

# Motor asymmetries and handwriting in children with unilateral crossbite: a novel approach.

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# Motor asymmetries and handwriting in children with unilateral crossbite: a novel approach.

**Running title:** motricity and unilateral crossbite in children.

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## Abstract

**Background:** Handwriting represents a cultural invention on which our society is based. According to western literate community, children acquire it during the primary school years, and it is based on mechanisms inherent in our biology from both a cognitive and motor perspective. Previous findings enhanced how handwriting skills are influenced by different ergonomic factors such as body posture. In this context there are various studies that attempt to show the relationship between the Stomatognathic Apparatus and the postural system through the different muscle chains. Among the oral malocclusions, Posterior crossbite is a disgnathic jaw relationship common in patients undergoing growth linked with postural changes.

**Aim:** Aim of this study is to evaluate changes in body posture and motor functions including handwriting in children with unilateral posterior crossbites who are treated with palatal expanders.

**Design:** 10 children (aged 6-12 years old) with unilateral posterior crossbite were enrolled in the study. Fine motor skills tests, handgrip strength and 3D body posture analysis were performed before and after the treatment with a Rapid palatal expander (RPE) at T0pre (before using RPE), t0Post (immediately after using RPE), T1 (after 21 days of RPE use) and T3 (after 6 months).

**Results:** After 6 months of treatment with the RPE all the tests were performed and there was a significant correlation between the improvement of the unilateral posterior crossbite and pathological conditions related to body posture.

**Discussion:** From our results, we can affirm that the intervention did not homogeneously affect across participants the development of motor function, nor the pressure exerted during grapho-motor tasks.

**Keywords:** symmetry, coordination, pediatric dentistry, posture, malocclusion

### 1. Introduction

Handwriting is defined as one of the communication tools on which our society hinges<sup>1</sup>. Despite being defined as a cultural invention, it is based on mechanisms inscribed in our biology<sup>2</sup>. It presents patterns of functioning that originate from both the field of neuropsychology<sup>3</sup> and motor control<sup>4</sup>. From both a cognitive and a motor point of view, previous findings agree on the modular hierarchical organization of writing and on the dissociation between 'high-level' syntactic, semantic, and orthographic processes, which converge in an orthographic working memory, and 'low-level' processes, which converge in predefined motor patterns<sup>5</sup>. This general architecture has remained relatively unchanged over the past 30 years<sup>6</sup>. Motor programmes are codes that specify the number of basic motor units and their spatio-temporal relationships in an abstract method that is independent of the ultimate effector<sup>7</sup>. Neuropsychological models have developed a similar notion of a 'graphic motor scheme' or 'motor engram'. These engrams originate from the observation of patients with apraxia, a condition in which writing is impaired despite intact spelling ability and normal sensory and motor skills<sup>3</sup>. To date, Plamondon's<sup>8</sup> kinematic model represents the most predictable model for handwriting production. In this model, each stroke made during handwriting is given by a coordinated activity of the muscular system and would be defined by a velocity vector<sup>9</sup>. Only the orientation and amplitude of each velocity vector would be encoded by the central nervous system<sup>10</sup>. In skilled adults, the main network is composed of five regions which display functional specificity for writing<sup>11</sup>:

- the left inferior frontal gyrus active in orthographic recall.

- the left fusiform gyrus (active in accessing or storing in orthographic long-term memory, it calculates an abstract representation of the letters that could, therefore, be accessed both when the letters are read and written).
- the left superior parietal lobule and the left superior frontal gyrus mobilized in relation to the motor control of writing.
- the right cerebellum involved in fine motor skills

The handwriting network of adults and of typical 8- to 11-year-old children is composed of the same five key regions. There is a major role of the cerebellum, primary motor cortex, and prefrontal regions in the acquisition of writing skills<sup>12</sup>. In addition, they highlight a complex pattern of maturation in the FuG with writing acquisition, and a specific lateralization profile for the words writing task in children<sup>13</sup>. Previous findings have mainly focused on the development of different geometric and kinematic aspects of writing, such as the length of the trace or the execution speed of the movement<sup>14</sup>. Less attention has received the dynamic component This body of research has shown that handwriting is done automatically and ballistically by the third grade of primary school<sup>14</sup>. Therefore, writing is not just a succession of isolated acts, but rather an organized, hierarchical process in which the time and space of each motor unit (i.e., strokes, letters, words) are contextually interdependent within a wider unit<sup>15</sup>. Moreover, handwriting skills are related to many different variables including ergonomic factors such as body posture<sup>17</sup>. Postural stability moderates the rate at which infants learn successful grasping<sup>18</sup> and reaching is comparatively impaired in infants who have not yet developed the compensatory head and trunk movements required to counterbalance their arm movements during such behavior<sup>19</sup>. Among the factors that influence body posture, dental occlusion has been identified, as previous studies have shown that nerve stimulation from the periodontium and the temporomandibular joint converge towards the trigeminal nuclei from which afferents also control body posture stability<sup>20</sup>, in fact, the first paper focused on potential correlation between stomatognathic apparatus and body posture was investigated by Rocabado et al. in 1982<sup>21</sup>. By entering the keywords #writing #dentalocclusion and #finemotorskills in the Pubmed database, a single study was found regarding how the systems of the jaw and neck are functionally related can influence the fine motor skills involved in writing<sup>22</sup>.

In fact, this study revealed that all handwriting parameters varied between resting, opening and clenching positions of the jaw on both solid and unstable surfaces, demonstrating that a change in the jaw motor system can potentially influence fine motor skills<sup>23</sup>. The aim of this study is to assess the general links between fine motor skill-dental malocclusion-posture in developmental age, assuming fine motor abilities fundamental in writing process. In this context, the more specific aim is to study whether there is a direct correlation between the fine motor control/handwriting and the resolution of a dental malocclusion, such as unilateral cross bite in children.

## **2. Materials and Methods**

### **2.1 Study Population**

10 patients (7 males; mean age:  $8.5 \pm 1.0$  years old; all right-handed; none of them were affected by developmental coordination disorders nor neuromotor dysfunctions) participated in this study; they were selected from a population of 116 patients diagnosed with a unilateral posterior crossbite (UPXB) at the Unit of Orthodontics of the Department of Innovative Technologies in Medicine & Dentistry between January 2021 and December 2022. The presence of cross-bite accounts for the possibility to evaluate the side-to-side symmetry in motor functions due to the biomechanical alterations on the frontal plane.

The study was approved by the Ethics Committee of the 'G. D'Annunzio' University of Chieti-Pescara, and informed consent was signed by the parents of the children. The study conformed to the Declaration of Helsinki.

## 2.2 Patients' inclusion criteria

The inclusion criteria were as follows:

- 1) systemically healthy.
- 2) no history of medications that may affect periodontal status in the previous 6 months.
- 3) An UPXB diagnosed.

## 2.3 Study Design

Children with an UPXB were treated with the application of a RME (rapid maxillary expansion)

Functional tests were performed on TG patients at:

**T<sub>0pre</sub>**: before the application of the RME,

**t<sub>0post</sub>**: immediately after the first activation of the RME

**T<sub>1</sub>**: after 21 days which correspond to the mean activation period of the RME

**T<sub>2pre</sub>**: after the 6 months retention period.

**T<sub>2post</sub>**: immediately after the removal of the RME

## 2.4 Orthodontic evaluations

The value obtained, in mm, from the distance between the midline of the face and the line joining the glabella and the center of the chin was analyzed to assess the resolution of the mandibular shift due to the presence of unilateral crossbite.

## 2.5 Fine motor skills evaluations

The writing tests are totally non-invasive and were performed in the Functional Evaluation Lab of the University «G.d'Annunzio» Chieti-Pescara. Considering that imposed spatio-temporal constraints affect young children's handwriting performances<sup>24</sup>, two different tests were used to assess the dynamics of handwriting of the dominant hand:

**Alphabet Writing Task-cursive mode**: children were instructed to write the alphabet in lower case cursive letters from memory in order as quickly as they could as shown in **figure 1**, but so that others could identify the letters out of word context; the score was the number of correct letters in 15 seconds (legible and in correct order) in writing the alphabet from memory, already used in children and adolescents<sup>25</sup>

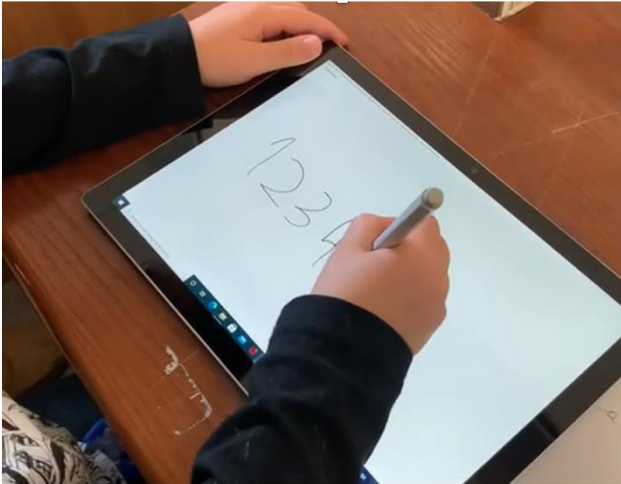
**M-task**: children were instructed to continue as quickly as possible a zigzag sequence without pen lifts to the end of the lines shown on the screen<sup>26</sup> as shown in **figure 2**.

Both tasks were conducted with a digital pen on a tablet pc screen, and pressure, speed, and number of strokes<sup>27,28</sup> will be registered through a home-made software (ScriptAN) implemented into the tablet pc (Microsoft Surface 6 pro). The analysis was focused on pressure.

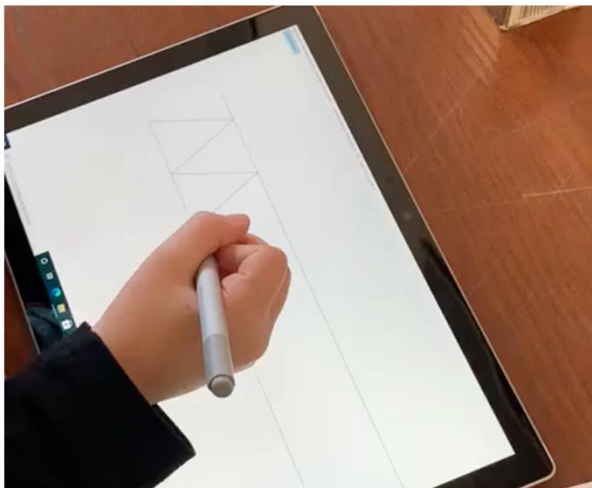
Two other tests were used to assess strength and fine motor skills for both hands:

**Handgrip strength test**: children remained seated and performed their maximal grip strength keeping the arm leaning to the chair's armrest<sup>28</sup>; one familiarization trial and two effective trials for each hand (best ones were registered) as shown in **figure 3**.

**Floppy test**: a transitive (tool-related) test measuring dexterity, which required participants to insert fourteen floppy disks one at a time in the proper case with one hand, as fast as possible, while the other hand controlled the case<sup>29</sup>; one familiarization trial and two effective trials for each hand (best ones were registered) as shown in **figure 4**.



**Figure 1: Alphabet Writing Task-cursive mode**



**Figure 2: M-task**



**Figure 3: Handgrip Test**



**Figure 4: Floppy Test**

## 2.6 Postural Analyses

4 of the 10 patients enrolled in this study performed postural examinations using the GOALS-E.G.G. (Bioengineering & Biomedicine Company Srl-Pescara, Italy) methodology designed by D'Amico et al<sup>30</sup>, which integrates an optoelectronic stereophotogrammetric system, a baropodometric treadmill and a telemetric surface electromyographic device (SEMG) Patients with a UPXB were subjected to a multifactorial analysis of posture and movement, integrating full 3D synchronized skeletal kinematics (including the spine), baropodometric assessments and electromyographic data. To describe trunk and global unbalancing, spinal offset, and global offset (i.e., displacements of each spine markers with respect to the vertical line passing through the S3 vertebra and with respect to the vertical line passing through the middle point between the heels, respectively) are used. Both global and spinal offset values are finally averaged to obtain descriptive data that summarize these parameters. The ASIS and PSIS positions provide the basis for the assessment of hip joint center positions and of pelvis width.

## 2.7 Statistical Analysis

Data from the software ScriptAN were exported into .xls files, and the median of pen's pressure was computed from a minimum of 0 to a maximum of 4096 customized units of pressure exerted by the digital pen on the tablet screen. The software ScriptAN was specifically developed by Dr. Anacleto Navangione and installed into a Microsoft Surface Pro 6 equipped with the digital pen. Statistical comparisons were performed using Jamovi Version 2.3.19.0 software (<https://www.jamovi.org>), and graphs were created with GraphPad Prism Version 9.3.1 (GraphPad Software, LLC).

To compare the results of Handgrip Strength test and *Floppy* test, both for absolute values and for symmetry, two series of general linear mixed model was used, after checking the assumptions of normality of residuals and Q-Q plots of residuals; participants were set as random variable, REML (residual maximum likelihood) method, Satterthwaite method for degrees of freedom, Bonferroni correction for multiple comparisons were used.

The same procedure was conducted for comparing the results of pen's pressure on the tablet screen, both for the Alphabet Writing Task-cursive mode and the M-task. Firstly, only the tests wearing the device were compared, setting time as fixed effect; then, the test at t1 was removed and the use of device was used as an additional fixed effect.  $R^2$  marginal and conditional were computed, along with p values. To compare the results of mandibular offset, a paired sample t-test was conducted, after checking the assumptions of normality of residuals; repeated measures Edges' g was computed as effect size (<https://effect-size-calculator.herokuapp.com>).



The 4 participants who completed the biomechanical analysis were included into the graphical reports of radar graphs, realized with Microsoft Excel Version 16.66.1. The following variables were included: 1) handgrip strength, 2) *Floppy* test time, 3) pressure on M-task, 4) pressure of W-task, 5) mandibular offset, 6) side-to-side percent changes on handgrip strength test, 7) side-to-side percent changes on *Floppy* test, and 8) global offset and 9) spinal offset from posturometric analysis. Variables 1-to-7 were computed as percent changes from t0 to t2, in both time points averaging the two results obtained with and without the mouth device. Specifically, variables were graphed after re-scaling results to the normalized percentiles computed from the minimum to maximum of each variable.

### 3. Results

The intervention was effective in reducing the mandibular offset on the frontal plane ( $p < 0.001$ , Hedges'  $g = 2.201$ ) as seen in **Table 1**. In particular, the reduction was observed in all the 7 participants who completed the time course, with a mean difference of 0.529 mm.

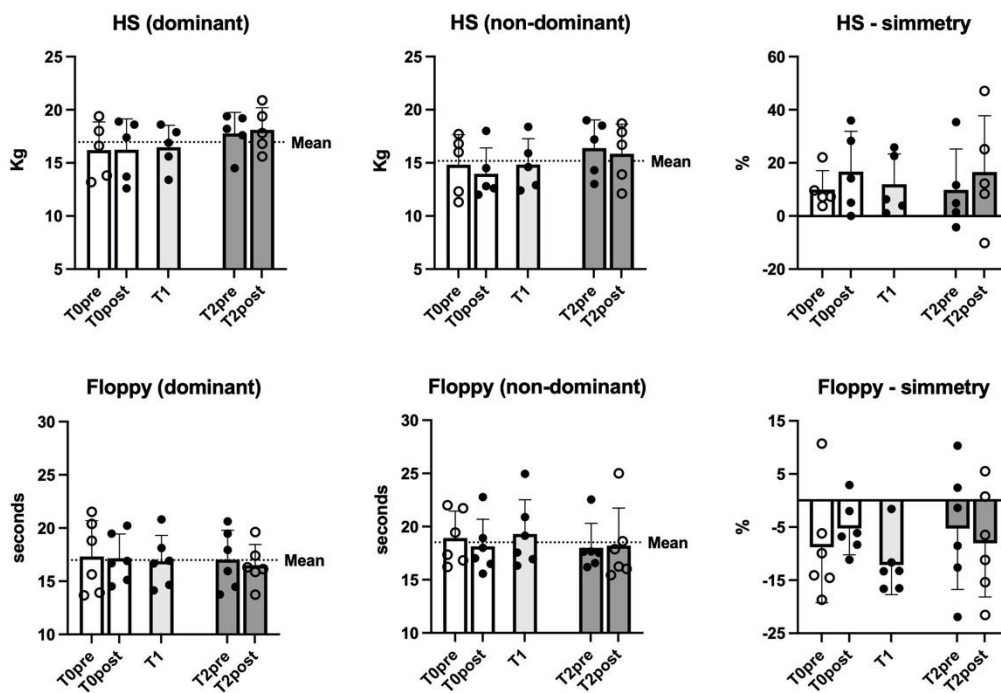
<i>Distance between the midline of the face (M) and the line joining the glabella and the center of the chin (GC)</i>		
d(M-GC) at Pre t0	d(M-GC) at t2	$\Delta$ d(M-GC)
0.5	0.1	0.4
0.3	0	0.3
0.7	0.3	0.4
1	0.4	0.6
1.2	0.6	0.6
0.9	0.2	0.7
0.8	0.4	0.1
0.6	0.1	0.5
0.5	0.3	0.2
0.7	0.1	0.6

**Table 1: Reduction of the Mandibular Shift from pre t0 to t2(in mm)**

For what concerns motor tasks, as expected in developmental age, handgrip strength increased over time, in both dominant and non-dominant hand (respectively:  $p = 0.007$ ,  $R^2$  marginal = 0.028,  $R^2$  conditional = 0.960;  $p = 0.002$ ,  $R^2$  marginal = 0.049,  $R^2$  conditional = 0.948), with robust increment at t2 compared to t0 (dominant hand:  $p = 0.015$ , non-dominant hand:  $p = 0.002$ ) and t1 (dominant hand:  $p = 0.011$ , non-dominant hand:  $p = 0.038$ ); these difference were not influenced by the immediate wearing or removal of the device (with/without device effect,  $p = 0.976$  and  $p = 0.689$  in the dominant and non-dominant hand, respectively). Instead, the performance on the *Floppy* test did not increase over time on either side, although a slight tendency emerged for the non-dominant hand ( $p = 0.166$ ,  $R^2$  marginal = 0.039,  $R^2$  conditional = 0.792; dominant hand:  $p = 0.896$ ,  $R^2$  marginal = 0.002,  $R^2$  conditional = 0.763); as before, the immediate wearing or removal of the device did not influence the performances (with/without device effect,  $p = 0.959$  and  $p = 0.453$  in the dominant and non-dominant hand, respectively) as shown in **Table 2**.

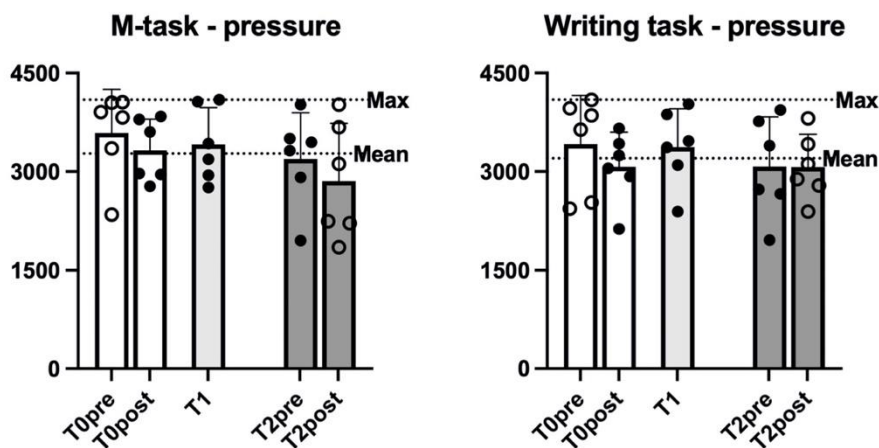
For what concerns the symmetry, the dominant hand obtained better performances in both tests, with higher values in the handgrip strength test (median percentage difference: +14.36%) and a lower time of completing in the *Floppy* test (mean percentage difference: -8.25%). These values remained quite stable over time, since no robust changes were observed on either handgrip strength ( $p = 0.430$ ,  $R^2$

marginal = 0.039,  $R^2$  conditional = 0.507) or *Floppy* test performances ( $p=0.213$ ,  $R^2$  marginal = 0.126,  $R^2$  conditional = 0.158); in both cases, the immediate wearing or removal of the device did not influence the performances (with/without device effect,  $p=0.959$  and  $p=0.453$  in the dominant and non-dominant hand, respectively as shown in **Table 2**)



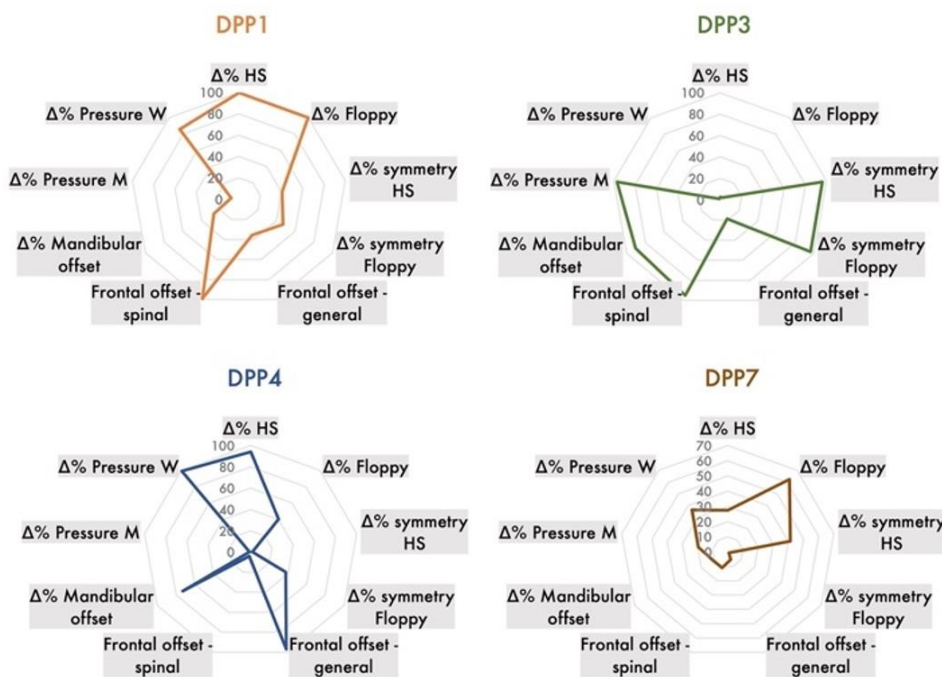
**Table 2:** Handgrip strength increased over time, in both dominant and non-dominant hand; with robust increment at t2 compared to t0 and t1. Instead, the performance on the *Floppy* test did not increase over time on either side, although a slight tendency emerged for the non-dominant hand.

For what concerns handwriting tasks, pen's pressure on the tablet screen during the tasks did not increase over time (M-task:  $p=0.721$ ,  $R^2$  marginal = 0.015,  $R^2$  conditional = 0.479; W-task:  $p=0.386$ ,  $R^2$  marginal = 0.021,  $R^2$  conditional = 0.785); the immediate wearing or removal of the device did not influence the performances (with/without device effect,  $p=0.866$  and  $p=0.591$  in the M-task and W-task, respectively). The pressure exerted during the tasks was similar during the two tasks as shown in **Table 3**.



**Table 3:** Pen's pressure on the tablet screen during the tasks did not increase over time and the immediate wearing or removal of the device did not influence the performances. The pressure exerted during the tasks was similar during the two tasks.

The radar graphs show that the different domains were not related homogeneously across participants (see **table 4**). One can observe that both the number of variations and the relationships across the domains greatly vary across the four patients.



**Table 4:** The following variables were included: 1) handgrip strength, 2) *Floppy* test time, 3) pressure on M-task, 4) pressure of W-task, 5) mandibular offset, 6) side-to-side percent changes on handgrip strength test, 7) side-to-side percent changes on *Floppy* test, and 8) global offset and 9) spinal offset from posturometric analysis. Variables 1-to-7 were computed as percent changes from t0 to t2, in both time points averaging the two results obtained with and without the RME.

In addition, regarding the plantar pressures, baropodometric analysis allows us to find out the distribution of the loads or pressures in various plantar zones and thereby evaluate the direct influences of the forces applied, its intensity and duration as shown in **table 5**.

Global Offset Frontal	Spinal Offsets Frontal	Differences L-R PSIS	Differences L-R HIP	mean support of the right foot in statics	mean support of the left foot in statics	mean support of the right foot in motion	mean support of the left foot in motion
7.3	- 11.5	00.8 ± 0.5	-00.2 ± 1.1	6.7	- 12.8	-3.8	-0.2
6.8	-2.2	01.6 ± 0.5	02.2 ± 1.4	1.4	-0.3	2.0	- 13.8
-8.9	- 11.9	08.3 ± 0.1	13.6 ± 0.3	3.3	0.2	13	-5
6.0	-3.1	01.8 ± 0.3	04.7 ± 0.5	4	3.6	8.1	-2.5

**Table 5:** Gait analyses

## 4. Discussion

The improvement on both motor performances over time was hypothesized<sup>31</sup>; such improvement emerged clearly for handgrip strength, but not for *Floppy* test. The low sample size and the huge age range of our study may have affected the results, possibly limiting the evidence of individual and gender-based development trajectories. In addition, the difference in the two tasks should be evoked: handgrip strength test is determined by a simple motor task, while the *Floppy* test, as a tool-related action test determined by manual dexterity and characterized by progressive difficulty, represents a more complex task. The greater the difficulty, the more the developmental trajectory is affected by dynamic interaction between person, task, and environment, along with the fact that improvement of fine motor skills is specific to practice of fine motor skills<sup>32</sup>.

More importantly, we studied the changes, if any, in the symmetry due to the intervention. As expected, the dominant side obtained better performances both in handgrip strength and in the *Floppy* test, confirming a pronounced functional lateralization in complex coordinative tasks and in maximal strength during developmental age<sup>33</sup>. Despite the intervention was effective in reducing the mandibular offset, this was not translated in altered functional symmetry on motor tasks. This confirms that motor imbalances are joint and task-specific, with compensatory mechanisms continuously counteracting such imbalances and individuality that define the responders' type concerning different motor tasks<sup>34</sup>.

The latter point has been crucial in our study, since we observed a very large heterogeneity, concerning the motor domain, in the response to the intervention. The radar graphs finely tuned this point, showing that the different domains, at least in this pilot sample, were not related, leading to individual trajectories of motor performances and individual responses to morpho-functional interventions. The neuromuscular and biomechanical effects due to temporo-mandibular system changes can be transmitted to the whole body via neural connections as well as active and passive tissues<sup>35</sup>, depicting a huge complexity of acute and chronic adaptations. The fact that the intervention did not affect the handwriting pressure on both tasks can rely on the complexity of neuro-motor drives during grapho-motor tasks, on the task-specific coordinative development<sup>36</sup>, on the exploitation of natural biomechanical tendencies of children while exerting grapho-motor tasks, and on the huge age range, since stronger perception–action coupling emerges as age increases<sup>37</sup>. The natural biomechanical tendency indeed emerges if considering that the pressure exerted was similar during the two grapho-motor tasks, despite the difference in the content and context of the performances, i.e., the W-task is constrained by the time while the M-task only requires to focus on the accurateness. To the best of our knowledge, only one previous study investigated the correlation between fine motor skills and occlusion in human subjects. The authors demonstrated how all handwriting parameters varied among the three jaw positions on both the firm and unstable surfaces, showing that changes in the jaw motor system may potentially affect the fine motor skills. However, there were no significant differences in the handwriting parameters among the resting, open, and clenched jaw positions between the firm and unstable surfaces. One of the limitations of the study was that the authors did not investigate any pathological conditions related to the patient's temporomandibular joint and occlusion, a key factor in assessing how the head/neck system may affect the fine motor skills involved in writing.

Our data showed that the dominant side performed better on both the handgrip test and the floppy test, resulting in a symmetry of the sides not altered in both forces exerted, and dexterity compared to the side where the crossbite was diagnosed with a direct correlation of the reduced mandibular deviation associated with this condition. This result is very important because it shows that the motor system of the mandible could influence fine motor activity, as shown by the correlation between the reduction of the functional shift of the mandible on the opposite side of the crossbite and the improvement of handgrip strength. Normal jaw function depends on a harmonic relationship between the different components of the masticatory system. This harmonic relationship may be interrupted in children with malocclusion, which may affect normal jaw development and function<sup>38</sup>. In fact, it

has been previously reported how dental malocclusion can affect the orofacial aesthetic perception<sup>39</sup>, oral functions<sup>40</sup> and psychological well-being<sup>41</sup> of affected individuals, thus affecting their oral health-related quality of life, therefore it is important to identify whether dental malocclusion can affect skills such as writing, which involves both high-level and low-level processes. From our results, we can affirm that the intervention did not homogeneously affect across participants the development of motor function, nor the pressure exerted during grapho-motor tasks. Therefore, the improvement obtained in the occlusion due to the RME, at least from these pilot results, did not clearly alter motor functions nor motor symmetry in those children who were not affected by coordination dysfunction. To date, handwriting is a one of the most important skills that children acquire during their primary-school years. In children, the sensorimotor systems must adapt to substantial morphological changes during growth and development. The orofacial area is particularly challenged by the growth and development of the jaws and the transition from primary to permanent dentition<sup>42</sup>. Studies on masticatory movements suggest that children have a characteristic chewing pattern that differs from that of adults and that certain movement parameters (jaw opening and closing velocities) change with age<sup>43</sup>. A series of well-controlled studies in children with normal occlusion have shown that chewing and jaw motor skills develop gradually with age<sup>44</sup>. The current study investigates whether orthodontic treatment contributes to the restoration of this neuromotor development while affecting motor functions and any muscular asymmetries leading to problems with writing. Poor posture and altered dental occlusion have been associated with increased muscle tension in the head and neck, leading to changes in the position of the shoulders, arms, and hands during handwriting. Altered dental occlusion has also been found to influence the strength and coordination of the muscles involved in handwriting, leading to changes in the quality and speed of writing.

The first limitation in this study concerns the low sample size and the heterogeneity of the patients enrolled in the study; in particular, it might be interesting to carry out postural assessments a priori both in a seated and standing position to make further integrative assessments. Moreover, the lack of case-controls affects the real predictability of changes in the parameters referring to writing before and after orthodontic treatment.

## **5. Bullet Points. Why this paper is important to pediatric dentists.**

- Our data showed how the use of a tablet computer in figure tracing could be pivotal in the daily clinical practice of the dentist to be able to intercept any problems related to children's fine motor skills, which is closely related to body posture. In fact, postural analyses showed a non-homogeneity of the plantar pressure on the side of the crossbite highlighting this direct correlation of alteration of the forces exerted due to the malocclusion.
- In the clinical practice of dentists, the use of these fine motor assessments can significantly serve as "sentinels" to detect, if any, early writing deficits or motor dysfunctions that can affect the quality of life of children, their cognitive abilities, and their developmental trajectories. It would be important to evaluate if orthodontic treatments would result in long-term adaptations in fine motor skills such as handwriting.
- It would be interesting to investigate whether scoliotic disorders and/or orthodontic diseases in children can affect the strength and coordination of the muscles involved in handwriting, leading to changes in the quality, kinematics, and dynamics of handwriting itself. Despite the extensive research on the links between handwriting, human posture and dental occlusion, there is still much to be understood about the mechanisms underlying these relationships.

## **Authorship**

Conceptualization, IR, DB, MDAt and TP; methodology, DB, EK, MDAm, MDAt and TP; software AN; formal analysis IR, DB, EK, MDAm, GDA; investigation IR, DB, EK, MDAm, CL, BDC, GF, MDAt; resources MDAm, SF, MDAt and TP; writing—original draft preparation IR, DB and TP.; writing—review and editing EK, MDAm, CL, BDC, GF, BS, AN, VV, SF, MDAt; visualization, IR, DB; supervision, MDAm, MDAt, GM and TP; funding acquisition, MDAm, MDAt, GM Aand TP. All authors have read and agreed to the published version of the manuscript.”

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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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## **Conflicts of interest disclosure**

The authors declare that they have no conflict of interest.

## **Ethics approval statement**

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of G d’Annunzio University Chieti-Pescara (protocol n4-04052017)

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