirit. Electrodes were placed parallel to the muscle fibers. For the anterior temporalis, they were placed vertically along the anterior margin of the muscle, just above the coronal suture, while for the masseter, they were placed parallel to the muscle fibers with the upper pole at the height of the intersection of the plane between the tragus and the angle of the lips and the plane between the gonion and the outer canthus of the eye. For each subject, five acquisitions lasting for 5 seconds each were performed in the position of maximum voluntary contraction (MVC). During these tests, the subjects were

verbally urged to clench as hard as possible. They were not shown the tracings during the test to avoid alterations because of visual biofeedback. In order to simulate the subjects' muscular activities as closely as possible to reality, the assessments were made with visual correction means usually used by the subjects examined. For the first three recordings, the subjects were made to sit on a wooden chair, assuming a straight posture, with their feet resting on the ground and their hands resting on their knees, their gaze directed toward the horizon plane. The additional two acquisitions, on the other hand, were taken by asking the subjects to acquire an orthostatic posture, with their gaze toward the horizon and their arms along their sides. In the first survey, a neutralization of the occlusal contact through "cotton clenching" was performed: in subjects seated according to the arrangements described above, with their eyes open and looking straight at the horizon, two cotton rolls, 10 mm in diameter, were interposed between the arches, symmetrically in the left and right premolar areas. After cotton clenching, the cotton rollers were removed, and the additional four recordings were made. The second recording was made with the subject sitting and eyes open. The third recording was made with the subject sitting and eyes closed. The fourth and fifth recordings, on the other hand, were made in the orthostatic position, with eyes open and then eyes closed, respectively. Thus, the electromyographic activity of the masseter muscles and the anterior temporalis muscles on both sides was recorded in different positions with eyes open and closed, to study the possible influence of postural and visual proprioceptive inputs. From electromyographic recordings, it was possible to evaluate raw and root mean square-derived standardized electromyographic signals (RDS) tracings to study the percentage of overlapping coefficient for masseter muscles (POC MM), percentage of overlapping coefficient for the temporalis anterior muscles (POC TA), IMPACT index (overall muscle work), BAR index (occlusal barycenter), and TORS index (muscle activity torsion).

The POC indices (masseters/anterior temporals) indicate the level of contraction symmetry of the individual pairs of muscles—masseters or anterior temporals. In case of perfectly symmetrical contraction, that is, if two muscles contract with perfect symmetry, we obtain a POC = 100%.

A malocclusion condition that reduces this symmetry is highlighted by a lower value at 83%. The BAR index refers to the overlap percentage between the clenching activity of the pair of anterior temporals, compared to the pair of masseters. When there is a prevalence of posterior contacts, the masseters do contract more than the anterior thunderstorms. Conversely, if the contacts are concentrated anteriorly, the occlusal barycentre will be anterior and the temporal contracts more. The lower limit of normal for the BAR value is 90%. The IMPACT index represents the electrical activity that each single muscle expresses during the test, compared to the cotton calibration values, expressed as a percentage; evaluate the intensity of the muscle work during maximum intercuspitation. The normal range is 85–115%. The TORS index measures the torsional attitude of the mandible (in the transversal plane) when this articulates in occlusion with the maxilla. Compare pairs "right anterior temporal to left masseter" and "left anterior temporal to masseter right." Normality varies between 90% and 100%. The presence of occlusal fulcrums can cause the prevalence of one couple over the other, resulting in a torsion of the jaw. The ASIM (asymmetry) index evaluates the symmetry of muscle contraction, of occlusal condition, between left and right-side muscles. In normal ranges, the index can vary between -10% and +10% (assuming positive values in case of occlusal prevalence in the right hemiarch and negative values in case of occlusal prevalence in muscles on the left). Over the evaluation of such electromyographic indices, it is possible to visualize the graphical representation of the occlusal condition of each individual subject with the fit bite algorithm of the software, which shows the areas in which the occlusion should be modified, suggesting possible modifications of the thickness necessary to obtain a better muscular activity (Figure 1).

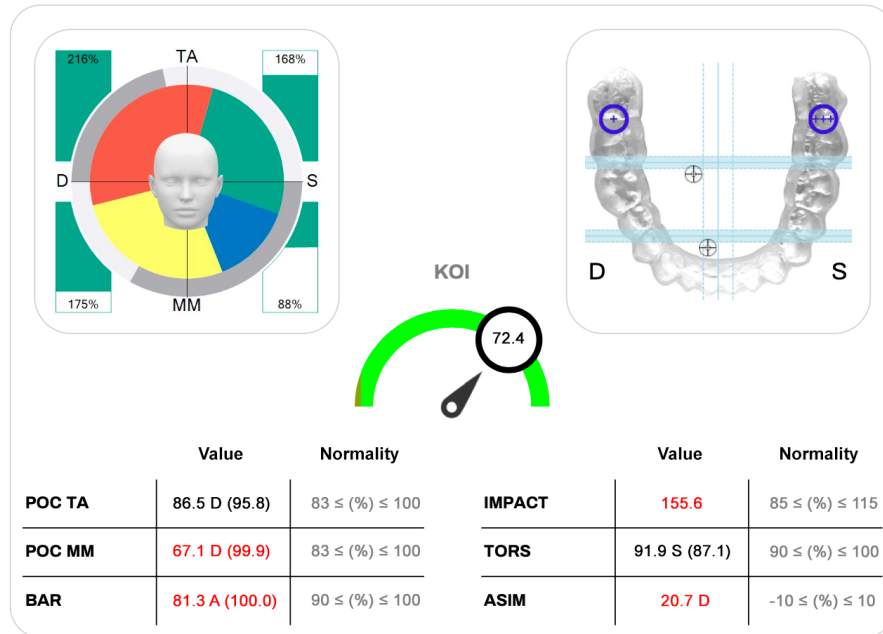


FIGURE 1. Indices obtained from an electromyographic recording, with muscle activity graph and fit bite algorithm (Kinlock electromyograph).

RESULTS

This study evaluated the influence of the arrangement of dental elements in the sagittal view on the contractile activity of the masticatory muscles.

The subjects examined presented curves of Spee with mild depth, zero depth, and in some cases, had a negative curve (inverted concavity), probably caused by previous orthodontic treatment. The study shows that in the arches where the curve of Spee is more flattened and leveled, the maximum depths of the curve are often reached at the level of the distal buccal cusp of the first molar.

A further observation pertains to the differences in the depth of the curves in two hemiarches, left and right, in the same subject.

It was noted that the curvatures of the two hemiarches are different, in a variably marked manner, and that the hemiarches in which the third molar is present have a greater curvature. In fact, the presence of the third molar in the arch causes a significant increase in the curve of Spee. The results show that the hemiarch in which the curvature is deepest is the hemiarch of the subject in which the occlusal contacts in maximum intercuspation are greater, as observed by colorimetric evaluations (Figure 2), and

in which the stress resulting from occlusion in maximum intercuspation appears as more distributed at the molar level and less localized at the premolar, canine and incisor levels. In contrast, in the arches or hemiarches in which the curve of Spee is flatter and almost level, stress appears to be more evenly distributed over anterior, lateral, and posterior elements.

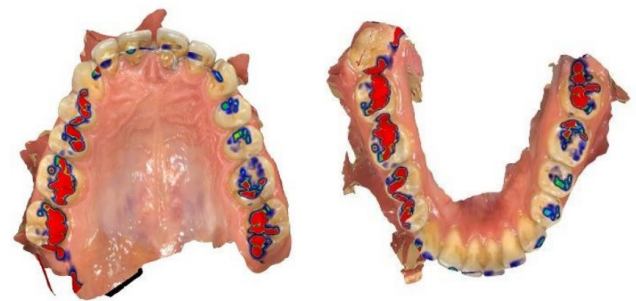


FIGURE 2. Colorimetric analysis of occlusal contacts (Cerec 5.2.4 software).

With these curve depth values (reduced, zero, or reversed), an increased activity was noted in the antero-temporalis. The BAR index (occlusal centre of gravity) was found to vary in all subjects and be delocalized anteriorly (Figure 3). In addition to the BAR index, the

electromyographic evaluations showed that IMPACT was another parameter which was often found to be lower than the normal range (Figure 4).

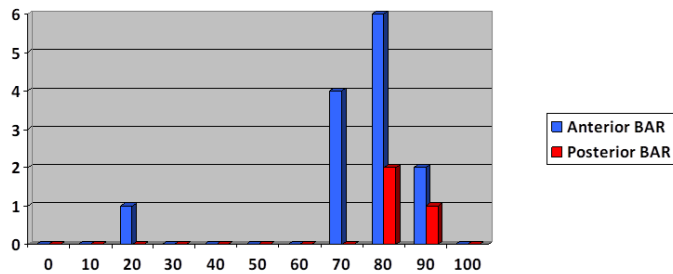


FIGURE 3. Histogram of the distribution of BAR index values in the studied subjects. The abscissa axis represents BAR values (%), and the ordinate axis represents the number of subjects. Red bars indicate posterior occlusal center of gravity, whereas blue bars indicate anterior occlusal center of gravity. Columns with values >90% indicate a good occlusal center of gravity. Most subjects showed BAR values <90%, with a predominance of anterior localization (blue bars), indicating an occlusal center of gravity frequently outside the normal range and shifted anteriorly.

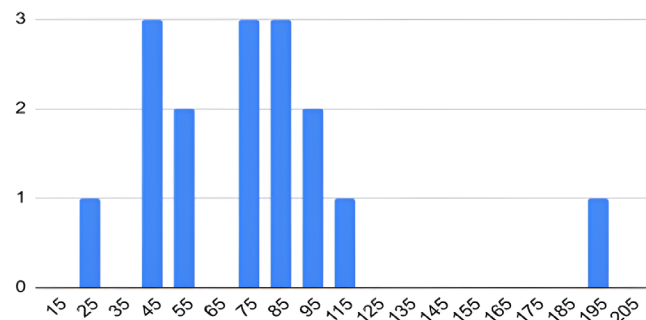


FIGURE 4. Histogram of the distribution of IMPACT index values in the studied subjects. On the abscissa axis is the IMPACT index value, and on the ordinate axis is the number of subjects. Columns between 85 and 115 indicate the IMPACT index. Values are found within the normal range. The prevalence of columns for values < 85 is noted; this indicates that most of the subjects have an IMPACT index altered beyond the normal range, often reduced.

In these cases, the system’s fit bite algorithm indicated the need for shim additions in posterior sectors (Figure 1). Changes in MVC activity recorded in the muscle activity while seated, and the activity measured in orthostatism, presented no significant differences; both tracings and

indices were found to be similar, slight differences presented were related to interpersonal variations. Visual inputs were found to be irrelevant in the muscle activity of the examined masticatory muscles. Slight differences in developed contractile activity (along the vertical y-axis of the ordinates of electromyographic tracing, measured in microvolts) reoccurred and were reflected in slight, non-significant differences at the level of the indices evaluated.

For this reason, in this study, the results of electromyographic recordings obtained while sitting and eyes opened were considered, as shown in the results (Table 1).

TABLE 1. Values obtained from measurements of the maximum depth of the curve of Spee and indices obtained by electromyographic examination.

Case	Maximum Depth of the Left Hemiarch Spee Curve	Maximum Depth of the Right Hemiarch Spee Curve	Bar 90 < (%) < 100	Impact 85 < (%) < 115	POC MM 83 < (%) < 100	POC TA 83 < (%) < 100	TORS 90 < (%) < 100	ASIM -10 < (%) < 10
1	2,219 mm	2,183 mm	89.6P	47.6	86.6L	82.3L	92.1L	-10.5L
2	2,779 mm	2,834 mm	76.8A	50.4	82.8R	91.6R	93.9L	9.8R
3	3,491 mm	1,663 mm	88.4A	64.0	89.4L	92.0R	92.2L	-1.4L
4	2,029 mm	1,842 mm	78.4A	27.0	86.3L	91.1L	92.8L	-5.0L
5	1,778 mm	4,154 mm	88.0A	81.1	93.5R	91.9R	94.5L	1.9R
6	1,474 mm	1,043 mm	87.6A	89.3	86.3R	84.7R	91.1L	11.0R
7	2,624 mm	2,159 mm	94.0A	98.3	89.7L	89.8L	94.1L	-5.8L
8	-1,257 mm	-1,403 mm	91.0A	119.2	91.4L	92.9L	95.3L	-3.3L
9	2,092 mm	2,302 mm	91.2P	112.9	88.3L	87.3L	92.2L	-3.7L
10	1,810 mm	2,927 mm	29.3A	52.2	62.5R	76.9R	84.7L	21.7R
11	4,281 mm	5,214 mm	88.7A	87.1	80.8L	88.8R	88.8R	-7.1L
12	2,784 mm	1,224 mm	80.8A	83.5	89.5R	82.5L	87.9L	-6.4R
13	1,412 mm	1,560 mm	89.0A	76.2	81.8L	89.0A	90.4L	0.1R
14	1,271 mm	1,035 mm	81.9P	198.7	62.6R	82.5L	71.1L	15.0R
15	4,214 mm	-1,082 mm	78.3A	59.7	74.3L	56.4L	82.9L	-36.2L
16	2,695 mm	3,783 mm	86.9P	90.2	93.9L	81.3L	93.1L	-10.5L

Footnote: Electromyographic recordings obtained while sitting and eyes opened were considered. Abbreviations: P, posterior; A, anterior; L, left; R, right.

DISCUSSION

The curve of Spee is in harmony with other factors that include the condylar guidance angle, the incisal guidance angle (overbite and overjet), and the occlusal plane angle. This harmony involves not only the dentoskeletal tissues but also the soft tissues.

The reduced, zero, or negative depth values of the curve of Spee assessed in the examined subjects imply a prevalence of anterior contacts (as visible in the colorimetric assessments) and correspond to an increased activity in the anterior temporalis, probably as a result of the reduced depth of the curve of Spee.

This finding is confirmed by the BAR index (occlusal center of gravity), which varied in all the subjects and was anteriorly delocalized in at least one of the five electromyographic recordings made in each subject.

This observation can be correlated with the images of the colorimetric evaluations of occlusal contacts carried out by the intraoral scanner software. Above all, in cases of leveled curves of Spee, occlusal contacts in maximum intercuspation are observed as well distributed along the arch, in a homogeneous manner, even at the level of the anterior incisal region, where, however, there should be a “grazing” contact and instead this contact is very much present, for guidance, during the protrusive excursive movements. Alteration in the BAR index can be correlated with the change in the IMPACT index. The latter index corresponds to the distal vessel occlusion (DVO) of the subject: an increase in DVO correlatively produces an increase in IMPACT index, which can be read as an index of chewing effectiveness.²⁰ The IMPACT index was found to be altered in almost all subjects: in most cases it was lower. In these cases, the system’s fit bite algorithm indicated the need for the addition of shims in the posterior sectors to restore an ideal curve of Spee, increasing its depth.

Information from the colorimetric analysis of occlusal contacts and from the fit bite software of the Kinelock electromyograph confirms the need for the addition of thickness at the posterior level.

If in a study it is observed that a mild and flattened curve of Spee is correlated (when the other values were normal) to low IMPACT, with a need for increased posterior DVO and an increase in the Spee curve, it is assumed

that leveling has resulted in a reduced biological vertical dimension of occlusion in the individual and a consequent reduced effectiveness of the masticatory muscle activity.

On the other hand, it is also possible that leveling alters the DVO by increasing its values.

The IMPACT index increases in subjects with increased DVO, for example because of leveling caused by excessive extrusion in the premolar/molar area: the curve of Spee should no longer be restored by adding thickness and height to increase posterior DVO, by adding hard tissue, or by extruding teeth and tilting them mesially, but rather by decreasing this DVO through selective intrusion or grinding.

Therefore, in the leveling of the curve of Spee, one of the issues that can arise is how to maintain a DVO in the ideal biological range for the subject and for the correct and the most effective muscular and postural functioning. Leveling the curve of Spee could in fact cause an alteration in DVO, which may increase or decrease.

When the mandibular curve is convex with negative depth values, DVO is increased, as is the IMPACT index. On the other hand, the leveling of the curve of Spee in the other cases result in reduced DVO, with low IMPACT index, and the consequent need, as indicated by the fit bite algorithm, to add shims at the posterior level, increasing DVO at the molar level, along with IMPACT, thus achieving a restoration of the curve of Spee. In addition, the fact that the maximum depth of the curve is often reached at the level of the distal buccal cusp of the first molar highlights how the mesiodistal inclination of these elements is different from normal, where the maximum depth is generally reached at the level of the buccal cusp of the second molar or at the mesiobuccal cusp of the first molar, because of their mesiodistal inclination. In the hemiarch in which the curve is deeper, the mesiodistal inclinations of the posterior elements are greater. In these cases, the occlusal stress is distributed more on these lateral-posterior dental elements and is less localized anteriorly. Moreover, the occlusal contact in the maximum intercuspation is greater. This arrangement of contacts and stress in the maximal intercuspation benefits both lateral and anterior elements, which are the ones assigned to bearing high occlusal loads, especially in movements with a greater vertical component (unlike the anterior and

lateral elements, which are usually used more in movements in which the horizontal component predominates: the antero-posterior and the latero-lateral, respectively).

Conversely, in the arches or hemiarches in which the curve of Spee appears to be shallower, stress appears to be more evenly distributed on anterior, lateral, and posterior elements, thus making both anterior and lateral elements susceptible to stress arising from the centric component of mandibular movements, and the posterior ones susceptible to stress from the eccentric and excursive components of the same mandibular movements during chewing.

In confirmation to this, it is observed from the electromyographic tracings that a deeper arch curve does not only correspond, as mentioned, to the arch in which the stronger occlusal contact occurs and is more localized at the molar level in maximum intercuspation but it is observed that it is also the arch in which a greater ipsilateral masseter activity occurs (within normal ranges). This is observed by analyzing both tracings and indices of the respective electromyographic evaluations.

Limitations of the Study

To better assess muscle function as a function of occlusion, it is proposed that dynamic electromyographic evaluations be made in future studies, especially for protrusive movements, with the relative muscles involved, in which the curve of Spee has been observed to play an important role.

The present study focuses its attention on the curve of Spee. It would be interesting if the evaluation was also applied to the curve of Wilson for a complete three-dimensional analysis of the occlusal plane.

An additional limitation of the study is the reduced size of the group of young adults on whom the test was performed, from which Angle class II and III subjects were excluded.

In addition, since many of the subjects evaluated had had previous orthodontic treatment, it would have been interesting to evaluate not only the post-treatment data but the pre-treatment data as well.

CONCLUSIONS

The curve of Spee represents a variable in dentistry treatments, in both orthodontic field and conservative prosthetic one, and it must be taken into account to achieve maximum masticatory efficiency and muscular balance in the stomatognathic apparatus.

In the orthodontic field, the flattening of the curve of Spee is expected in both restorative and prosthetic fields. Such a compensatory curve plays an indispensable role in positive outcomes, success, stability, and long-term prognosis of the element. Its alteration or the production of a curve that is not in harmony with the balance, both static and dynamic, of the neuro-musculo-skeletal (NMS) apparatus of the subject may lead to various difficulties. Proposing a treatment with an alteration of such a curve should raise questions, not only about the stability of the treatment but also about the occlusal and overall harmony of the stomatognathic system.

INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of University of Palermo-Policlinico Paolo Giaccone n. 04/2023 (19/04/2023).

AUTHOR CONTRIBUTIONS

Conceptualization, G.A.S., and I.B.; Methodology, G.A.S., I.B., and E.M.C.; Validation, M.I.K., A.M., G.G., and G.B.; Formal analysis, I.B.; Investigation, I.B. and G.A.S.; Data curation, I.B.; Writing—original draft preparation, G.A.S.; Writing—review and editing, P.M.; Visualization, A.M., M.I.K., and L.C.; Supervision, P.M.

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CONFLICTS OF INTEREST

The authors declared no conflict of interest.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Informed consent was obtained from all the subjects involved in the study.

FUNDING

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FURTHER DISCLOSURE

Not applicable.

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