

# An electromyographic investigation of masticatory muscles symmetry in normo-occlusion subjects

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**SUMMARY** The influence of occlusal conditions on stomatognathic function can be assessed by electromyography. Electromyographic activity of left and right temporal and masseter muscles was recorded in 30 young healthy adults with a normal occlusion during: (1) a 3-s maximum voluntary clench on cotton rolls positioned on the posterior teeth (standardization recording); (2) a 3-s maximum voluntary clench in intercuspal position; and (3) a 3-s alternate 'maximum' voluntary contraction and relaxation with a 1 Hz frequency. All potentials were standardized as a percentage of the maximum potential of test 1. Waveforms of paired muscles were compared by computing a percentage overlapping coefficient (ratio between each 50-ms overlapped areas and the total areas, up to 100% for

symmetric muscles). Waveforms were also analysed for a laterodeviating effect on the mandible given by unbalanced muscular couples, and a torque coefficient (up to 100% for a significant laterodeviating couple on the mandible) was computed. In all subjects, both tests were performed with symmetric muscular patterns (more than 88%) and with negligible laterodeviating couples on the mandible (lower than 10%). The two coefficients allow an assessment of muscular asymmetry during static and dynamic clenching tests, and, together with the standardization of the potentials, could be a useful tool to detect functionally altered occlusal conditions, i.e. conditions where an apparent good morphological situation is not related to a correct neuromuscular status.

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## Introduction

Asymmetry is a common finding in man. Apart from unpaired and asymmetric organs, such the liver or the heart, both morphology and function of paired structures differ in the left and right sides of the body. Morphological evaluations of craniofacial asymmetry have become a usual part of the characterization of both normal subjects and patients (Peck, Peck & Kataia, 1991; Schmid, Mongini & Felisio, 1991; Ferrario *et al.*, 1993b, 1994; Mattila, Könönen & Mattila, 1995; O'Bryn *et al.*, 1995). The evaluation of functional symmetry of the craniofacial complex usually involves the patterns of jaw movements and the activities of masticatory muscles (Humsi *et al.*, 1989; Naeije, McCarroll & Weijs, 1989; McCarroll *et al.*, 1989a,b; Fer-

rario *et al.*, 1992, 1993a, 1996; Abekura *et al.*, 1995a,b; Ferrario, Sforza & Serrao, 2000).

The patterns of contraction of paired muscles can be investigated using surface electromyography (EMG), which allows the monitoring of some of the main masticatory muscles (masseter, temporalis, suprahyoid muscles), with results that do not significantly differ from those obtained with intra-muscular recordings (Belser & Hannam, 1986) and that has been found to be well reproducible when performed with well-standardized protocols (Ferrario & Sforza, 1996; Karkazis & Kossioni, 1997). Obviously, surface EMG is more vulnerable to extra-muscular factors that may alter and distort the true electric signal. Therefore, to reduce the 'biological noise', and to allow useful comparisons between different subjects and different studies, the EMG

potentials should be standardized (normalized). In most studies, the potentials are expressed as a percentage of a maximal voluntary contraction (Belser & Hannam, 1986; Blanksma & van Eijden, 1995; van der Bilt *et al.*, 1995; Ferrario & Sforza, 1996; Ferrario *et al.*, 2000).

The EMG symmetry of contraction of homologous muscles of both the left and right sides of the body is usually assessed by calculating the mean voltages over a selected time span and using these mean values in indices such as the asymmetry index by Naeije *et al.* (1989). Unfortunately, not only does this index estimate the muscular pattern of a more or less long span by a single value, where more detailed evaluations that take the whole waveform into account should be provided (Basmajian & De Luca, 1985), but, also, it is usually highly variable both between and within individuals (Ferrario *et al.*, 1993a), making cross-sectional and longitudinal evaluations difficult.

In clinics, EMG may be used to assess the influence of occlusal conditions on stomatognathic function. For instance, occlusal stability has been found to be related to muscular performance, i.e. subjects with a higher occlusal stability showing shorter contraction times and larger EMG potentials during chewing than subjects with a lower occlusal stability (Bakke, Michler & Moller, 1992). Unfortunately, biological noise may partly mask the effect of occlusal alterations, and suitable EMG protocols should be developed.

In the current investigation, the EMG activity of left and right masseter and temporalis anterior muscles during the performance of standardized tests has been studied in a group of young healthy individuals with a normal occlusion. A new method for the within-subject standardization of EMG potentials has been developed, and muscular asymmetry was expressed by a new index that takes account of the whole morphology of the EMG wave as a function of time.

## Materials and methods

### Sample

Fifteen men and 15 women aged 18–19 years (mean 18.4, s.d. 0.2) participated to the experiment. All subjects had a sound full permanent dentition including the second molars (at least 28 teeth), with bilateral angle class I first permanent molar and canine rela-

tionship ( $\pm 1$  mm), overjet and overbite ranging from 2 to 5 mm, absence of anterior or lateral crossbite, no cast restorations or cuspal coverage, no previous craniofacial trauma or surgery and no temporomandibular or craniocervical disorders. All subjects gave their informed consent to the experiment.

### Instrumentation

The masseter (M) and temporalis anterior (T) muscles of both sides (left and right) were examined. Bipolar surface electrodes were positioned on the muscular bellies parallel to muscular fibers as previously described (Ferrario & Sforza, 1996; Ferrario *et al.*, 2000): temporalis anterior, vertically along the anterior margin of the muscle (about on the coronal suture); masseter, parallel to the muscular fibers, with the upper pole of the electrode at the intersection between the tragus-labial commissura and the exocanthion-gonion lines. To reduce skin impedance, the skin was carefully cleaned prior to electrodes placement, and recordings were performed 5–6 min later, allowing the conductive paste to adequately moisten the skin surface.

During testing, disposable silver/silver chloride bipolar electrodes\* with a diameter of 10 mm and an inter-electrode distance of  $21 \pm 1$  mm were used, while a disposable reference electrode was applied to the right ear.

EMG activity was recorded using four of the eight channels of an instrument†. The analogic EMG signal was amplified, digitized and digitally filtered. The instrument was directly interfaced with a computer that presented the data graphically and recorded them on magnetic media for further quantitative and qualitative analyses. The signals were averaged over 50 ms, with muscle activity of the four tested muscles [right masseter (MR), left masseter (ML), right temporalis anterior (TR), left temporalis anterior (TL)] assessed as the root mean square (r.m.s.) of the amplitude (unit:  $\mu$ V). EMG signals were then recorded for further analysis. Details about the EMG apparatus and raw data analysis can be found in Ferrario & Sforza (1996).

\* Duo-Trode; Myo-Tronics Inc., Seattle, WA, U.S.A.

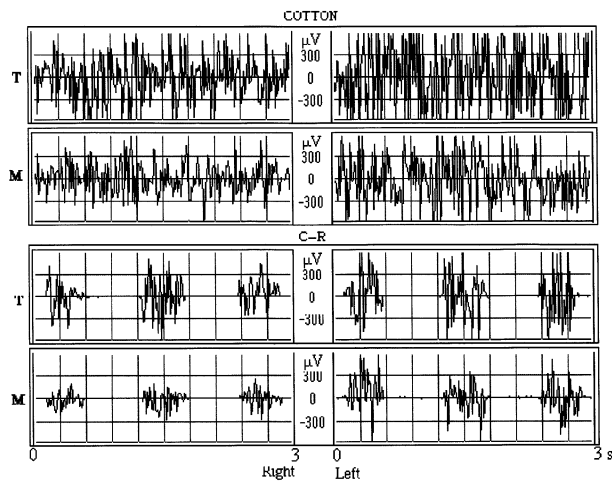
† De Götzen srl; Legnano, Milano, Italy

### Measurement protocol and EMG data analysis

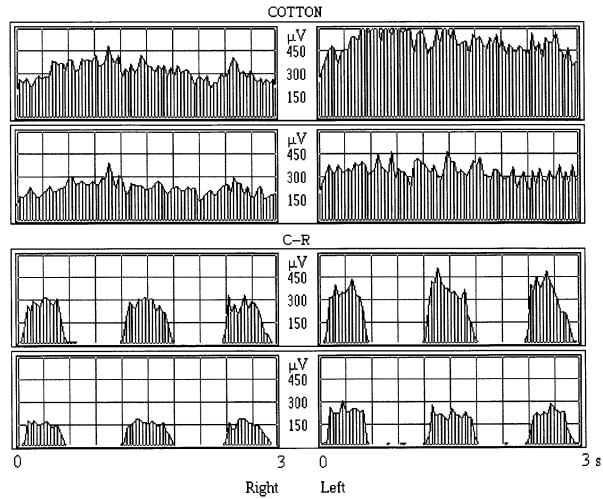
A first recording for the standardization of EMG potentials was made as detailed by Ferrario *et al.* (2000). In brief, two 10-mm thick cotton rolls were positioned on the mandibular second pre-molar and molars of each subject and a 3-s maximum voluntary clench (MVC) was recorded (Fig. 1, upper panel). For each muscle, the maximum EMG potential was set at 100%, and all further EMG potentials were expressed as a percentage of this value (unit:  $\mu\text{V}/\mu\text{V} \times 100$ ). The relative percentage EMG values found in the experiment should thus be affected only by the occlusal surfaces because this kind of standardization should annul the variability due to skin and electrode impedance, electrode positioning and relative muscular hypo- or hypertrophy.

EMG activity was then recorded during two different tests, each lasting 3 s:

1. MVC in intercuspal position (static isometric test); the subject was invited to clench (without cotton rolls) as hard as possible and to maintain the same level of contraction for all the test.
2. Alternate 'maximum' voluntary contractions and



**Fig. 1.** Electromyographic potentials recorded over the left and right masseter and temporalis anterior muscles during the 3-s MVC on cotton rolls (upper panel) and the 3-s clench-relax test (lower panel) in a girl aged 18 years with good occlusal conditions. *X* axis: time, 3 s. Average r.m.s. potentials: MVC, right temporal 319  $\mu\text{V}$ , left temporal 512  $\mu\text{V}$ , right masseter 224  $\mu\text{V}$ , left masseter 333  $\mu\text{V}$ ; clench-relax, right temporal 173  $\mu\text{V}$ , left temporal 236  $\mu\text{V}$ , right masseter 98  $\mu\text{V}$ , left masseter 147  $\mu\text{V}$ . In the MVC test, some potentials appear graphically clipped, but they have all been considered in the digital calculations.



**Fig. 2.** Electromyographic waves computed with the raw data shown in Fig. 1. MVC on cotton rolls (upper panel) and clench-relax test (lower panel). *X* axis: time, 3 s.

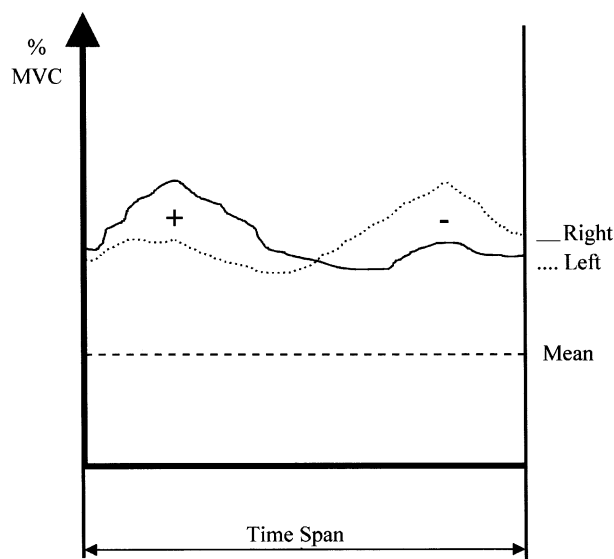
relaxations with a 1 Hz frequency (dynamic clench-relax test); the subject looked at the computer monitor where a pointer gave him/her the frequency and was asked to clench as hard as possible maintaining the given frequency (Fig. 1, lower panel).

For both tests, the subjects sat with their heads unsupported and were asked to maintain a natural erect position (Bakke *et al.*, 1992; Balkhi *et al.*, 1993). To avoid any effect of fatigue, a rest period of at least 3 min was allowed between the standardization recording and each test.

For each subject, muscle and test, 60 data points (r.m.s. EMG potentials) were thus available, and used to plot the relevant EMG waves (Fig. 2). The EMG potentials were then expressed as per cent of the MVC on the cotton rolls and the EMG waves of paired muscles of each subject were compared by computing a percentage overlapping coefficient (*POC*, unit: %). The two EMG waves were superimposed and the ratio between the superimposed areas and the total areas was computed as:

$$POC = \left[ 1 - \frac{\sum_{i=1}^{60} (\text{right muscle}_i - \text{left muscle}_i)}{\sum_{i=1}^{60} (\text{right muscle}_i + \text{left muscle}_i)} \right] \cdot 100$$

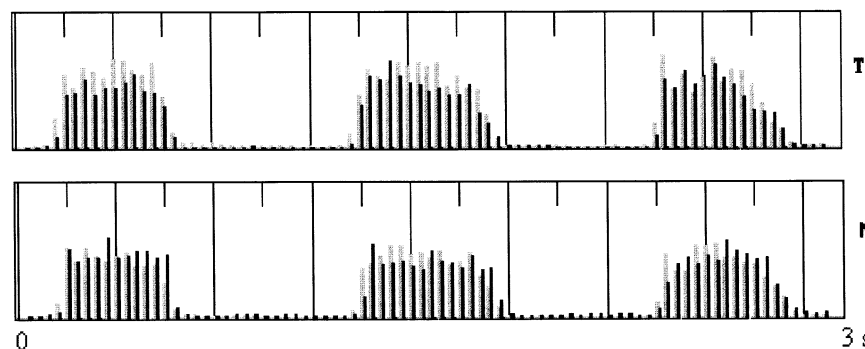
where *i* are the r.m.s. potentials averaged over each 50 ms.



**Fig. 3.** Example of calculation of the percentage overlapping coefficient (*POC*) for a couple of left (dotted line) and right (continuous line) masticatory muscles. For the calculation of *POC*, the sum of the non-overlapped areas ('+' and '-') is divided by the total areas under the two curves:  $POC = 91.75\%$ . The percentage contribution to the asymmetric part of *POC* (8.25%) is 50.21% for the right side muscle and 49.79% for the left side muscle. The classic asymmetry index (*As*) by Naeije *et al.* (1989) would be nearly 0 (the mean values of the left and right side muscle potentials are superimposed). *X* axis: time.

If the two muscles contract with perfect symmetry, a *POC* up to 100% is to be expected. For the 'asymmetric' part of the index ( $100 - POC$ ), the percentage contribution of the left and right side muscles is computed. A very prevalent muscle coupled with a very weak muscle will have a percentage contribution equal to or very next to 100%, a couple of muscles with oscillating contractions will have percentage contributions around 50% each (Figs 3 and 4).

#### Percent of MVC on cotton rolls



**Fig. 4.** Percentage overlapping coefficients (*POC*) for the masseter (lower panel,  $POC = 87.63\%$ ) and temporalis anterior (upper panel,  $POC = 88.29\%$ ) muscles computed during the clench-relax test, potentials standardized as per cent of MVC on cotton rolls. r.m.s. data shown in Figs 1 and 2. *X* axis: time, 3 s; *Y* axis: per cent of MVC on cotton rolls, 100%. Black bars: right side muscle; grey bars: left side muscle.

Calculations were performed for each couple of muscles (masseter and temporalis anterior) and for each subject, thus obtaining for each test a temporal and a masseter *POC* and relevant percentage left/right contribution to the asymmetry.

The EMG waves of the left and right masseter and temporalis anterior muscles of each subject within test were further analysed to assess the presence of a possible laterodeviating effect on the mandible during the test given by unbalanced TR and ML and TL and MR couples. As detailed by Ferrario *et al.* (1993a), considering the direction of the resultant forces of the masseter and temporal muscles, a couple with a laterodeviating effect on the mandible may be produced. Indeed, while the resultant of the temporal muscle of one side is directed upward and backward, the resultant of the contralateral masseter is directed upward and onward. A force couple that may deviate the mandible on the temporal side could thus be produced, and its presence was assessed by calculating a torque coefficient (*TC*, unit%):

$$TC = \sum_{i=1}^{60} \left| \delta T_i - \delta M_i \right| / \sum_{i=1}^{60} (MR_i + ML_i + TR_i + TL_i) \cdot 100$$

where *i* are the r.m.s. potentials averaged over each 50 ms and  $\delta M_i = MR_i - ML_i$ ,  $\delta T_i = TR_i - TL_i$  are the non-overlapped left and right muscular areas of the masseter (M) and temporal (T) muscles. *TC* ranges between 0% (no torque during the test) and 100% (a significant laterodeviating couple on the mandible). *TC* is obviously null when the test is performed with perfectly symmetric muscular contractions ( $\delta T = 0$ ,  $\delta M = 0$  and  $POC = 100\%$ ). If the difference ( $\delta T - \delta M$ ) is negative (i.e. lower than 0), the resultant couple will have a left side laterodeviating effect. If it is positive (i.e. larger than 0), the laterodeviating effect will be

Parameter	Unit	MVC			Clench-relax		
		Mean	s.d.	CV	Mean	s.d.	CV
TR and TL	$\mu\text{V}/\mu\text{V}\%$	91.10	13.49	14.80	41.05	9.76	23.78
MR and ML	$\mu\text{V}/\mu\text{V}\%$	85.45	26.57	31.08	38.63	14.61	37.82
$POC_T$	%	89.34	3.90	4.36	88.20	3.42	3.87
$POC_M$	%	88.06	4.55	5.16	88.28	3.12	3.53
$TC$	%	6.36	1.18	18.50	8.30	2.27	27.38

TR and TL, MR and ML: average between paired muscles;  $POC_T$ ,  $POC_M$ : percentage overlapping coefficients for the temporalis anterior and masseter muscles;  $TC$ : torque coefficient.

right sided. The percentage contribution of the left (negative differences) and right (positive differences) side laterodeviating couples is consequently computed.

#### Reproducibility of EMG measurements

Reproducibility of surface EMG measurements of the same muscles has already been tested in our laboratory and found to be good (Ferrario *et al.*, 1991).

Consistency of EMG patterns during the two tests performed according to the present protocol was tested in three subjects randomly chosen from the whole sample (one male, two females). The subjects repeated the tests within a 1-week interval, data were analysed as previously detailed and Dahlberg's error (Cooke & Wei, 1991) ( $\text{Error} = \sqrt{[\sum (\text{first measurement} - \text{second measurement})^2/6]}$ ) was computed for each coefficient ( $POC$ ,  $TC$ ) and test. The errors were 1.949 ( $POC_T$ ), 1.851 ( $POC_M$ ) and 0.218 ( $TC$ ) for the clench-relax test, and 0.48 ( $POC_T$ ), 0.447 ( $POC_M$ ) and 0.64 ( $TC$ ) for the MVC test.

## Results

When standardized on the MVC on cotton rolls, male and female young adults had similar EMG patterns without differences in the average potentials (data not shown). All subsequent calculations were thus performed regardless of gender. The indices computed using the 60 r.m.s. EMG potentials of the four analysed muscles as per cent of maximum voluntary clench on cotton rolls (unit:  $\mu\text{V}/\mu\text{V} \times 100$ ) recorded in the two 3-s tests, as well as the average muscular potentials, are reported in Table 1.

In the clench-relax test, the average standardized

**Table 1.** Descriptive statistics of EMG mean potentials as per cent of MVC on cotton rolls, and EMG indices, in 30 healthy young adults

EMG potentials were about 40% of the MVC on cotton: not only the given frequency of 1 Hz hindered the subjects to reach a maximum value of contraction, but also the r.m.s. mean potentials computed over the entire test comprised both the 'clench' (muscular contraction) and the 'relax' (no contraction) parts of the test (Figs 1 and 2, lower panels). In both clench-relax and MVC tests, the average standardized r.m.s. potentials were larger in the temporalis anterior muscle than in the masseter muscle. Both tests were performed with symmetric muscular patterns ( $POCs$  larger than 88%). For the 'asymmetric' part of the contraction, there was a prevalent side (left or right side) for both masseter (MVC test: average percentage contribution 82.37%, s.d. 15.28; clench-relax test: 77.17%, s.d. 16.38) and temporalis anterior (MVC test: 82.43%, s.d. 16.49; clench-relax test: 79.11%, s.d. 15.03) muscles, but this side was not constant within the sample.

The torque coefficient  $TC$  was low, especially during the MVC test. Within individual, there was a prevalent left-side or right-side laterodeviating couple (MVC test: average percentage contribution 76.77%, s.d. 15.43; clench-relax test: 78.85%, s.d. 15.19), but both couples were similarly represented within the group.

Within-sample variability was limited for the  $POC$  (coefficients of variation up to 5%) and somewhat larger for the torque coefficient and for the EMG potentials (coefficients of variation between 15 and 38%).

## Discussion

The asymmetry index proposed by Naeije *et al.* (1989), or some mathematical modifications of its original for

mula, have been widely used for the evaluation of healthy subjects (Ferrario *et al.*, 1993a; Abekura *et al.*, 1995a), of patients with several craniomandibular disorders (Humsi *et al.*, 1989; Abekura *et al.*, 1995a) or after modifications of the occlusal surfaces (McCarroll *et al.*, 1989a,b; Abekura *et al.*, 1995b). These indices usefully compare the EMG activity of paired masticatory muscles during the performance of standardized activities (usually a clench), but they provide just a first, rough estimation of the phenomenon because they do not assess the entire waveforms but only single average values, as shown in the Appendix.

Indeed, the index can be satisfactory for the analysis of very brief tasks performed with EMG potentials that remain constant (or nearly constant) during the entire analysed time span. On the contrary, the index introduced in the present study (*POC*) could be used for other kinds of tests where the patterns of muscular contraction vary during the analysed time span (e.g. dynamic phenomena). *POC* analysed muscular asymmetry with a very frequent sampling (50 ms). The intra-sample variability, moreover, was limited, and it might be possible to define a threshold of normality for selected muscular tasks for subjects with a normal occlusion.

Similar considerations could be made when comparing the classic torque index (Ferrario *et al.*, 1993a) and the currently proposed *TC*, which is not limited to average values but takes the entire waveform into account (see the Appendix). *TC* was somewhat larger in the clench-relax test (a more dynamic activity, involving rapid modifications of the contracted muscles) than in the MVC test. A similar finding had been reported by Ferrario *et al.* (1993a) for their torque index.

Indeed, the clench-relax test was introduced to detect borderline occlusal instability, those transitory pre-contacts usually cancelled in a (relatively) long MVC, but that can be disclosed during the performance of tasks demanding rapid modifications of occlusion.

Within individuals, the asymmetric part of the contraction (100 – *POC*) was explained by a prevalent left or right side for both muscles, as well as the torque effect (*TC*) on the mandible was accounted for by a prevalent left-side or right-side laterodeviating couple. In both cases, the two opposite muscles or couples were similarly represented within the group. Even in the present highly selected subjects whose occlusion

was good from a morphologic point of view, the presence of a prevalent side or couple seems to be an intrinsic asymmetric characteristics of occlusion, independently from biological noise.

A consideration on the average muscular potentials reported in Table 1 should be made: in both the clench-relax and MVC tests, the standardized potentials were larger in the temporalis anterior muscle than in the masseter muscle. Indeed, it has to be noted that the potentials were standardized against a MVC performed with cotton rolls positioned on the posterior teeth: in this condition, the temporalis anterior usually contracts with a lower intensity (Ferrario *et al.*, 1993a), making the reference (denominator) value lower (and the resulting percentage potentials larger).

Within-subject repeatability was good and the EMG patterns recorded after a 1-week interval were well reproducible. This variability contains two sources of variation: biological differences and possible technical difficulties (for instance, the thickness and texture of the soft tissues located between the electrode and the muscle, the electrode positioning and impedance, the amplifier and filters of the EMG apparatus and possible electrical interferences) (Blanksma & van Eijden, 1995; Macaluso & de Laat, 1995; van der Glas *et al.*, 1996; Ferrario *et al.*, 2000). The normalization of EMG potentials used in the present investigation (MVC on cotton rolls performed just before the recording of the actual test, i.e. with the same electrodes, cables and EMG apparatus, and on the same cutaneous area) should cancel all biological and technical noise. Indeed, the height of the cotton roll might slightly modify the vertical dimension (and consequently the length of muscular fibers and the inter-electrode distance), but, when clenched, it becomes so thin to make the effect negligible. The resulting standardized EMG potentials should therefore be determined only by the muscular contraction as it correlates to the occlusal surfaces (for instance, in all subjects the MVC on cotton rolls was larger than the MVC on the free teeth, see the standardized EMG potentials in Table 1). As shown in Figs 1, 2 and 4, the muscular asymmetry recorded for both the MVC on cotton rolls (larger left side potentials in both muscles, Fig. 1) and the clench-relax test (left side muscles larger, Fig. 2) cancelled when standardized potentials were used to compute the *POC* (Fig. 4). Whatever the cause of the detected asymmetric muscular potentials (biological or technical), the occlusion

could be excluded. Indeed, the same standardization protocol has already been used for the analysis of chewing patterns in unilateral crossbite patients who were found to have an altered EMG coordination of masticatory muscles (Ferrario *et al.*, 2000).

### Clinical implications

(1) The proposed method for the standardization of the EMG potentials could allow the separation of the effects of an altered occlusion from that of other pathologies (for instance, temporomandibular joint problems, muscular diseases).

(2) The indices proposed in the present investigation could be a useful tool to detect functionally altered occlusal conditions, i.e. conditions where an apparent good morphological situation is not related to a correct neuromuscular status. Morphologically correct prosthetic reconstructions and orthodontic treatments could thus be evaluated also from a functional point of view.

### Appendix A

The asymmetry index ( $As$ ) by Naeije *et al.* (1989) and torque index ( $To$ ) by Ferrario *et al.* (1993a) can be computed with both the single  $N$  r.m.s potentials  $i$  computed in the analysed time span and with the average muscular potentials. In fact, for a couple of left (L) and right (R) masticatory muscles:

$$As = \frac{\sum_{i=1}^N (R_i - L_i) / \sum_{i=1}^N (R_i + L_i) \cdot 100}{\left( \frac{\sum_{i=1}^N R_i - \sum_{i=1}^N L_i}{\left( \sum_{i=1}^N R_i + \sum_{i=1}^N L_i \right) \cdot (N/N)} \right) \cdot 100}$$

$$As = (\text{mean R} - \text{mean L}) / (\text{mean R} + \text{mean L}) \cdot 100$$

and, for the left (L) and right (R) masseter (M) and temporalis anterior (T) muscles:

$$To = \frac{\sum_{i=1}^N [(TR + ML) - (TR + MR)]_i / \sum_{i=1}^N [(TR + ML) + (TR + MR)]_i \cdot 100}{\left( \frac{\sum_{i=1}^N TR_i + \sum_{i=1}^N ML_i - \sum_{i=1}^N TL_i - \sum_{i=1}^N MR_i}{\left( \sum_{i=1}^N TR_i + \sum_{i=1}^N ML_i + \sum_{i=1}^N TL_i + \sum_{i=1}^N MR_i \right) \cdot (N/N)} \right) \cdot 100}$$

$$To = \frac{\sum_{i=1}^N TR_i + \sum_{i=1}^N ML_i - \sum_{i=1}^N TL_i - \sum_{i=1}^N MR_i}{\left( \sum_{i=1}^N TR_i + \sum_{i=1}^N ML_i + \sum_{i=1}^N TL_i + \sum_{i=1}^N MR_i \right) \cdot (N/N)} \cdot 100$$

$$To = \frac{[(\text{mean TR} + \text{mean ML}) - (\text{mean TL} + \text{mean MR})] / (\text{mean TR} + \text{mean ML} + \text{mean TL} + \text{mean MR}) \cdot 100}{\left( \frac{\sum_{i=1}^N TR_i + \sum_{i=1}^N ML_i - \sum_{i=1}^N TL_i - \sum_{i=1}^N MR_i}{\left( \sum_{i=1}^N TR_i + \sum_{i=1}^N ML_i + \sum_{i=1}^N TL_i + \sum_{i=1}^N MR_i \right) \cdot (N/N)} \right) \cdot 100}$$

Conversely, the currently proposed percentage overlapping coefficient ( $POC$ ) and torque coefficient ( $TC$ ) are computed with the absolute (signless) r.m.s differentials computed for each sample interval  $i$ . The  $N$  differentials are then summed over the entire time span:

$$POC = \left[ 1 - \frac{\sum_{i=1}^N |R_i - L_i| / \sum_{i=1}^N (R_i + L_i)}{\left( \frac{\sum_{i=1}^N R_i - \sum_{i=1}^N L_i}{\left( \sum_{i=1}^N R_i + \sum_{i=1}^N L_i \right) \cdot (N/N)} \right) \cdot 100} \right] \cdot 100$$

$$TC = \frac{\sum_{i=1}^N |(TR + ML)_i - (TL + MR)_i|}{\sum_{i=1}^N [(TR + ML) + (TL + MR)]_i \cdot 100}$$

$$TC = \frac{\sum_{i=1}^N |(TR - TL)_i - (MR + ML)_i|}{\sum_{i=1}^N (TR + ML + TL + MR)_i \cdot 100}$$

$$TC = \frac{\sum_{i=1}^N |\delta T_i - \delta M_i| / \sum_{i=1}^N (MR_i + ML_i + TR_i + TL_i) \cdot 100}{\left( \frac{\sum_{i=1}^N TR_i + \sum_{i=1}^N ML_i - \sum_{i=1}^N TL_i - \sum_{i=1}^N MR_i}{\left( \sum_{i=1}^N TR_i + \sum_{i=1}^N ML_i + \sum_{i=1}^N TL_i + \sum_{i=1}^N MR_i \right) \cdot (N/N)} \right) \cdot 100}$$

The absolute difference at the numerator makes impossible to use the mean values computed over the analysed time span (Fig. 1).

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