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TEMPOROMANDIBULAR JOINT IN HEALTH AND DISEASE A 3D MORPHOFUNCTIONAL ANALYSIS

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ABSTRACT

Quantitative, objective and accurate evaluation of masticatory muscle activity and jaw movement is mandatory for a better understanding of the normal function and dysfunction of the stomatognathic apparatus.

A non-invasive recording protocol, integrating an electromyographic system and an optoelectronic 3D-motion analyzer, has been developed and used to perform multifactorial estimations of TMJ functioning. The masticatory system has been objectively quantified, assessing bite stability, mandibular border movements and chewing performance, in both healthy and pathologic individuals. Three separate investigations have been made.

In the first study, functional symmetries of the craniofacial complex involving the patterns of jaw movements and the activities of masticatory muscles were assessed in a control group of clinically healthy subjects. Data were evaluated separately for men and women, and a gender-related effect was tested, together with the potential influence of mandibular dimensions.

In the second study, the same complete protocol was employed to analyze the masticatory function in patients with mild-moderate temporomandibular disorders, in order to provide new insight concerning a still controversial pathology.

The aim of the third study was to assess the recovery of mandibular range of motion in border movements, focusing on the potential changes in mandibular condylar motion, analyzed in untreated patients with skeletal Class III malocclusions, and patients who had received orthognathic surgery for the correction of this dentoskeletal deformity.

The outcomes suggest that the proposed method could be a useful tool to evaluate the neuromuscular coordination during the performance of static and dynamic masticatory activities, and to detect functionally altered stomatognathic conditions.

Diagnosis of alterations of the stomatognathic apparatus, and assessment of the effects of therapy, would both profit from this quantitative approach, thus reducing the discordance among several clinical examinations.

Key words

Human temporomandibular joint; 3D motion analysis; electromyography; health; disease.

OVERVIEW

In comparison to other musculoskeletal systems of the human body, the masticatory system presents special characteristics that make it an interesting object of study in the field of biomechanical movement. A great variety of precise and coordinated movements such as chewing, speaking and laughing, depend on its correct functioning, and several other vegetative and relational functions more or less are based on its performance. In all these processes, coordinated by the central nervous system and under the influence of peripheral inputs, there are mandibular motor requests and, at the same time, movements of the tongue, hyoid bone, soft palate, lips and other structures, involving the masticatory muscles.

The movement of the mandible (lower jaw) is linked inherently to the morphofunction of the temporomandibular joint (TMJ), whose malfunction could impair the aforementioned fundamental processes.

Actually, the study of mandibular movements (kinematics of the mandible) is of key importance in the clinical analysis of mastication. No current imaging systems can provide a complete threedimensional (3D) evaluation of TMJ motion: conventional radiographic images lack the third dimension; both spiral and helical computed tomography (CT) and magnetic resonance imaging (MRI) can be used to reconstruct 3D joint morphology, but lack the necessary dynamic information, the former ones being also invasive. Current ultrafast MR imaging shows the 3D morphology of the TMJ as continuous, high-resolution, moving images without exposing the subject to radiation; however, this technology distinguishes poorly between teeth and bone, because of low contrast between these 2 hard tissues. Furthermore, the patient must lie down during MRI imaging, altering normal jaw movements.

The recording of the six degrees of freedom of free jaw movements can be carried out with three-dimensional non invasive motion analyzers, which allow the recordings to be done while the patient sits upright in a chair. By means of these instruments, jaw movements have been analyzed extensively in the past for prosthodontic reasons, or to study the function of the masticatory system (Palla et al., 2003). Early investigations analyzed the movement of a single mandibular point of simple detection, usually the lower interincisal one, which was used to calculate the distance of maximum mouth opening (MMO). The analyzed point was identical to the recorded landmark, for example a magnet or a light emitting diode (LED); thus, the registered path simply corresponded to the actual movement of the recorded point. Though, interincisal path alone cannot provide enough information on TMJ function (Travers et al., 2000; Naeije, 2002; Mapelli et al., 2009): people can significantly vary the relative amounts of

condylar translation and rotation and still have similar amounts of opening at the incisors (Salaorni and Palla, 1994; Monteverdi et al., 2006).

Nowadays, TMJ kinematic behaviour can be efficiently and accurately detected by optoelectronic tracking systems, which allow the non invasive direct/ indirect recording of multiple mandibular points in all six degrees of freedom (Piehslinger et al., 1993; Salaorni and Palla, 1994; Koolstra and van Eijden, 1995; Yatabe et al., 1995, 1997; Lotters et al., 1996; Zwijnenburg et al., 1996; Gallo et al., 1997; Merlini and Palla, 1988; Catic and Naeije, 1999; Lobbezoo et al., 2000; Travers et al., 2000; Lewis et al., 2001; Naeije, 2002; Ferrario et al., 2005; Mapelli et al., 2009). In particular, the three-dimensional condylar motion can be picked out, thus offering more insight for TMJ functional evaluation. Though, the analysis of the movement of a condylar point is more complex, as it is impossible to record its trajectory directly; consequently, this must be geometrically reconstructed on the basis of biomechanical models and mathematical calculations. Accordingly, it is also possible to investigate the relative contribution of rotation (condyle-disc compartment) and translation (mandibular fossa-disc compartment) along all condylar paths in both mouth opening and closing, allowing a deeper understanding of the normal joint motion (Mapelli et al., 2009).

From a clinical point of view, it would be interesting to perform such a detailed assessment of condyle-disc motion in patients with joint alterations (Merlini and Palla, 1988; Catic and Naeije, 1999), quantifying the different performance of the mandibular fossa-disc and condyle-disc TMJ compartments from normal individuals. For instance, Sforza et al. (2009) observed that patients rehabilitated after a condylar fracture showed modification of the rotation/translation components of mouth opening despite a good recovery of total mandibular movement. Findings like that can be of help in redirecting treatment plans.

Beside multiple investigations which have evaluated the diagnostic potential of the mandibular motion analyzers (Karlsson and Carlsson, 1990; Travers et al., 2000; Wintergerst et al., 2004; Hansdottir and Bakke, 2004; Miyawaki et al., 2004; Bianchini et al., 2008; Rilo et al., 2009; Wang et al., 2009; Sforza et al., 2009, 2010b), other researchers have proved the reliability of surface electromyography in the stomatognathic functional analysis (Pinho et al., 2000; Ferrario et al., 2007; Ries et al., 2008; Tartaglia et al., 2008a, 2008b; Ardizone et al., 2010; Forrester et al., 2010).

Surface electromyography (EMG) has been used since the early 1950s for studying the action of the superficial masseter and temporal muscles during mastication. Currently it is a part of patient assessment in dentistry (Ferrario et al., 2006b), providing quantitative data on the function of superficial muscles with minimal discomfort to the patient and without invasive or dangerous

procedures. Indeed, when well-standardized protocols are used (in order to solve problems like the wrong positioning of the electrodes, the difference of impedance of the patient's skin, the muscle cross-talk, etc.), surface EMG of the head muscles has been reported to be an effective method for the functional assessment of the stomatognathic apparatus (Farella et al., 2003; Garcia-Morales et al., 2003; Ciuffolo et al., 2005), with a good repeatability (Kogawa et al., 2006; Ferrario et al., 2006b; De Felicio et al., 2009b).

Diagnosis of alterations of the stomatognathic apparatus, and assessment of the effects of therapy, would both profit from a quantitative approach, thus reducing the discordance among several clinical examinations (Schmitter et al., 2005; Manfredini et al., 2006). Objective measurements are also needed by insurances and forensic medicine (Tartaglia et al., 2008a).

Over the last 20 years, the Functional Anatomy Research Centre (FARC) of the Dipartimento di Morfologia Umana e Scienze Biomediche "Città Studi", Università degli Studi di Milano (Italy), has devised and developed, in parallel, a protocol for TMJ kinematic analysis (latest version in Mapelli et al., 2009) and a protocol for the characterization of masticatory muscles activity (latest version in Tartaglia et al., 2008b). The former is currently applied to both mandibular border movements (Ferrario et al., 2005; Mapelli et al., 2009; Sforza et al., 2009, 2010b) and gum chewing; the latter is used for both jaw clenching (Ferrario et al., 2004b, 2006b, 2007; Tartaglia et al., 2008b, 2011; De Felicio et al., 2009b) and chewing (Ferrario and Sforza 1996; Ferrario et al., 1999, 2004b; Dellavia et al., 2007; Tartaglia et al., 2008a).

In the present investigation, the two protocols have been applied to analyse both the mandibular kinematics and the electromyographic characterization of the masticatory muscles in:

- healthy subjects (Study I)
- patients with moderate temporomandibular disorders (Study II)
- patients with diagnosis of class III dentoskeletal deformity, before and after orthognathic surgery (Study III).

The general purpose was to investigate the stomatognathic morphology and function during the performance of standardized static (clenching) and dynamic (border movements and chewing, neuromuscular coordination) tasks.

ANATOMY and FUNCTION

THE TEMPOROMANDIBULAR JOINT

The temporomandibular joint (TMJ), one of the most complex joints in the body, is the bilateral synovial articulation between the mandible and the skull, composed of the condylar heads of the mandible and the articular eminence of the right and left temporal bones, covered by dense, fibrous connective tissue and surrounded by several ligaments. Interposed between the incongruent articulating surfaces is an articular disc, contoured to fit over the head of the condyle and into the concavity of the mandibular fossa, which compartmentalizes the joint into two separate synovial-lined cavities (fig.1).



Figure 1. Sagittal section through the left temporomandibular joint (Williams et al., 1989).

The mandible (or lower jaw), the unpaired bone of the stomatognathic apparatus, is the largest, strongest and lowest bone in the face. It has a horizontally U-shaped body, convex forward, and two broad rami, ascending posteriorly. The mandibular ramus is quadrilateral, with two surfaces, four borders and two processes: the coronoid process and the condyloid process (fig. 2). The mandibular notch, separating the two processes, is a deep semilunar depression, and is crossed by the masseteric vessels and nerve.



Figure 2. The skull: norma lateralis. The mandible with its two processes in gray (Williams et al., 1989, modified).

The condyloid process is apically enlarged as a head or **condyle**, covered by fibrocartilage. The condyles, which are convex in all directions, are elliptical and measure about 20 mm through the long mediolateral axis and 10 mm through the anteroposterior axis. The angle between the condylar main axes of the left and right joint varies between 150 and 160 degrees. The long axis is also at right angle to the ramus of the mandible. Anteriorly the condyle is strongly convex, whereas posteriorly the convexity is reduced to medial and lateral slopes. Its lateral aspect is a blunt projection, palpable in front of the auricular tragus. It is important to remember, however, that there is an inter-individual variability in size, shape and position of the mandibular condyle

that may be caused by any one or a combination of factors, including heredity and functional adaptation.

On the temporal bone, the TMJ occupies the inferior surface of the zygomatic process, on the posterior surface of the articular eminence. The articular eminence is the strongly convex bony elevation on the root of the zygomatic process representing the anterior-most boundary of the articular or mandibular fossa (also referred to as the **glenoid fossa**). In particular, the articular tubercle is the bony knob on the lateral aspect of the articular eminence, where the fibrous capsule and the temporomandibular ligament attach.

The articular surfaces of the TMJ are covered by dense, collagenous connective tissue overlying a thin proliferative layer of cells associated with the underlying hyaline cartilage. It is reported that the hyaline cartilage of the condyle is present while the individual is still growing, until about 20 years of age, whereas the cartilage covering the articular eminence has a shorter life span. At the termination of growth, this cartilage layer is replaced by compact bone. In the adult, the compact bone of the condyle is covered by a layer of fibrocartilage that, in turn, is covered by a thin layer of proliferative tissue. Cells of the proliferative layer may become activated to function in remodeling of the joint as a result of changes in function, wear, and tooth movement. Superficial to the proliferative layer is a relatively thick layer of dense, irregular collagenous connective tissue whose deeper layers house fibroblasts. Although the articular structures are avascular, they are bathed in synovial fluid, which provides lubrication and nourishment for the cellular coverings.

The **articular disc** is the primary mechanism of stress distribution and lubrification within the TMJ. It is a deformable, dense, and fibrous connective tissue plate that is oval and contoured to fit between the mandibular condyle and the articular eminence of the temporal bone. The inferior surface of the disc is concavely contoured to fit the convex condyle of the mandible. Superiorly, its surface is sagittally concavo-convex. The convex posterior portion conforms to the concave mandibular fossa, whereas anteriorly, the disc becomes concave to fit the convex posterior aspect of the articular eminence. The disc is thicker at its periphery and thinner at the loadbearing area of the joint (that occasionally, especially in older individuals, becomes perforated). Peripherally, the disc becomes less dense as it merges into the surrounding fibrous capsule and, in front, it blends with the tendon of lateral pterygoid muscle (fig. 3).



Figure 3. Form, subdivisions and thickness variations of the intra-articular disc in the TMJ. Lateral aspect (a) and sagittal section (b) (Williams et al., 1989).

The peripheral regions of the disc are very vascular, whereas the central, stress-bearing portion is devoid of blood vessels. Posteriorly, the disc is attached to a highly vascular connective tissue known as the retrodiscal tissue: a venous plexus separates upper and lower layers, the upper band of fibro-elastic tissue is attached to the fossa posterior margin, while the lower band (non-elastic fibrous tissue) is attached to the posterior surface of the condyle. During mandibular movements, the geometric relationships of the TMJ articular surfaces vary, so that the disc undergoes stress concentrations that change with time and location. The primary function of the deformable visco-elastic articular disc is to permit these activities while reducing the risk of trauma.

The TMJ **capsule**, composed of dense, irregular collagenous connective tissue, encloses the entire articulating region of the temporal bone, disc, and mandibular condyle, sealing the joint space. The joint capsule is evident only laterally (fig. 4), while medially, anteriorly and posteriorly is not a recognizable entity independent from the disc connections with the temporal and condylar surface (Sforza et al., 2010a).



Figure 4. Lateral aspect of a right human TMJ capsule (courtesy of prof. Simone C.H. Regalo, University of Sao Paulo, Brasil).

It attaches laterally to the longitudinal root of zygomatic process, medially to the sphenoid spine, inferiorly to the neck of condyle, anteriorly to the front edge of articular tubercle (the limit is not clear and seems to continue with the lateral pterygoid muscle), posteriorly to the front edge of the petrosquamous suture. Above the articular disc the capsule is loose and it is taut below it. The placement of the disc between the two articulating bones and its peripheral attachments to the walls of the capsule causes the capsular space to be divided into two separate superior and inferior synovial compartments. The larger, superior compartment between the disc and temporal bone permits some freedom of movement between the disc and articular eminence. Anteriorly, the capsule and disc are tightly fused, permitting the insertion of some fibers of the lateral pterygoid muscle into the disc. Medially and laterally, the capsule and disc are attached to the condyle margins, thus necessitating associated simultaneous movement of the condyle and disc. The inferior compartment encloses the entire neck of the mandible and is more firmly attached to the disc. This attachment prohibits excessive movement between the disc and condyle. The joint capsule is richly endowed with sensory endings from the mandibular division of the trigeminal nerve, most of which are supplied from its auriculo-temporal and masseteric branches. Vascular supply to the joint is provided by branches of the superficial temporal and maxillary arteries as they approximate the joint.

Several ligaments strengthen the joint and limit the movements.

Two short, strong collateral ligaments (discal ligaments) serve to anchor the medial and lateral borders of the articular disc to the poles of the condyle, ensuring that disc and condyle move together in protraction and retraction.

Reinforcements of the joint capsule along its lateral margin by obliquely oriented bundles of collagenous fibers are responsible for naming this pronounced lateral portion of the capsule, the lateral ligament or temporomandibular ligament (fig. 5).



Figure 5. The left temporomandibular joint: lateral aspect (Williams et al., 1989).

The temporomandibular ligament possesses two separate bands of fibers, whose directions are oblique to each other. The superficial layer, which is more extensive, arises as a broad band from the lateral surface of the articular eminence at the articular tubercle. The ligament narrows as it passes obliquely inferior and posterior to be inserted on the posterolateral aspect of the mandibular neck just inferior to the lateral pole of the condyle. The smaller, medially situated portion of the lateral ligament arises from the crest of the eminence to pass almost horizontally to insert into the lateral aspect of the condyle. The lateral ligament in the anteroinferior direction, but its superficial portion prevents lateral movement, whereas the deeper horizontal portion prevents posterior displacement of the condyle.

Two additional ligaments are considered accessory to the TMJ. The sphenomandibular ligament (fig. 6), medial to and separate from the capsule, is a flat, thin band descending from the sphenoidal spine and widening to reach the lingula of the mandibular foramen. Superolateral to it there are the lateral pterygoid muscle and auriculo-temporal nerve; inferior to this it is separated from the mandibular neck by the maxillary vessels, below which the inferior alveolar vessels and nerve and a parotid gland separate it from the mandibular ramus.



Figure 6. The left temporomandibular joint: medial aspect (Williams et al., 1989).

The stylomandibular ligament, the other accessory ligament, is a specialization of the deep cervical fascia. This medial ligament extends as a thin band from the apex of the styloid process of the temporal bone to the posterior border of the angle and ramus of the mandible.

Although the precise functions of these two accessory ligaments are not fully understood as they relate to the TMJ, it has been suggested that the sphenomandibular ligament assists in limiting lateral mandibular movement, whereas the stylomandibular ligament apparently assists in limiting the anterior extent of protrusion of the mandible (Williams et al., 1989; Hiatt and Gartner, 2010).

THE MASTICATORY MUSCLES

The muscles of mastication are a set of four bilateral muscles (the temporalis, medial pterygoid, lateral pterygoid, and masseter) whose function is to move the mandible about the temporomandibular joint as it occurs in phonation, chewing (mastication), and swallowing. All of these muscles, excepting the masseter muscle, originate from either the temporal or infratemporal fossae and insert upon the medial aspect of the mandible. The masseter muscle, in contrast, originates on the zygomatic arch and inserts upon the lateral aspect of the mandible. The epimysia that cover these muscles become the fascia encircling the masticator compartment, which contains the four muscles of mastication and the ramus of the mandible. Prolongations of the buccal fat pad fill in the spaces between the muscles of mastication deep to the mandible.

The **temporalis muscle** is a fan-shaped muscle originating on the bones of the broad temporal fossa (fig. 7). Specifically, the site of origin extends inferiorly from the inferior temporal line over the entire temporal fossa, including parts of the parietal and most of the squama of the temporal bones, and the greater wing of the sphenoid, including its infratemporal crest and the temporal surface of the frontal bone. Occasionally, some fibers arise from the posterior temporal surface of the frontal process of the zygoma. The muscle bundles converge to insert as a tendon on the coronoid process of the mandible and down along its anterior surface and the anterior border of the ramus as far anteriorly as the third molar. The anterior fibers of this muscle are vertically directed from origin to insertion, whereas the middle fibers are oblique and the posterior fibers are almost horizontal.

The muscle is primarily an elevator of the mandible; however, because of the directional alignments of the muscle fibers, the posterior and middle portions of the muscle are reported to act also in retracting the mandible.

The temporalis muscle is innervated by anterior and posterior deep temporal nerves from the mandibular division of the trigeminal nerve. The nerves enter the muscle from its deep aspect in the temporal fossa. Vascularization is supplied via branches of the superficial temporal and maxillary arteries. Arising from the former is the middle temporal artery, which enters the muscle on its superficial aspect. Anterior and posterior deep temporal arteries, arising from the maxillary artery, accompany the like-named nerves and enter the deep aspect of the muscle, where they anastomose with the middle temporal artery.



Figure 7. Model of the skull with left temporalis and masseter muscles.

The shape of the posterior region of the jaw is due to the quadrangular form of the **masseter muscle** overlying the angle and ramus of the mandible (fig. 7). The masseter originates on the zygomatic arch and inserts into the lateral surface of the mandible. This muscle possesses, from its origin, a superficial portion and a smaller, deep portion. The superficial portion arises, via a tendinous aponeurosis, from the zygomatic process of the maxilla and the anterior two-thirds of the inferior border of the zygomatic arch. The smaller, deep portion arises from the inferior border of the posterior one third of the zygomatic arch and from along its entire medial aspect. The fibers of the superficial and deep portions of the muscle fuse to insert on the mandible, broadly covering the angle, along with some of the ramus and the body, as far anteriorly as the region directly below the last molar. Some fibers derived from the deepest portion insert as far superiorly as the base of the coronoid process. It is in this region that fibers of the temporalis muscle, arising from the inner surface of the zygomatic arch, may be fused with those of the deep portion of the masseter.

The masseter muscle functions as a powerful elevator of the jaw. The superficial fibers act to protract the condyle, stabilizing it against the articular eminence, and to direct a powerful force on the molars; whereas the deep fibers, more vertically directed, effect a retractive force, especially in closing the jaws.

The muscle is innervated by the masseteric nerve derived from the mandibular division of the trigeminal nerve. This motor nerve enters the muscle on its deep aspect adjacent to the mandibular notch, through which it gains access from its origin in the deep face. Vascular supply to the muscle is provided by the masseteric branch of the maxillary artery. The artery and vein accompany the nerve in its path to the muscle.

The **medial (internal) pterygoid muscle** originates in the deepest aspect of the deep face, and inserts on the inner aspect of the ramus and angle of the mandible, mirroring the insertions of the masseter (fig. 8). Thus, it is anatomically and functionally a counterpart to the masseter muscle. The specific sites of origin are the pyramidal process of the palatine bone in the pterygoid fossa and the medial surface of the lateral pterygoid plate. The medial pterygoid muscle is directed inferiorly, posteriorly, and laterally to be inserted onto the medial surface of the ramus of the mandible.

The medial pterygoid muscle functions primarily as an elevator of the mandible. Its fibers are directed in an oblique fashion; however, the force is more pronounced in a vertical direction. The medial pterygoid muscle receives its motor innervation from a like-named nerve branching from the mandibular division of the trigeminal nerve and entering the deep surface of the muscle. The muscle is vascularized by a branch of the maxillary artery.



Figure 8. Left pterygoid muscles: the zygomatic arch and part of the ramus of the mandible have been removed (Williams et al., 1989).

The **lateral** (external) pterygoid muscle is a short muscle, filling the remainder of the infratemporal fossa and covering much of the medial pterygoid muscle. This muscle possesses two heads of origin (fig. 8). The smaller, superior head originates from the infratemporal region of the greater wing of the sphenoid bone as far laterally as the infratemporal crest. The larger, inferior head originates from the lateral surface of the lateral pterygoid plate. The fibers of the superior head course posteriorly and laterally in an almost horizontal direction from the infratemporal crest. Fibers of the inferior head are directed posteriorly, laterally, and slightly superiorly on their way to the mandible. Though the two heads of origin are separated from each other, their fibers converge as they approach the site of insertion on and about the mandible. The superior head inserts into the articular capsule of the TMJ, the anterior border of the articular disc, and the superior part of the mandibular neck.

The lateral pterygoid muscle is described classically as the jaw opener, which protrudes the mandible and moves the mandible from side to side when functioning unilaterally. In particular, the superior head, attached to the articular capsule and disc, functions in stabilizing the mandibular condyle, whereas the inferior head is reported to function in pulling the mandible and disc forward and down, effecting jaw opening (Ferrario and Sforza, 1992).

The lateral pterygoid muscle is innervated by a branch entering its deep surface from either the anterior division separately or as a branch of the buccal nerve from the mandibular division of the trigeminal nerve. Vascular supply is provided by a branch from the maxillary artery as it passes either superficial or deep to the muscle.

The origins and insertion sites of these muscles on the mandible dictate the joint function. Generally, the functions are for opening or closing the jaw; however, subtle variations exist when muscles are acting antagonistically or synergistically with other muscles on one side or the other or on both sides (Williams et al., 1989; Hiatt and Gartner, 2010).

MANDIBULAR KINEMATICS

The bilateral TMJs are connected through the mandible and, therefore, the articulations function as a single unit rather than independently. The TMJ is composed essentially of two convex structures opposed to each other, with an intermediate articular disc placed between them. Considering the anatomy of the disc, it becomes clear that movement within the TMJ is basically of two types. Ginglymus (hinge) movement is possible between the condyles of the mandible and the inferior surface of the discs. The other permitted movement within the joint is an arthrodial (gliding) motion (Ferrario et al., 2005). This is possible as the superior surface of the articular disc slides down at the articular eminence. Therefore, the TMJ is considered a **ginglymo-arthrodial joint**: the condyle/disc movement is rotatory, whereas the disc/temporal bone motion is translational.

Functionally, the mandible can be depressed or elevated, protruded or retracted and, since both joints always act together but may differ in actual movement, some lateral rotation may occur.

The **resting position** is defined as having the patient's head in the anatomic position (in an upright posture). This places the masticatory musculature at rest, permitting the teeth of the upper and lower jaws to be slightly apart (2-5 mm between incisors), but having the upper and lower lips touching. It is in this attitude that the mandibular condyle is positioned so that its anterosuperior articulating surfaces is opposite the posterior slope of the articular eminence of the temporal bone (fig. 9), with the disc between the two bones.



Figure 9. Right TMJ bones at resting position (Rampello, 2004).

It is apparent that the resting position is independent from shape, number, position and even presence or absence of the teeth; indeed, it depends a lot on the muscular tone of the elevator muscles, as well as the gravity force to counteract.

The position of **centric occlusion** is obtained when the cusps of the mandibular and maxillary teeth are in contact and interdigitate maximally; indeed, it is also referred to as intercuspal position (ICP). The condyles are slightly rotated backwards and retracted with respect to the rest position.

Mouth opening involves the translational (gliding) movement of the disc and condyle down the slope of the articular eminence coupled with rotatory (hinge) movement of the mandibular condyles against the disc (Mapelli et al., 2009). This results in a rototranslation, which is due to the attachments that link the disc to the condylar head. Thus, the posterior portion of the angle of the mandible moves slightly backwards, and the mandibular body moves inferiorly, steered by its own weight (fig. 10).



Figure 10. Right TMJ bones at maximum mouth opening (Rampello, 2004).

The lateral pterygoid muscles initiate the depression, drawing heads and discs onto the articular tubercles, aided, when the mouth is widely open or against resistance, by the digastric, geniohyoid, and mylohyoid muscles. This assumes that the hyoid bone has been fixed by the stylohyoid muscle. Discal sliding ceases when its posterior fibro-elastic attachments to the temporal bones are stretched to their limit. Further hinging and gliding of the condyles bring them into articulation with the most anterior parts of the discs as the mouth opens fully. The maximum mouth opening (MMO) is achieved when the elevator muscles cannot be stretched anymore.

In **mouth closure** movements are reversed: each head glides back and hinges on its disc, still held by the lateral pterygoid, which relaxes to allow the disc to slide back and up into the mandibular fossa, assisted by the masseter, temporalis and medial pterygoid muscles which raise the mandible.

Mandibular protrusion is accomplished by contracting the lateral and medial pterygoid muscles together with the superficial fibers of the masseter muscles and the anterior portion of the temporalis muscles, which draw the condyle-disc complex forward and down the articular eminence, whereas the inferior incisors project in front of the upper ones.

Mandibular retraction, in contrast, returns the mandible to a position posterior to the resting position. This action is accomplished by the medial and posterior fibers of the temporalis muscles, assisted by middle and deep parts of the masseters, digastric and geniohyoid muscles.

Mandibular lateral rotation (i.e. a lateral deviation on one side) is achieved by the condyledisc complex of the controlateral side (working condyle), which slides inferiorly and anteriorly on the articular eminence while moving medially. The result of this active process effects a passive lateral rotation of the condyle head on the ipsilateral side (balancing condyle). The lateral pterygoid muscle of the side opposite the lateral rotation effects the movement, acting together with its ipsilateral medial pterygoid muscle.

The **chewing** or grinding movement is produced by one condyle-disc complex, which glides alternately forward and backward, while the other one moves simultaneously in the opposite directions; at the same time the condyles undergo a vertical rotation on the discs. The grinding movement is caused by the alternate action of the pterygoid muscles of either side.

Such a list, though useful, obscures the complex integrations of simultaneous contraction and lengthening of many muscles. In fact, it must be pointed out that the entire masticatory and accessory muscles are involved in producing any one or combinations of these movements.

Furthermore, condylar movements are controlled not only by the shape of the articulating surfaces and the contraction patterns of the muscles, but also by the dentition. This, indeed,

determines the end position as well as the movement of the condyle-disc complex when jaw movements are performed with the teeth in contact (Hiatt and Gartner, 2010).

MATERIAL and METHODS

INSTRUMENTATIONS

Optoelectronic Motion Analyzer

Mandibular kinematics was recorded using an optoelectronic three-dimensional motion analyzer, the SMART-E system (BTS S.p.a, Garbagnate Milanese, Italy), one of the most advanced optoelectronic motion capture systems currently available.

High-precision infrared sensitive CCD video cameras (fig. 11) are coupled with the video processor with up to 120 Hz sampling ratio. The 3D positions of lightweight, passive and retro-reflective markers are instantly recorded with a spatial accuracy of up to 0.1 mm.



Figure 11. Detail of a camera.

In brief, stroboscopic infrared light (wavelength, 880 nm) is emitted by an array of LED (light emitting diodes) mounted around the lens of each camera, and the CCD sensor detects the reflection from the markers placed on the body.

The process of recognizing passive markers in the 2D video frames is performed via enhanced blob analysis. The 3D coordinates of each marker are finally computed based upon the 2D data of at least two cameras. This process, called spatial triangulation, needs the system to be previously calibrated.

Indeed, calibration allows the system to estimate the capture volume, the relative position and orientation of the cameras (external parameters), their geometric and optical characteristics (internal parameters).

BTS SMART system requires two calibration phases. The "static calibration" sets the position

and the orientation of the global reference system: all cameras simultaneously record a still, special reference device (fig. 12), whose marker reciprocal distances are known to the system. The "dynamic" calibration exploits the epipolar constraint between a 3D point and its 2D projections on the sensor of two cameras: all cameras simultaneously record a rigid bar (Y axis) in motion throughout the working volume.



Figure 12. Global reference system.

At the end of the metric calibration and correction of optical and electronic distortions, the system provides the current accuracy level, which will characterize the following acquisition sessions.

Once a movement has been recorded, special software provides the spatial configuration of the marker set (fig. 13).



Figure 13. 3D graphic representation of the marker set at issue.

The operator has to label the markers of interest in one frame, opening the corresponding model previously created; afterward, the system should be able to recognize all moving markers, tracking their pathways.

To record mandibular movements (Ferrario et al., 2005; Mapelli et al., 2009; Sforza et al., 2009, 2010a), 6 cameras (approximately, 1 meter high) were deployed around a stool (fig. 14), and calibrated to create a 77 (width) cm x 66 (height) cm x 77 (depth) cm working volume.

Mean dynamic accuracy and precision lower than 0.15 mm on a 20 cm-bar (full scale accuracy, 0.02%) were required before the recordings.

A 60 Hz capture rate was used for all acquisitions.



Figure 14. Setting of the cameras with respect to the subject sat on a stool. Top view.

Electromyographic System

Electromyography (EMG) is a technique that allows an objective recording of muscle function and dysfunction, enlightening the processes that generate force and produce joint movement.

Two principal kinds of EMG can be used in clinics: needle (or intramuscular) EMG, where a slender detecting wire is inserted inside the muscular belly, and surface EMG (sEMG), where the detection of the signal is obtained by a non-invasive bipolar electrode positioned superficial to the skin, overlaying the muscular belly.

In surface EMG, a bipolar probe measures the voltage difference between two electrodes, which is the sum of the electrical contributions of the active motor units; thus it reflects both the muscle membrane properties and the central control strategies. With small inter-electrode distance with respect to the muscle size, the activity conducted from adjacent muscles is similar on the two leads, and therefore is partly rejected (Castroflorio et al., 2008).

The BTS FREEEMG system (BTS S.p.a, Garbagnate Milanese, Italy) is a wireless sEMG device with active probes, weighting just 8 grams, for signal acquisition and wireless transmission (fig. 15). The probes amplify the differential EMG signals captured by disposable pre-gelled

silver/silver chloride bipolar surface electrodes, digitize them and communicate with a portable receiving unit. The complete absence of cables allows for quick and comfortable preparation of the patient, without affecting in any way the motor pattern. This system is easily connectable with the motion analyzer, permitting the real time recording of synchronized kinematic and electromyographic data.



Figure 15. Detail of an EMG probe clipped on a pair of electrodes.

In controlled experimental conditions, surface EMG has been shown to be a powerful tool for physiological investigations of the jaw elevator muscles (Kumar et al., 2001, 2003; Ferrario et al., 2007; Castroflorio et al., 2008; Tartaglia et al., 2008a,b, 2011; Tecco et al., 2011; De Felicio et al., 2009b; Forrester et al., 2010; Sforza et al., 2010a, 2011).

ANALYZED STOMATOGNATHIC FUNCTIONS

Maximum Voluntary Contraction (MVC)

Maximum voluntary bite force is an important variable to assess the functional state of the masticatory system (van der Bilt, 2011). Unfortunately, the direct assessment of bite force is technically difficult, and with several biological limitations. For instance, several of the instruments used for its detection are very bulky, and significantly modify the oral and mandibular conditions, either increasing the vertical dimension of occlusion, or providing unnatural proprioceptive and tactile information. A positive and near linear relationship has been shown to exist between surface EMG of the jaw elevator muscles and a steady level of bite force during isometric contractions (Ferrario et al., 2004a). Also, the relationship between EMG and bite force as obtained during clenching experiments (static condition) may be used to estimate bite forces from recordings of EMG during function (dynamic condition).

One of the principal problems that limit a widespread use of sEMG in clinics is the necessary normalization of its recordings (DeLuca, 1997). Indeed, to compare EMG recordings among different subjects it is mandatory to relate all measurements to the electrical muscle activity detected during some standardization recording, like a maximum voluntary contraction (Castroflorio et al., 2005). In the dental field, sEMG potentials collected during an MVC on both cotton rolls and in intercuspal position have been reported to have the best inter- and intra-individual repeatability (Ferrario et al., 2006b; De Felicio et al., 2009b; Suvinen et al., 2009; Forrester et al., 2010; Hellmann et al., 2011), and diagnostic tests based on MVC standardization have been in use for the last 10 years (Ferrario et al., 2004b, 2006b, 2007; Tartaglia et al., 2008b, 2011; De Felicio et al., 2009b; Tecco et al., 2011).

Mastication

Mastication is a complex task that mixes voluntary and automatic motor pathways controlled by central nervous system pattern generators, located in the brainstem, and is regulated by the feedback from several receptors (extero-, proprio- and viscero-receptors) (Rilo et al., 2007). Mastication requires well-controlled repeated separation and contacts of the maxillary and mandibular teeth, characterized by rhythmic up and down motion, protrusive-retrusive movement, rotation in the horizontal plane, and lateral shifting of the mandible.

The concomitant assessment of chewing kinematics, morphology and EMG activity of the masticatory muscles provides important information on the chewing function (Kohyama et al., 2008; Piancino et al., 2008). The exact process varies between the individuals but, once the pattern is established, it remains fairly constant for that particular person. This is not to imply that the process is static; indeed it is continually altered, since changes within the stomatognathic system are constant and dynamic throughout life (Hiatt and Gartner, 2010).

Among the others, the chewing pattern is generally thought to be one of several useful parameters for objectively evaluating chewing function, and unilateral gum chewing is the test most commonly used for obtaining standardized data (Yamashita et al., 1999). The typical adult chewing pattern, as represented in the frontal plane, is a teardrop shape with the opening phase medial to a more lateral closing phase. Akiyama et al. (1991) analysed the masticatory movements of the mandible in the frontal plane and classified the chewing patterns into eight different types according to the opening and closing paths of the interincisal point (fig. 16).



Figure 16. Schematic illustration of typical chewing patterns of the interincisal point in the frontal view (Takeda et al., 2009).

Pattern I was defined as smooth tracings in both directions, often teardrop shaped, or lenticular. Pattern II was defined as opening patterns that were inclined to the chewing (or working) side, combined with the characteristic closing movements resembling a mirrored "s". Pattern III was defined as opening patterns that were inclined to the nonchewing (or balancing) side, combined with closing movements resembling convexity. Pattern IV was defined as opening patterns that were inclined to the nonchewing side, combined with characteristic closing movements resembling a mirrored "s". Pattern V was defined as opening patterns that were inclined to the nonchewing side, combined with characteristic closing movements resembling a mirrored "s". Pattern V was defined as opening and closing patterns that were drawn from the same chewing stroke. Pattern VI was defined as having both sections inverted (reversed pattern). Pattern VII was defined as crossed opening and closing patterns. Finally, pattern VIII was defined as a linear opening and closing patterns I, II, and III are the grinding patterns and are usually observed in subjects with normal occlusion. Patterns IV and VI are characteristically observed in subjects with a posterior cross-bite. Patterns V, VII, and VIII are observed in subjects with mandibular prognathism and temporomandibular disorders, and in particular, pattern VIII is referred to as a chopping pattern (Takeda et al., 2009).

Modifications in the masticatory movements can point to alterations in several structures: the masticatory muscles, the temporomandibular joints, the teeth and parodontium, the nervous afferent and efferent pathways (Buschang et al., 2000). Direct in-vivo observations of chewing movements are therefore mandatory for a better understanding of the normal function and dysfunction of the stomathognathic apparatus.

Border movements

The border excursions of the human lower jaw with respect to the skull are limited by constraints within the masticatory system. These limitations are considered to be a fundamental portion of the functional performance of the masticatory system. The constraints that determine the envelope of border movements may be passive (TMJ surfaces, ligaments, passive tensions of the muscles) and/or active (reflexes of muscles to protect the articular capsule). The surrounding soft structures, such as skin and glands, also may limit mandible excursions (Koolstra et al., 2001). Aside from maximum mouth opening (MMO), lateral and forward excursions with occlusal contacts (contacts between upper and lower teeth) are a fundamental part of the mandibular border movements used clinically to evaluate function, and they have received considerable study (Peck et al., 1999). The condylar pathways during lateral excursions have also been given special attention because abnormalities in these pathways may be related to temporomandibular disorders (Hayasaki et al., 2008).

RECORDING PROTOCOL

For each subject, the recordings took approximately 30 minutes (considering also the time needed for subject's preparation). The protocol did not involve dangerous or painful procedures, and it was preventively approved by the ethics committee of the Department of Human Morphology, University of Milan.

After the methods and aims of the investigation had been completely described, written informed consent was obtained from each participant.

The subject sat on a stool in the middle of the working volume, with his/her head unsupported, and was asked to maintain a natural erect position.

Electromyographic assessment of Maximum Voluntary Contraction

To reduce skin impedance, facial epidermis was carefully cleaned with alcohol prior to the 10 mm-electrodes placement (like Kendall Arbo; Tyco Healthcare, Neustadt, Germany). Five minutes later, when the conductive paste had moistened the skin surface adequately, the left and right masseter and temporalis anterior muscles were examined with the aforementioned electromyographic system, positioning four probes on their muscular bellies parallel to muscular fibres: vertically along the anterior muscular margin, about on the coronal suture, for the temporalis anterior; with the upper pole of the electrode at the intersection between the tragus-labial commissura and the exocanthion-gonion lines for the masseter (fig. 17).



Figure 17. Orientation of right temporalis anterior and masseter probes.

At first, two 10 mm-thick cotton rolls were positioned on the mandibular second premolar/first molars of each subject, and a 5 s-maximum voluntary contraction (MVC, fig. 18) was recorded (COT). Then, the subject was invited to clench as hard as possible with the maxillary and mandibular teeth in maximum contact (intercuspal position, ICP), and to maintain the same level of contraction for 5 s (CLENCH).



Figure 18. EMG signals of the 4 muscles during MVC.

Electromyographic assessment of mastication

EMG activity was recorded during unilateral, left and right, chewing of sugarless gum (Ferrario and Sforza, 1996). The EMG potentials exerted in the first 15 s of each mastication trial were recorded on the same muscles without any modifications of the setup (fig. 19). Thus, about 20 chewing cycles were detected for each trial.



Figure 19. EMG signal of the right masseter muscle during 4 right chewing strokes.

Kinematic and electromyographic assessment of mastication

Maintaining electrodes and wireless probes on the skin, other two unilateral chewing cycle sequences were recorded using the optoelectronic motion analyzer, together with the electromyographic system. To this scope, three head passive markers (diameter: 6 mm) were added on the nasion and the left and right frontotemporali, defining a cranial reference plane, by means of biadhesive tape. These three markers were insensitive to skin motion artefacts during jaw movement. Other three passive markers (diameter: 6 mm) were positioned on the three corners of an equilateral triangular stainless steel extra oral device (side 40 mm; weight 2 g); this tool was fixed on the mandibular anterior gingiva just out of dental contact using a surgical adhesive (Stomahesive; Convetec Inc, Deeside, United Kingdom), and provided a mandibular reference system. This rigid body was positioned to be as unobtrusive as possible and to allow each participant to move in and out of maximum intercuspation freely (fig. 20).



Figure 20. Complete measurement setup of a subject.

In a single reference frame, a further passive mandibular marker (diameter: 3 mm) was located on the midline incisor edge (inter-incisor point, IP); it identified a dental (occlusal) landmark, relative to the extraoral system (Ferrario et al., 2005). Similarly, two condylar reference points (CRPs) were firstly individuated by palpation and secondly detected by means of a marked pointer while the subject was keeping his mouth closed in ICP.

Kinematic assessment of mandibular border movements

With the same configuration of markers, a border movement sequence of free maximum mouth opening (MMO) and closing, followed by mandible maximum unilateral laterotrusions and protrusion was performed three times by each subject. Each trial had to be started and concluded with the jaws in ICP. In particular, subjects were instructed to naturally open their mouth and to slowly move the mandible to the right/left side and forward from ICP as far as comfortable, with sliding tooth contacts.

MEASUREMENT PROTOCOL

Both raw EMG signals and marker coordinates constituted the input data for the protocol calculations, which were implemented on Microsoft Excel, Matlab or C-code. Descriptive and inferential statistics were evaluated by means of SPSS Statistics.

EMG signals - MVC

For each of the four analyzed muscles, the mean EMG potential evaluated on the most constant 3 s-interval of COT trial (mean of the root mean squared, RMS, calculated in 25 ms-temporal windows) were set at 100%, and all EMG potentials obtained during both MVC directly performed on the occlusal surfaces (CLENCH) and mastication (see below) were expressed as a percentage of this value (unit: mV/mV*100). According to this protocol, normalized EMG data may inform on the influence of occlusion (teeth contact) on the neuromuscular activity, avoiding individual variability (anatomical variations, relative muscular hypo- or hypertrophy, physiological and psychological status, etc.) and technical variations (muscle cross-talk, electrode position, variability due to skin and electrode impedance, etc.).

The EMG waves of paired muscles were compared by computing a percentage overlapping coefficient (POC, unit: %). POC is an index of the symmetric distribution of the muscular activity as determined by occlusion. The index (fig. 21) ranges between 0% (no symmetry) and 100% (perfect symmetry).



Figure 21. Graphic representation of POC calculation for a pair of muscles. The sum of non-overlapped areas is divided by the total area under the two curves.

Masseter and temporalis POCs were obtained for each subject. To individuate the most prevalent side of masticatory muscles, the asymmetry index (ASIM, unit: %) was also computed as the percentage ratio of the difference between the mean right and left standardized potentials, and the sum of the same standardized potentials. This index is positive (up to +100%) when the right muscles standardized potentials are larger than the left ones, negative (down to -100%) when the left muscles potentials are larger, and null when they are equal.

Because an unbalanced contractile activity of contralateral masseter and temporalis muscles, for instance, right temporalis and left masseter, might prompt a potential lateral displacing component, the torsion coefficient (TORS, unit: %) was calculated by superimposing the right temporalis plus left masseter normalized EMG amplitudes over the left temporalis plus right masseter normalized EMG amplitudes: the area of the superimposition was assessed as a percentage of the total EMG amplitudes. TORS ranges between 0% (complete presence of lateral displacing force) and 100% (no lateral displacing force). The add-on TORQUE index was calculated to express with a positive or negative sign the respectively prevalence of right or left displacing component, similarly to what ASIM index did for POC index.

To individuate the most prevalent pair of masticatory muscles, the activity index (ATTIV, unit: %) was also computed as the percentage ratio of the difference between the mean masseter and temporalis standardized potentials, and the sum of the same standardized potentials. This index is positive (up to +100%) when the masseter muscles standardized potentials are larger than the temporalis muscles ones, negative (down to -100%) when the temporalis muscles potentials are larger, and null when they are equal. When standardized muscular potentials are not balanced between the two analyzed masticatory muscles, the occlusal centre of gravity might be displaced onwards (temporalis prevalent) or backwards (masseter prevalent).

Finally, the mean (masseter and temporalis) total standardized muscle activities was calculated as the integrated area of the EMG potential over time (std. IMPACT, unit: mV/mV %).

EMG signals - Mastication

EMG signals of gum chewing were normalized on COT trial in the same way explained for MVC. From the 600 RMS potentials recorded from the four tested muscles during each 15 s chewing test, two main parameters were computed: the masticatory frequency and the confidence ellipse (Hotelling's 95%) of the simultaneous maximum differential right-left masseter and temporalis standardized activity extracted from each cycle (Lissajous's plot).

The confidence ellipse is a statistical tool to assess the repeatability of the masticatory muscle pattern of contraction during the execution of a standardized movement (e.g. unilateral gum chewing). The differential right-left masseter activity serves as the x-coordinate, and the differential temporal activity as the y-coordinate, in a Cartesian graph representation (fig. 22).



Figure 22. Right and left side gum chewing in a normal subject. Surface EMG data are plotted according to a Lissajous's plot.

From the pairs of coordinates, the position of the unknown population centre is estimated by the sample centre. The phase angle gives the inclination of the ellipse relative to the coordinate axes, whereas the amplitude gives the distance of the centre of the ellipse from the centre of the coordinate axes. To assess if the left- and the right-side chewing tests were performed with symmetrical muscular patterns, using the centres of the two confidence ellipses (left and rightside chewing) calculated in each subject, a further index, the symmetric mastication index (SMI, unit: %), was computed. In subjects with a normal neuromuscular coordination, the centres of the ellipses describing unilateral chewing plotted as a Lissajous's figure should be located in the first (right side) and third (left side) quadrants (Kumai, 1993), with about the same amplitude and a 180° difference between the phases. A symmetrical muscular pattern, provided that the ellipses are statistically significant, would then produce a SMI equal (or very close) to 100%. Conversely, an asymmetrical pattern would produce a SMI close to 0%. To directly compare right- and left-side chewing, then, this latter's phase was mirrored, subtracting 180° to its value. Furthermore, the mean (masseter and temporalis) total muscle activities during chewing was assessed as the integrated areas of the standardized EMG potentials over time (IMPACT, unit: %*s). For each patient, both the activity normalized on the number of performed cycles, and its percentage referred to the working side, were also computed.

Kinematics

The extraoral mandibular markers (Mk1, Mk2, Mk3) individuated the plane of mandibular motion, given that both mandibular dynamic deformations (Yatabe et al., 1997; Catic and Naeije, 1999; Chen et al., 2000; Naeije, 2003; Ferrario et al., 2005) and instability at the device-gingiva interface were negligible (Ferrario et al., 2005; Mapelli et al., 2009). The relative motion

between the head reference system (Mk4, Mk5, Mk6) and the mandibular one was computed by means of mapping operators, which allow analysing mandibular pathway relative to the head (fig. 23).



Figure 23. Global view of the marker set (a) and the two reference systems (b).

Hence, neck and trunk movements were subtracted from the raw motion of the mandible. Subsequently, the displacements of the dental (Mk0) and condylar points (Mk7, Mk8) were reported, frame by frame, in the global reference system (head system), with their paths being evaluated in the three anatomical planes (horizontal, frontal, sagittal planes).

The right-left coordinates of the condylar points were further arbitrarily corrected of 15 mm in the medial direction (Merlini and Palla, 1988; Salaorni and Palla, 1994; Gallo et al., 1997) to better represent the head of the condyles (condylar reference points, CRPs) (fig. 24).


Figure 24. Spatial coordinates of a subject's right CRP during a sequence of mandibular border movements. Positive/negative values.

The data were mathematically smoothed with the use of a second-order Butterworth's low-pass filter (cut-off frequency of 8 Hz). Indeed, according to Miles (2007), the mandible voluntary movements together with its continuous tremors can reach a peak frequency of 7 Hz. In fact, voluntary movements of the mandible are interspersed with small accelerations and decelerations of 6-7 Hz, which are the result of alternating activity in antagonistic masticatory muscles superimposed onto the muscle activity that is responsible for the voluntary movements.

A mandibular radius (r) was estimated as the distance between the dental marker (IP) and the midpoint of the intercondylar axis, while the mandibular width (w) was estimated as the distance between the right and left condylar markers (fig. 25).



Figure 25. Mandibular anthropometric parameters.

In each motion frame, the rotational angles made by the extraoral device (i.e. the mandible) around the three global axes were calculated using Cardan angles; this method provided a description of joint movements nearer to the common concepts of flexion/ extension, abduction/ adduction, and internal/ external rotation used in clinical practice.

The sagittal mandibular movement during mouth opening and closing was further divided into its rotation and translation components; in each frame of motion, the relative percentage contribution of the two components to the total movement was calculated. In order to compare different patients, the mandibular movement was normalized on MMO distance (sagittal projection): mouth opening and closing were sampled in 10% steps, and in each step the rotation and translation components were further considered. To find the rotational component of the mandibular movement, the mandibular radius (r) was used together with the sagittal plane mandibular angle of rotation: the circumference arc (s) that r described turning around the CRP was the rotational component, whereas CRP pathway was the gliding component (fig. 26). Therefore, both components were expressed with the same unit (length), allowing their comparison (Mapelli et al., 2009). Another advantage of this approach is that mandibular dimension does not affect the relative contribution of condylar rotation and translation: the normalization, in fact, is included in the calculations.



Figure 26. Sagittal view of the rotation and translation components of a mandibular motion step.

To assess mastication kinematics, each individual's cycles were detected by a specific algorithm code written in Matlab. In particular, the three-dimensional coordinates of the first frame for each sequence defined the starting point of the cycle sequence. The program did not include the first cycle; it searched along the trace of the IP until it identified the starting frame of each

subsequent cycle. The starting frame was defined as the frame at which the three-dimensional distance from the initial point of the cycle attained a minimum (i.e., stopped decreasing and started increasing). Having identified the starting frame, the program continued along the trace until it identified the next, which marked the end of the cycle. The following frame was the starting frame of the next cycle. To be included as a valid cycle, each cycle had to last more than 250 msec in duration and to be more than 3.0 mm long vertically. Then, each chewing cycle detected at the IP was broken down into two phases (open-close), and each path length, time duration and velocity were extracted. Then, the total area delimited by the IP in the frontal plane was evaluated, together with its percentage subdivision in the working and balancing sides. Moreover, the morphology of the chewing cycles (fig. 27) was assessed classifying the pathway of each stroke into 1 of 8 standardized categories (Akiyama et al., 1991): pattern I, II and III were pooled together and referred to as "ideal" cycle shapes, whereas the other 5 (pattern IV, V, VI, VII, VIII) were considered "anomalous" (Takeda et al., 2009).



Figure 27. Frontal view of a typical Pattern I chewing cycle.

Method error

Measurement variability in EMG data was tested by repeated analyses of seven subjects chosen at random; for all MVC variables the intraclass correlation coefficient ranged between 0.629 and 0.977, without significant differences among the measurement sessions (Ferrario et al., 2006a). A good reproducibility of the same indexes was reported also in another test-retest examination (De Felicio et al., 2009b).

The method error of mandibular movements was assessed in the reference subjects, and has been previously reported (Ferrario et al., 2005): the intraclass correlation coefficients (five subjects, three independent sessions) ranged between 0.571 and 0.760, without significant differences among repeated sessions.

Furthermore, to check the stability of the extraoral framework, we assessed the difference in the position of the two lateral cranial markers relative to the framework reference system between the initial intercuspal position and the final one that was reached after a wide range of mandibular movements. For this experiment, two additional patients (not included in the current study) who had a fixed orthodontic appliance were enrolled. Three tests with the framework fixed to the fixed orthodontic appliance were compared to other three tests with the standard positioning (framework attached to the anterior gingiva using surgical adhesive). The framework fixed to the orthodontic appliance gave a mean difference of 0.35 mm (SD, 0.04), whereas in the second configuration the mean difference was 0.49 mm (SD, 0.02). Overall, the differences in the two configurations were similar, showing that the adhesive interface can be considered satisfactory for the current measurements.

Study I – HEALTHY SUBJECTS

INTRODUCTION

A detailed knowledge of normal jaw function is necessary for a better understanding of TMJ disorders, dento-facial malocclusions, and for their treatment.

The aim of the current study was to quantitatively assess, in healthy subjects, the range of motion of mandibular border movements, the morphology and kinematics of chewing cycles, and the electromyographic activity of masticatory muscles during MVC and chewing. The relative contribution of rotation and translation of the condyle-disc assembly during both opening and closing movements was also assessed.

Data were evaluated separately for men and women, and a gender-related influence was tested, considering also a potential effect of mandibular dimension. The comparison of male and female data may offer a further contribution to the assessment of functional and anatomical sexual dimorphism in the human stomatognathic apparatus, a still debated question (Lewis et al., 2001; Naeije, 2002).

Furthermore, asymmetry is a common finding in humans. Apart from unpaired and asymmetric organs, both morphology and function of paired structures differ in the left and right sides of the body. Morphological evaluations of craniofacial asymmetry is a usual part of the characterization of both normal subjects and patients (Naeije et al., 1989; Ferrario et al., 2000). In the present study, functional symmetries of the craniofacial complex involving the patterns of jaw movements and the activities of masticatory muscles were also tested.

METHODS

Subjects and data collection

Nineteen volunteer healthy subjects (9 men and 10 women), aged 21–49 years, were analyzed in this study. They were recruited from the students and staff attending the Dipartimento di Morfologia Umana e Scienze Biomediche "Città Studi", Università degli Studi di Milano (Italy). History and clinical examination were used to select the subjects. The inclusion criteria to be recruited in the control group were: a sound, complete, permanent dentition with bilateral canine and molar Angle Class I jaw relationships; anterior teeth with vertical and horizontal overlap between 0 and 3 mm; maxillary and mandibular interincisal lines without lateral deviations

larger than 2 mm; no cast restorations or cuspal coverages, no anterior or lateral reverse occlusion; no previous history of craniofacial trauma or congenital anomalies; no TMJ or craniocervical disorders, based on the RDC/TMD and ProTMDmulti questionnaire (see Study II).

Data of one man was lost for technical reasons.

Statistical analysis

Descriptive statistics of subjects' age, mandibular radius and width were calculated separately for men and women. The same was done for the 3 Cardan angles and the 3D pathways of IP and CRPs during MMO, maximum right and left laterotrusions and protrusion of the mandible; for the kinematic parameters of gum chewing; for all the EMG indices of MVC and gum chewing. The Chi-squared test was used to test the homogeneity of chewing pattern distributions both between men and women and between right and left working sides.

The normal distribution of data was checked with the Kolmogorov-Smirnov test, and Levene's test was used to test for homogeneity of variances.

Then, male and female age, mandibular dimensions and electromyographic indices in MVC were compared by Student's t-test for independent samples. One-way analysis of covariance (ANCOVA) was used to compare unilateral data (between-subject fixed factor: gender), and 2-way mixed-model ANCOVA (between-subject fixed factor: gender; within-subject fixed factor: side) for bilateral data (right and left condyles, laterotrusions, chewing cycles). 3-way ANCOVA was adopted to test both condylar translation and its percentage relative to mandible rotation during mouth opening, and also for length, velocity and duration of chewing cycles (between-subject fixed factor: gender; within-subject fixed factor: side and phase).

For kinematic evaluations, mandibular radius was included in the fixed part of the model as a covariate, to evaluate the dependence of sex differences on mandibular size. The first order interactions between the factors were also computed.

Since main effects of repeated-measures factors are independent of the between-participant covariate of mandible size, pure repeated-measures effects were reported from an analysis that excluded the covariate.

The significance level was set at 5% for all statistical analyses (p < 0.05).

RESULTS

All data within each subgroup (men, women) were normally distributed (Kolmogorov–Smirnov tests, p > 0.05) and the inter-group homogeneity of variances was always agreeable (Levene's test, p > 0.05).

No significant difference was found between male and female mean ages, whereas the mean dimensions of the mandible (radius and width) were significantly larger in men (tab. 1).

	Male (n = 9)		Female (n =	Female (n = 10)		
Measure	Mean	SD	Mean	SD	p-value	
Age [y]	28.2	7.6	26.5	8.3	NS	
Mandible radius [mm]	91.3	5.8	82.6	8.5	0.018	
Mandible width [mm]	128.5	5.4	118.2	5.4	0.001	

Table 1. Analyzed subjects, age and mandibular dimensions.

NS, not significant, p > 0.05.

	Male (n = 9)		Female (r	t-test	
Measure	Mean	SD	Mean	SD	p-value
POC mass. [%]	84.5	5.4	84.2	2.2	NS
POC temp. [%]	85.3	3.5	84.7	2.2	NS
ASIM (abs.) [%]	5.0	5.6	3.8	2.6	NS
TORS [%]	89.7	2.3	88.7	1.6	NS
TORQUE (abs.) [%]	3.6	4.2	5.0	3.8	NS
ATTIV [%]	-6.9	13.6	-5.8	11.7	NS
STD. IMPACT [%]	113	37	106	35	NS

Table 2. EMG indices in maximum voluntary contraction (MVC).

abs. = absolute value. NS, not significant, p > 0.05.

No sex related differences were found for any of the EMG indices evaluated during MVC (tab. 2). In both male and female groups the mean standardized clenching was symmetric, with only a weak torque component; temporalis muscles had a slightly larger normalized activity than the masseter muscles.

During the opening movements, the incisor moved caudally and dorsally, whereas the condyles moved caudally and ventrally (tab. 3). The vertical component of MMO was larger in men, but the difference with women was not statistically significant. Overall, mouth opening resulted remarkably symmetric, regardless of gender; mean coronal and horizontal angles were negligible.

	Male (n	= 9)	Female (n :	= 10)	ANCOVA
Measure	Mean	SD	Mean	SD	p-value
IP MMO [mm]	50.8	5.7	47.8	4.4	NS #
IP caudal component [mm]	43	5.5	39.2	4.2	NS #
IP dorsal component [mm]	26.4	6.4	26.6	6.7	NS #
IP lateral component [mm]	-1.2	1.3	-1.4	1.9	NS #
Sagittal angle [°]	33.5	5.1	34.6	2.3	NS #
Coronal angle [°]	0.7	3.8	-0.5	3.1	NS #
Horizontal angle [°]	1.3	1.4	2.3	1.6	NS #
CRP caudal component [mm]	9.8	4.1	8.9	3.9	NS &
CRP ventral component [mm]	14.5	6.8	11.8	6.6	NS &
CRP lateral component [mm]	-0.2	1.1	-0.2	1.5	NS &

Table 3. Kinematic range of motion of the mandible at Maximum Mouth Opening (MMO).

#, 1-way ANCOVA. &, 2-way ANCOVA, since the effect of side was not significant, right and left values were pooled; positive lateral components correspond to right displacements. NS, not significant, p > 0.05, refers to main factors and interactions.

During mandibular lateral displacement (tab. 4), the balancing condyle moved significantly more than the working condyle. The former showed greater vertical (downward) and sagittal (onward) ranges of motion (orbitant condyle), while the latter was almost still (pivoting condyle).

	Male (n	= 9)	Female (n	= 10)	ANCOVA
Measure	Mean	SD	Mean	SD	p-value
IP laterotrusion [mm]	9.9	2.1	8.9	1.9	NS &
wCRP caudal component [mm]	0.2	1.9	0.7	1.9	NS &
wCRP dorsal component [mm]	0.8	1.9	0.4	1.5	NS &
wCRP lateral component [mm]	1.1	1.6	1.3	1.1	NS &
bCRP caudal component [mm]	8.3	2.0	7.0	2.3	NS &
bCRP ventral component [mm]	8.1	3.0	6.9	2.2	NS &
bCRP medial component [mm]	1.8	1.4	1.8	0.8	NS &

Table 4. Kinematic range of motion of the mandible at maximum laterotrusion.

&, 2-way ANCOVA. Since the effect of side was not significant, right and left values were pooled. NS, not significant, p > 0.05, refers to main factors and interactions. w = working, b = balancing.

Similarly to mouth opening, mandibular protrusion was quite symmetric and a little larger in men than in women, even not significantly (tab. 5).

		= 9)	Female (n = 10)		ANCOVA
Measure	Mean	SD	Mean	SD	p-value
IP protrusion [mm]	9.1	1.5	8.0	2.3	NS #
CRP caudal component [mm]	6.6	2.3	5.3	1.9	NS &
CRP ventral component [mm]	8.6	1.9	7.6	3.2	NS &
CRP lateral component [mm]	0.1	0.7	-0.5	1.4	NS &

Table 5. Kinematic range of motion of the mandible at maximum protrusion.

#, 1-way ANCOVA. &, 2-way ANCOVA, since the effect of side was not significant, right and left values were pooled. NS, not significant, p > 0.05, refers to main factors and interactions.

Tables 3-5 describe the border movements of the mandible, reporting the ranges of motion of its reference points (lower interincisal point and condyles). No sex and side significant effects were found.

During mouth opening and closing standardized as a percentage of MMO distance (fig. 28), the relative contributions of mandibular rotation and translation were almost always similar in men and women, with differences ranging between 2 and 3% (2-way ANCOVA, p > 0.05).



Figure 28. Translation component of the condylar movement in mouth opening and closing as a percentage of the total mandibular movement. Since there was no significant sex effect at any step, male and female values were pooled.

The rotation component was prevalent during all the movement of mouth opening and closing, in particular at MMO; the peaks of relative condylar translation were reached near ICP. At the maximum displacement of the interincisal point, the overall percentage of mandibular movement explained by pure condylar translation was similar between men (mean, 29%; SD,

5%) and women (mean, 28%; SD, 7%); also their mean absolute values were close to each other (men, 21.8±6 mm; women, 20±6.5 mm). The effect of gender was not statistically significant, as well as side (right/left condyle), phase (opening/closing) and covariate (mandible radius) effects (3-way ANCOVA: p > 0.05).

During mastication, on average, chewing cycles were characterized by similar parameters of frequency and area between men and women, regardless of the working side (2-way ANCOVA). As shown in table 6, opening and closing had the same length, but the latter was significantly faster (3-way ANCOVA, phase factor: p < 0.0001 for both duration and velocity dependent variables). No significant sex, inter-side difference or covariate effects were found.

			E I . /		
	iviale ((n = 9)	Female (n = 10)	ANCOVA
Measure	Mean	SD	Mean	SD	p-value
Frequency [Hz]	1.23	0.34	1.26	0.29	NS &
IP area [mm ²]	26	13	17	8	NS &
IP ipsilateral area [%]	73	13	77	20	NS &
Opening length [mm]	14.1	2.5	11.8	2.6	NC Ć
Closing length [mm]	14.4	2.7	11.9	2.4	Ç CN
Opening duration [ms]	375	74	380	75	0 00 Ś
Closing duration [ms]	291	80	310	43	0.00 \$
Opening velocity [m/s]	0.04	0.011	0.033	0.008	0 00 Ś
Closing velocity [m/s]	0.07	0.045	0.048	0.017	0.00 \$

 Table 6. Kinematic parameters of unilateral gum chewing.

&, 2-way ANCOVA; \$, 3-way ANCOVA. Since the effect of side was not significant, right and left values were pooled. NS, not significant, p > 0.05, refers to main factors and interactions.

ANOVA <u>p-value</u> NS & NS & NS &

NS &

NS &

NS &

	Male (n	= 9)	Female (n = 10)		
Measure	Mean	SD	Mean	SD	
amplitude [%]	99	55	95	69	
Hotelling's ellipse area [% ²]	946	545	1069	1075	
Impact [%s]	632	308	688	380	

36

67

67

Table 7. Electromyographic parameters of unilateral gum chewing.

Impact/cycle [%s]

%w Impact [%]

SMI [%]

&, 2-way ANOVA. Since the effect of side was not significant, right and left values were pooled. NS, not significant, p > 0.05, refers to main factors and interactions. % w = percentage of the working side.

Table 7 shows that male and female subjects had also similar EMG parameters of mastication, regardless of the working side (2-way ANOVA, p > 0.05 for both the main effects and interaction). Overall, a large inter-subject variability was apparent.

17

9

21

37

69

67

21

11

17

Although the centres of the ellipses describing unilateral chewing were located in the first quadrants of the Lissajous diagram (left side chewing data were previously mirrored), thus indicating a prevalent activity of the working-side muscles, right working side chewing was characterized by a statistically significant lower phase than left working side chewing (fig. 29).



Figure 29. Lissajous phases of unilateral chewing, mean \pm SD. Two-way ANOVA: side, p = 0.005; sex and interaction, p > 0.05. The horizontal dashed line indicates the bisector of the first quadrant.

The qualitative analysis of chewing cycle morphology found that the percentage distribution of "ideal" (patterns I, II, III) and "anomalous" (patterns IV, V, VI, VII, VIII) frontal shapes (fig. 16), either between men and women or between right and left working sides, was similar (Chi-squared test: p > 0.05).

DISCUSSION

In the present control group, mandibular dimensions resulted on average larger in men than in women, in agreement with previously literature findings (Naeije, 2002; Ferrario et al., 2005; Mapelli et al., 2009).

The ranges of mandibular motion well compare with distances previously published for maximum mouth opening, laterotrusions and protrusion in healthy adults (Piehslinger et al., 1993; Travers et al., 2000; Buschang et al., 2001; Lewis et al., 2001; Ferrario et al., 2005). Nevertheless, MMO distance was in disaccord with other investigations, being both superior

(Gallo et al., 1997; Gallagher et al., 2004) and inferior (Merlini and Palla, 1988; Zwijnenburg et al., 1996) to what obtained by other investigators. Differences in sample composition (age, sex, ethnic origin, Gallagher et al., 2004), and in the measuring device, may explain the discrepancies. For instance, electromagnetic trackers were reported to create distortions for MMO larger than 40 mm (Lewis et al., 2001).

Saitoh et al. (2007) and Hayasaki et al. (2008) reported a less caudal movement of the balancing condyle during unilateral laterotrusion, but their subjects were instructed to achieve habitual lateral excursion.

Accordingly to previous investigations (Zwijnenburg et al., 1996; Naeije, 2002), but in contrast with others (Salaorni and Palla, 1994; Gallo et al., 1997; Lewis et al., 2001; Ferrario et al., 2005), no sex-related differences in mandibular kinematics were found. Our use of a condylar reference point located 15 mm medial to the digitized point previously palpated may partially explain some of these differences.

In the current investigation, no right-left side differences were found, in accord with other studies (Travers et al., 2000; Hayasaki et al., 2008). In contrast, Buschang et al. (2001) reported significantly larger movements of the left than of the right condyle during protrusion and laterotrusion in healthy adults. Similar amounts of left-side dominance in condylar protrusive movements have been already reported (Harper, 1990; Piehslinger et al., 1993; Theusner et al., 1993; Gsellmann et al., 1998). The authors related these findings to normal morphological asymmetry or asymmetrical constraints, thus, not necessarily diagnostic of craniomandibular disorders (Kenworthy et al., 1997).

In mouth opening and closing, mandibular translation was present during the whole motion (Lotters et al., 1996; Lewis et al., 2001; Mapelli et al., 2009), it was always smaller than rotation, and decreased during mouth opening. A similar finding was reported by Lotters et al. (1996), although they also showed a relatively reduced translation near ICP. In contrast, a radiographic study found a rotating component of about 14–30% in the initial steps of jaw motion (Wu et al., 1988). Leader et al. (2003) found a larger translation than rotation in the first phases of mouth opening. Anatomically, movement decomposition reported in figure 28 during mouth opening could be due to the progressive passive block provoked on the head of mandible by the ligament tension (Matsumoto et al., 1995; Lotters et al., 1996), which impedes further antero–inferior translation. During mouth closing, after the first steps in which the blockage remains, the translation component progressively increases: the elastic recall of the ligaments outclasses the active blocking system (Lotters et al., 1996; Yatabe et al., 1997).

The present total opening translation did not differ from the closing one, in accord with some previous studies (Travers et al., 2000; Lewis et al., 2001; Mapelli et al., 2009), but in contrast with other ones (Lobbezoo et al., 2000); our findings do not support the notion that the condylar opening pathway is shorter than the closing pathway (Yatabe et al., 1997).

Again, no sex-related differences in condylar path lengths were found, according to previous reports (Salaorni and Palla, 1994; Yatabe et al., 1995; Lotters et al., 1996; Zwijnenburg et al., 1996; Naeije, 2002). The current results do not support the theory of a more mobile TMJ in women than in men (Gallagher et al., 2004). According to that theory, the longer component of condylar translation in women is due to the greater laxity of the sphenomandibular ligament, which would be stressed later in motion (Lotters et al., 1996).

A pre-softened gum was chosen to obtain a standardized and constant (volume and weight) bolus all over the chewing test. This food should maintain its characteristics during the complete chewing trial. Moreover, no significant modifications of its texture are to be expected in this short duration (15 s for each trial).

The form or shape of the human masticatory cycle has been of interest for many years (Buschang et al., 2000). Several morphological classifications have been proposed for the analysis of masticatory pattern. Proschel and Hofman (1988) analyzed the masticatory movement in detail and classified it into 196 patterns. It would have been difficult, however, to classify less than 50 strokes counted per each subject into 196 patterns. In contrast, Akiyama et al. (1991) proposed a more simple and practical classification system, which consists of 8 patterns (see Materials and Methods section). This latter classification, which was used in the present study, contains typical masticatory patterns, such as grinding (patterns I, II, and III), reverse (pattern VI), and chopping (pattern VIII). Pattern I, II, III were deemed "ideal", whereas the other 5 were referred to as "anomalous". In the current study, no difference was found between the distribution frequencies of "ideal" and "anomalous" patterns in healthy subjects. Actually even "normal" subjects show some anomalous strokes between the ideal teardrop-shaped patterns to accomplish several tasks. And, of course, controversy remains regarding what constitutes an "ideal" chewing pattern (Yamashita et al., 1999).

It has been indicated that the masticatory pattern movement should be examined not only morphologically, but also in terms of masticatory rhythm, because this last is often influenced by malocclusion (Youssef et al., 1997). Hence, cycle frequency, opening and closing phase duration and velocity were examined. Neither left-right, nor sex differences were found on mean values of these kinematic parameters. The only significant difference was between phase velocities,

being closure faster than mouth opening, probably due to the elastic recall of the ligaments and the concentric contraction of the elevator muscles of the mandible.

The mean frontal area of chewing cycles was similar to that found by Evans and Lewin (1986), who reported an average value of 20.6 mm². By contrast, the present values are notably lower than the ones obtained by Chew et al. in 1988 (33.0 mm²). However, it should be outlined that all these studies have in common the detection of a great inter- and intra-subject variability (Throckmorton et al., 2001; Hayasaki et al., 2003) even in healthy subjects, which makes the simple consideration of the mean value unreliable. In 2006, Ferrario and co-workers quantitatively analyzed the variability of unilateral chewing movements in young adults. The highest between subjects/ between sessions variance ratios were found for cycle duration and shape, whereas the spatial characteristics of gum chewing cycles had a large within-subject variability (Ferrario et al., 2006a).

Among the jaw elevator muscles, the masseter and temporalis muscles are those most often assessed in clinical evaluations because they are the most superficial, and they are the only accessible to surface EMG examination. In contrast, the medial and lateral pterygoid muscles can be evaluated only with needle EMG. Indeed, in the assessment of stomatognathic dysfunction and several head disorders, the analysis of masseter and temporalis muscles can provide quantitative functional data with minimal discomfort to the patient and without invasive or dangerous procedures (Visser et al., 1995; Sato et al., 1998; Liu et al., 1999; Burnett et al., 2000; Pinho et al., 2000; Ferrario et al., 2002, 2004b; Suvinen et al, 2003; Landulpho et al., 2004).

Unfortunately, as underlined by several researchers, this simple, low cost, and fast exam also has many limitations that must be carefully considered and eventually removed (DeLuca, 1997). For instance, technical artifacts (the instrumental noise), the thickness of the skin fat layer, crosstalk from different muscles. Therefore, a correct EMG assessment should be performed only with standardized (normalized) potentials, thus removing most of biological and technical noise (DeLuca, 1997).

In the current study, to reduce patient variability, the EMG protocol comprised a normalization record (a MVC on cotton rolls performed just before the recording of the actual tests, i.e. with the same electrodes, cables, and EMG apparatus, and on the same cutaneous area) that should limit biologic and technical noise (DeLuca, 1997; Burnett et al., 2000).

Standardized EMG potentials can allow one to verify and quantify the muscular equilibrium, both between the muscles of the two sides of the body (symmetry: POC and ASIM indices for

MVC; SMI index for mastication) and between couples of muscles with a possible laterodeviant effect on the mandible (TORS and TORQUE indices for MVC).

Also, EMG can allow the measurement of the actual impact of morphology on stomatognathic function (Visser et al., 1995; Sato et al., 1998; Liu et al., 1999; Burnett et al., 2000; Pinho et al., 2000; Ferrario et al., 2002, 2006b; Landulpho et al., 2004). From the standardized electric potentials produced by the single masticatory muscles, the muscular activity (Impact, integrated value in time) can be calculated to assess the actual effort made by the muscles (Sato et al., 1998; Burnett et al., 2000; Ferrario et al., 2004b, 2006b; Tartaglia et al., 2008a). Quantitative analyses of the patterns of muscular contraction during standardized dynamic activities allow one to assess neuromuscular coordination (Ferrario and Sforza, 1996).

In the current study, sex did not seem to influence patterns of contraction of the analysed muscles, either during MVC or mastication, as previously reported (Ferrario et al., 2006b; De Felicio et al., 2009b; Tartaglia et al., 2011). Indeed, this aspect has been scanty analyzed in previous studies; in some occasions, only one sex was assessed (Kroon and Naeje, 1992; Gay et al., 1994; Farella et al., 2002), no information about sex was given (Bazzotti, 1999), or no comparisons were made (Koyano et al., 1995). A possible explanation for the lack of sex specificity may have to do with the use of gum (Gerstner and Parekh, 1997); previous studies reported that gum reduces the level of sex specificity in mastication (Neill and Howell, 1986; Howell, 1987).

Current mean values of MVC parameters were in agreement with data obtained in previously analysed control groups (De Felicio et al., 2009b; Tartaglia et al., 2011), indicating low asymmetry between right and left muscle pairs and balanced contractile activities of contralateral masseter and temporalis muscles (low lateral displacing force). The activity value was low, indicating a good antero-posterior charge distribution at the mandible. The standard deviation for each index was limited. In addition, the Impact value was compatible with that observed for control subjects (De Felicio et al., 2009b; Tartaglia et al., 2011).

During mastication, EMG activity of the masseter and anterior temporalis muscles of the chewing side was higher than that of the other side, as previously reported (Miyawaki et al., 2000, 2001; Piancino et al., 2008). The right and left Lissajous's figures, as reported by SMI index, were overall symmetrical, with similar position within the Cartesian axes, and similar size and shape, indicating comparable patterns of right- and left-sided mastication with regard to the chewing forces and cooperation between muscles. The lack of significant right-left side differences is in good accord with other literature data (Kumar et al., 2001, 2003; Farella et al., 2002), even if some investigations reported asymmetric findings (Gay et al., 1994).

However, right working side chewing was characterized by statistically significant lower phase than left working side chewing. The right Lissajous's figure closer to the masseter axis indicates that, on average, the temporal and masseter muscles of the working (right) side contracted more strongly in gum mastication, but the difference in masseter activity between the working and the balancing sides was larger than the difference in the temporal activity.

Even in the present highly selected subjects whose occlusion was good from a morphofunctional point of view, the presence of this asymmetric behaviour seems to be an intrinsic characteristic of occlusion, independently from biological noise.

Overall, even when standardized, coherent boluses were used in well standardized conditions, chewing in normal subjects seems to be highly variable (Hayasaki et al., 2003), as it is reported in the current study. A large variety of movement patterns can be found even within a single individual chewing the same coherent bolus (Proschel, 1987; Ferrario et al., 2006a). The findings of the present investigation are obviously strictly inherent to the extremely standardized protocol and cannot be directly extended to natural chewing (free movements of bolus in both sides of mouth) or to other foods with different mechanical characteristics.

The principal limitation of this study is the number of analysed subjects, which was under the proper sample size to avoid type II errors. Indeed, from a starting group of more than a 50 young adults, less than half of them fulfilled the inclusion criteria. In particular, a large number of men and women had received or were receiving orthodontic treatment.

These normal data provide a first reference for the assessment of patients with alterations in the cranio-mandibular system (Study II and III).

Study II – PATIENTS WITH MODERATE TMD

INTRODUCTION

Temporomandibular disorders (TMD) consist of a number of clinical problems that involve the masticatory musculature, the temporomandibular joint and associated structures or both. Approximately 7–15% of the adult population is affected, with higher prevalence in women at reproductive ages. The most frequent complaints reported by subjects with TMD are pain in the TMJ and/or masticatory muscles, TMJ sound and difficulty to chew (Michelotti and Iodice, 2010). Additionally, some patients do not come to observation with acute problems, and chronic disabilities increase the diagnostic problems (Epker et al., 1999).

In an effort to make diagnosis as objective as possible, several protocols for history taking and clinical assessment have been proposed (De Felicio et al., 2009a); among the most used, there are the Research Diagnostic Criteria for temporomandibular disorders (RDC/TMD).

Multiple treatments have been employed, although the pathophysiology is not fully understood and the mechanisms of action of the treatments may be not clear (Cairns, 2010; Michelotti and Iodice, 2010). According to literature, in certain situations, an adequate muscular training may allow a better function, reducing pain and disability (Sforza et al., 2010a). The orofacial myofunctional therapy has demonstrated positive effects on the reduction of symptoms and clinical signs of TMD, as well as improved swallowing and chewing functions (De Felicio et al., 2010). The classic dental and skeletal etiologic theories of TMD have been challenged and refuted by recent studies conducted around the world (Klasser and Greene, 2009). The new approaches are focusing on the relationship between oral motor function and TMD (Lobbezoo et al., 2006; Ardizone et al., 2010; Douglas et al., 2010). Mastication may be hampered by TMD (Bakke and Hansdottir, 2008); as a result, limited masticatory function is one of the problems that patients with TMD encounter. Rehabilitation to improve masticatory function is therefore one of the goals in the treatment of TMD. Thus, it seems important to understand the link between pain-related injury and the stomatognathic status. Furthermore, studying coordination patterns under normal and pain circumstances may be possible to unfold "efficient" versus "inefficient" movement strategies, which knowledge can in turn be integrated into training and rehabilitation strategies (Cote and Hoeger Bement, 2010).

Most of the TMD studies rely on self-assessed masticatory function obtained from questionnaires, whereas only a limited number of studies has measured the masticatory performance objectively.

The current gold standard to identify the presence or absence of TMD still remains mainly based on clinical examination supplemented, when deemed appropriate, with imaging (Klasser and Okeson, 2006). Therefore, other objective, quantitative methods are needed to supplement the diagnosis of TMD, and to monitor the effectiveness of the relevant treatments.

Indeed, the mandibular movement changes are often perceptible in the presence of TMJ disorders (Merlini and Palla, 1988); thus, the detection of movement changes could be used as both a TMD diagnostic tool and a success criterion for treatment (Lemoine et al., 2007).

Currently, also surface electromyography (EMG) can make an objective recording of the masticatory muscle function and dysfunction (Gay et al., 1994; Suvinen et al., 2003; Castroflorio et al., 2005, 2008; Ferrario et al., 2007; Tartaglia et al., 2008a, 2011; De Felicio et al., 2009b). Most of the previous EMG investigations made on symptomatic TMD patients analyzed severe pain conditions, finding that their masticatory muscles were more asymmetric and more easily fatigued, less efficient and coordinated, and produced reduced electric potentials and bite forces when compared to those of healthy subjects (Suvinen et al., 2003; Ferrario et al., 2007; Tartaglia et al., 2008a).

In the current study, masticatory function of patients with mild-moderate TMD, who did not seek treatment, has been objectively determined, assessing bite stability, mandibular border movements and masticatory performance. Both EMG and jaw kinematic parameters were evaluated and compared to those of the normal subjects collected in Study I.

METHODS

Subjects and data collection

Twenty patients with mild-moderate TMD (5 men and 15 women), aged 22–56 years, were analyzed in this study, and compared to the control group previously assessed (Study I). To be recruited in the pathologic group, patients had to present TMD according to the Research Diagnostic Criteria for TMD (RDC/TMD, Dworkin and LeResche, 1992), short lasting (at most 6-month duration) mild-moderate severity, according to ProTMDmulti protocol (De Felicio et al, 2009a), and they should not have been seeking treatment.

All subjects were evaluated by the same experienced examiner, specialist in TMD and orofacial pain, according to the RDC/TMD; the clinical assessment featured auscultation for joint noise and inspection of tenderness to palpation in the masseter, temporal, supra-hyoid muscles and by the TMJ.

The ProTMDmulti questionnaire was used to determine the subjective perception (presence and severity) of TMD signs and symptoms. The questionnaire is divided in two parts. The first part checks the presence of TMD signs and symptoms with a series of 12 questions requiring a positive or negative reply. In the second part, the subjects are asked to indicate the severity of 9 signs and symptoms (muscular pain, TMJ pain, neck pain, otalgia, tinnitus, ear fullness, tooth sensitivity, joint noise, difficulty to swallow) felt (or not) according to the situation (when waking up, during mastication, when speaking, at rest). Severity is indicated on a printed 11 point numerical scale, where zero corresponds to the complete absence of the symptom, and 10 corresponds to the highest possible severity. The total severity score is obtained by summing all the single severity scores (total range, 0-360).

According to RDC/TMD (axis I), the patients with TMD were classified as follows: 4 with miofascial pain and arthralgia, 16 manifesting disc displacement with reduction (14, bilateral DDR; 2, unilateral DDR) together with miofascial pain and/or arthralgia.

According to ProMultiTMD protocol, all subjetcs reported joint noise and one or more symptoms like headache (14), difficulty in mandibular movements (12), muscle pain (10), ear fulness (6) and tinnitus (4). The mean total severity score was 33.3 for the TMD group and 3 for the control group.

Furthermore, all the TMD patients had a permanent dentition, with at least one molar maxillarymandibular contact per dental hemiarch, no dental pain or periodontal problems, no previous or current orthodontic, tumors or traumas in the head and neck region, no neurological or cognitive deficit.

Statistical analysis

Descriptive statistics were calculated for all the parameters of the complete protocol, as already done in Study I.

The normal distribution of data was checked with the Kolmogorov-Smirnov test.

Then, all the parameters were compared between control (Ctrl) and TMD groups by means of Student's t-test for independent samples. The Fisher's exact test and the Chi-squared test were used, respectively, to test the homogeneity of sex and chewing pattern distributions in the two groups.

The significance level was set at 5% for all statistical analyses (p < 0.05).

RESULTS

All data were normally distributed within each group (Kolmogorov–Smirnov tests, p > 0.05). Patients and healthy subjects had similar estimated age and mandibular dimensions (tab. 8). The sex distribution was not significantly different in the two analyzed groups (Fisher's exact test: p = 0.19).

	Healthy (n = 9 M + 10 F)		TMD (n = 5 N	t-test	
Measure	Mean	SD	Mean	SD	p-value
Age [y]	27.3	7.8	30.0	10.4	NS
Mandible radius [mm]	86.7	8.4	83.5	6.8	NS
Mandible width [mm]	123.1	7.5	121.2	8.3	NS

Table 8. Analyzed subjects, age and mandible dimensions.

NS, not significant, p > 0.05.

Table 9.	EMG indice	es in	maximum	voluntary	contraction	(MVC).	
						· · · / ·	

	Healthy (n = 9	9 M + 10 F)	TMD (n = 5 M + 15 F)		t-test
Measure	Mean	SD	Mean	SD	p-value
POC mass. [%]	84.4	3.9	78.8	11.6	0.052
POC temp. [%]	85.0	2.8	81.0	8.4	0.055
ASIM (abs.) [%]	4.4	4.2	10.4	9.1	0.013
TORS [%]	89.2	2.0	85.3	8.9	0.075
TORQUE (abs.) [%]	4.4	4.0	9.8	10.9	0.047
ATTIV [%]	-6.4	12.3	-5.1	21.5	NS
STD. IMPACT [%]	109	35	108	39	NS

abs. = absolute value. NS, not significant, p > 0.08.

Seemingly, TMD patients were less symmetric (smaller POC of masseter and temporalis muscles) and had a larger torque component than healthy subjects (p-value close to the significance threshold). The TMD group had an overall standardized activity (STD. IMPACT) nearly identical to that measured in the control group.

The inter-individual variability was always greater in the TMD group.

For all variables of interincisal point and condylar reference points, at MMO, protrusion and lateral excursions, there were no differences between the groups (fig. 30 and 31, t-test: p > 0.05). In particular, at MMO, the absolute values of IP lateral displacement (mean±SD, C: 1.7 ± 1.0 mm; TMD: 1.5 ± 1.1 mm), coronal angle (C: $2.7\pm2.0^{\circ}$; TMD: $2.4\pm2.3^{\circ}$) and horizontal angle (C: $1.9\pm1.4^{\circ}$; TMD: $2.5\pm2.0^{\circ}$) were not different between the two groups.



Figure 30. IP displacement and sagittal angle at MMO, mean+SD. t-test: p > 0.05.



Figure 31. IP maximum lateral and ventral displacements, mean+SD. t-test: p > 0.05.

During mouth opening and closing, standardized as a percentage of MMO distance, the relative contributions of mandibular rotation and translation were not significantly different between healthy subjects and patients (t-test: p > 0.05).



Figure 32. Translation component of the right condyle in opening and closing. Case report of a 32-years-old TMD female patient.

Figure 32 illustrates a case report of a female patient with DDR; the reduction of the disc is recognizable as the peak of almost pure translation that occurred during closing.

During mastication, the kinematic characteristics obtained in patients were well comparable to those of healthy individuals (tab. 10). Again, the closing phase was faster than the opening phase. EMG parameters, instead, revealed some differences between the two groups (tab. 11): TMD patients had significantly larger standard activity of the 4 analyzed muscles and intrasubject variability (the mean Hotelling's ellipse had a doubled area). Overall, also the intersubject variability was increased relative to the control group.

	Healthy (n =	Healthy (n = 9 M + 10 F)		M + 15 F)	t-test
Measure	Mean	SD	Mean	SD	p-value
Frequency [Hz]	1.25	0.30	1.27	0.23	NS
IP area [mm ²]	21	11	19	13	NS
IP ipsilateral area [%]	75	17	73	20	NS
Opening length [mm]	12.9	2.7	13.1	2.5	NS
Closing length [mm]	13.1	2.8	13.2	2.5	NS
Opening duration [ms]	378	73	381	66	NS
Closing duration [ms]	301	62	298	59	NS
Opening velocity [m/s]	0.036	0.010	0.036	0.009	NS
Closing velocity [m/s]	0.058	0.034	0.052	0.014	NS

Table 10. Kinematic parameters of unilateral gum chewing.

Right and left values are averaged. NS, not significant, p > 0.05.

 Table 11. Electromyographic parameters of unilateral gum chewing.

	-	•
	Healthy $(n = 9 M + 10 F)$	TMD (

	Healthy (n = 9 M + 10 F)		TMD (n = 5 M + 15 F)		t-test
Measure	Mean	SD	Mean	SD	p-value
amplitude [%]	97	61	110	50	NS
Right phase [°]	27	22	20	41	NS
Left phase [°]	50	20	39	37	NS
Hotelling's ellipse area [% ²]	1010	845	2275	2734	0.061
Impact [%s]	662	340	1099	526	0.004
Impact/cycle [%s]	37	19	57	28	0.013
%w Impact [%]	68	10	64	13	NS
SMI [%]	67	18	60	30	NS

Right and left values are pooled (except for the phase). % w = working side %. NS, not significant, p > 0.07.

In the control group, all subjects had the centres of the ellipses describing unilateral chewing located within the first (right-side chewing) and third (left-side chewing) quadrants of the Cartesian coordinate system, thus indicating a prevalent activity of the working-side muscles. Among TMD patients, instead, 9 subjects had at least one of their unilateral chewing cycles with the centre of the ellipse positioned outside the correct quadrant.

For what concern the chewing cycle morphology (tab. 12), the patients performed significantly more anomalous patterns than ideal ones (Chi-squared test: p = 0.000).

	ideal	anomalous
Healthy	281	311
TMD	141	421

Table 12. Chewing pattern distribution frequency.

Right and left side cycles are pooled. Chi-squared test: p = 0.000.

DISCUSSION

In the present study, signs and symptoms were classified according to the RDC/TMD criteria (Dworkin and LeResche, 1992), the reliability of which has been demonstrated in a multicentre international study (John et al., 2005).

Even not significantly heterogeneous, sex distribution was not the same in the two analyzed groups; female patients were remarkably more than men in TMD group. The larger percentage of women experiencing TMD is well-known (Epstein and Klasser, 2011; Mobilio et al., 2011), and may be somehow related to inherent sex-related differences in jaw and cervical muscular composition, activity, contraction and recovery (Mobilio et al., 2011). Another theory asserts that women probably adapt to orofacial pain differently than men do, and this could be one reason why TMD appears to be more common in women (Gerstner and Parekh, 1997).

However, in the current study, the effect of sex was assumed negligible, since it has never resulted significant in the control group (Study I). Age and mandibular dimensions were well comparable between the two groups.

Moreover, since signs and symptoms were overall bilateral for all patients, no "healthy" or "pathologic" sides were separately evaluated for patients.

Kinematic data of mastication and mandibular border movements resulted quite the same in both TMD and control subjects; patients' movements were also as symmetric as in healthy individuals. Admittedly, mastication was done with a chewing gum, which is easy to masticate and performed without crushing, breakdown, or selection of food particles; such a simple pattern of jaw closing for compression of the bolus might remain unaffected by the TMD in some patients (Yashiro and Takada, 2005).

Notwithstanding, the patients performed significantly more anomalous unilateral chewing patterns than ideal ones. In general, the most frequent chewing pattern in healthy subjects is a smooth, uncrossed, teardrop-shaped pattern (Yamashita et al., 1999); TMD anomalous cycle shapes were three times more frequent than ideal ones, whereas in the control group there was an almost balanced distribution.

During mouth opening and closing, the relative contributions of mandibular rotation and translation were not significantly different between healthy subjects and patients. However, it should be noticed that this kind of assessment, when different subjects are pooled, mostly in pathologic group, might conceal highly relevant individual features, as shown in figure 32.

The quantitative EMG characteristics of the masticatory muscles of TMD patients during standardized teeth clenching were found to differ from those recorded in healthy control subjects

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without TMJ alterations. Overall, TMD patients showed more asymmetry between the standardized activity of muscle pairs (POC temporal and masseter, ASIM) and larger unbalanced contractile activities of controlateral masseter and temporal muscles (TORS and TORQUE). Both findings are in accord with the data previously collected on patients with moderate to severe TMD by Ferrario et al. (2007). Asymmetry of muscle activities have already been reported also in long lasting (Santana-Mora et al., 2009; Tartaglia et al., 2011) and acute TMD patients (Tartaglia et al. 2008a). While the current findings on POC indices well parallel the previous data, the lack of significant differences on the total muscle activity (standardized IMPACT) is in accord with some (Tartaglia et al., 2011), but not all investigations (Ferrario et al., 2007; Tartaglia et al., 2008a), and may be related to the different TMD level of the patient groups.

Consequently, according to the current and previous experimental findings, the temporalis and masseter muscle asymmetries seem to be useful parameters in differentiating between patients and healthy people when MVC is performed.

In the chewing test recorded in the current study, both larger Hotelling's ellipses (lower coordination) and larger standardized muscular activities (likely higher energy) were found in patients with TMD. Overall, when a larger energy is spent for the same effort, efficiency decreases, and muscles may become fatigued earlier. Several reports found out that mean contractile not-standardized activity at rest was higher in TMD patients than healthy subjects, indicating basal hypertonia; during clenching, by contrast, it was about half that observed in healthy subjects (Visser et al., 1995; Sato et al., 1998; Pinho et al., 2000). Since our impact index is normalized on cotton rolls recordings, it may conceal this occurrence. Accordingly, the larger Impact found during mastication of TMD patients may not mean necessarily higher energy cost.

The right and left Lissajous figures of gum-chewing were not symmetrical in many TMD patients, and their total pattern varied greatly (high inter-subject variability); this aspect was also outlined by the wide range of the SDs. The asymmetrical figures indicate a difference of chewing patterns between right- and left-sided mastication. Deformation of the normal figure, the extent of which is represented by the deviation of phase angle from 45°, is a reflection of either imbalance in basic activity between the paired muscles or great differences between the activity of the temporalis and masseter muscles, indicating jaw movement in a biased direction. Ramfjord and Ash (1983) reported that most patients with unilateral pain in the TMJ try to chew on the involved side, and conjectured that this was due to less pressure on the working condyle than on the balancing condyle. Biomechanical calculations confirmed that altered muscular activities, with asymmetric contractions and increased torque, could increase TMJ load (Ferrario

and Sforza, 1994). However, the continuous unilateral chewing could result in abnormally high levels of stress on the joint apparatus or on the muscles on the working side, which in turn could lead to dysfunction on the habitual working side (Kumai, 1993).

From all these findings, it could be surmised that in cases of mild-moderate TMD, the change in muscle recruitment may be a compensatory mechanism for pain relief, and asymmetrical muscle recruitment may precede the muscle pain symptoms (Lobbezoo et al., 2006). The strategy of differential activation, which is related to the functional complexity of the sensory–motor system and to the multidimensional nature of pain (Peck et al., 2008), protects the injured muscle/joint while simultaneously maintaining optimal function (i.e. no apparent kinematic differences with respect to the control group). In case of depletion or insufficiency of such adaptive capacity, the system will produce a failure in some point, which will be manifested as a sign or a symptom (Douglas et al., 2010). The sequence events may vary but, once the TMD springs up, the stomatognathic system is no longer able to withstand the functional loads without the occurrence of some discomfort, pain and/or compensation.

Study III – PATIENTS WITH DIAGNOSIS OF CLASS III DENTOSKELETAL DEFORMITY, BEFORE AND AFTER ORTHOGNATHIC SURGERY

INTRODUCTION

Patients with diagnosis of Class III dentoskeletal deformity are characterized by mandibular prognathism and/or maxillary deficiency, with a more anterior mandible (or lower jaw) relative to the maxilla (or upper jaw). Before treatment (orthognathic surgery), which consists in setting back the mandible and/or advancing the maxilla (figure 33), these patients suffer not only from morphological abnormalities but also from a number of functional deficits such as reduced maximum bite force, altered occlusal contact areas, reduced ability to break down food, altered mandibular kinematics and masticatory muscles deficit (Throckmorton, 2006). Indeed, dysfunction and aesthetics are the two major indications for orthognathic surgery.

While aesthetic problems are widely investigated, the literature on dysfunctional deficits is poor because mainly consists of retrospective uncontrolled studies. In the last 25 years several authors investigated different aspects of jaw function recovery after orthognathic surgery: occlusal contact areas, muscle activity, masticatory efficiency, mandibular range of motion, maximum bite force, neurosensory function, Helkimo Index and questionnaires on patients' perceived function (Melsen et al., 2010). These studies have shown that the prevalence of functional disorders is larger among patients submitted to orthognathic surgery than in the normal population.

While overall function may appear clinically normal (for instance, a normal amount of mouth opening), on a detailed examination the pattern of motion is often altered (Sforza et al., 2010b). Unfortunately, most of the examinations that have been proposed in literature cannot provide a three-dimensional reconstruction of the motion of the mandibular (occlusal) plane. This information may provide useful insight about temporomandibular joint (TMJ) function in III class patients, and the changes following surgery, complementing the clinical examination.

In a previous pilot study (Sforza et al., 2010b), the rotation and translation component of the mandible at maximum mouth opening (MMO) were assessed in a group of patients successfully rehabilitated after orthognathic surgery. Notwithstanding mandibular motion was clinically well restored after orthognathic surgery, the kinematics of the joint was modified. Unfortunately, data of pre-surgery patients were not available, and no information about condylar movements were provided.

The aim of the current study was to assess the recovery of mandibular range of motion in border movements, its rotational and gliding components during mouth opening and closing, and to provide information on the changes in mandibular condylar motion, analyzed before (Presurgery group) and after (Post-surgery group) orthognathic surgery for the correction of skeletal Class III malocclusions.



Figure 33. Graphic representation of maxillary advancement coupled with bilateral sagittal split mandibular osteotomy (website [1], modified).

METHODS

Subjects and data collection

The "Pre-surgery" group was composed of 10 patients (3 men, 7 women; age range 17-27 years) with a skeletal Class III malocclusion, all scheduled for orthognathic surgery (Le Fort I maxillary advancement coupled with bilateral sagittal split mandibular osteotomy, BSSO). All subjects were recruited by a private practice dentist from Pavia (Italy). The inclusion criteria were Skeletal Class III malocclusion, no history of surgery on both jaws, complete set of dentition, no syndromic or medically compromised patients.

The "Post-surgery" group was composed of 9 patients (3 men, 6 women; age range 22-48 years) surgically treated for skeletal Class III malocclusion. All subjects were recruited by the same operator. The inclusion criteria included Skeletal Class III malocclusion prior to surgery, no other history of surgery on both jaws and complete set of dentition, no syndromic or medically compromised patients. Preoperatively no patient had TMJ disorders. Preoperative orthodontic

treatment had been given before Le Fort I maxillary advancement (all patients), coupled with bilateral sagittal split mandibular osteotomy (BSSO, 5 patients). The treatment was finished with postoperative orthodontics. The patients were assessed 12-30 months after the surgery (on average, 20±6 months). They all had clinically satisfactory healing and restoration of function: pain-free, absence of sounds in the TMJ, good mandibular opening with negligible latero-deviation, laterotrusive movements with canine disclusion, good protrusive movement, and symmetrical position of the gonia (Sforza et al., 2010). Both groups underwent only mandibular border movement recordings.

Statistical analysis

In both *Pre* and *Post* groups, descriptive statistics were calculated for all the parameters describing mandibular border movements.

For each dependent variable, Kruskall-Wallis test (K-W-test) was applied among the control group (Ctrl) previously assessed (Study I), and the current *Pre* and *Post* groups. Post hoc Mann-Withney U-tests were computed to deepen statistically significant K-W-tests.

One-sample t-tests were also adopted to test the hypothesis of null values of Cardan angles in maximum mouth opening (MMO) and protrusion.

The significance level was set at 5% for K-W-test and t-test (p < 0.05); at 1.7% for post hoc U-test, after Bonferroni's correction (p < 0.017).

RESULTS

Patient groups and healthy subjects had similar estimated mandibular dimensions, whereas presurgery patients were significantly younger than post-surgery patients (post hoc U-test: p = 0.012, tab. 13).

 Table 13.
 Analyzed subjects, age and mandibular dimensions.

	Control (n = 19)		Pre (n =	Pre (n = 10)		Post (n = 9)		U-test
Measure	Mean	SD	Mean	SD	Mean	SD	p-value	(p< 0.017)
Age (y)	27.3	7.8	22.3	4.1	35.7	10.8	0.021	Pre vs Post
Mandible radius (mm)	86.7	8.4	89.3	3.1	88.8	5.9	NS	
Mandible width (mm)	123.1	7.5	118.1	6.7	123.4	6.8	NS	

NS, not significant, p > 0.05. Pre vs Post: U-test, p = 0.012.

During laterotrusions and protrusion, the ranges of motion evaluated by the lower interincisor and the two condylar reference points were not significantly different among the three groups (K-W-test: p > 0.05).

Several differences were found at maximum mouth opening for both general mandibular motion (tab.14, figure 34) and condylar pathways (tab. 15, figure 35).

		MMO	IP caudal	IP dorsal	Sagittal
	Measure	[mm]	component [mm]	component [mm]	angle [°]
Control (n = 19)	Mean	49.2	41.0	26.5	34.1
	SD	5.1	5.1	6.4	3.8
Pre (n = 10)	Mean	42.8	32.8	26.9	28.8
	SD	8.7	6.0	8.6	7.5
Post (n = 9)	Mean	49.2	40.0	28.2	33.8
	SD	7.5	5.0	8.1	6.6
K-W-test	p-value	0.022	0.004	NS	NS
Post hoc U-test	Ctrl vs Pre	0.007	0.002		
	Pre vs Post	0.050	0.013		
	Ctrl vs Post	0.961	0.446		

Table 14. Kinematic range of motion of the mandible at Maximum Mouth Opening (MMO).

NS, not significant, p > 0.05.



Figure 34. IP displacement and sagittal angle at MMO, mean+1SD.

Pre-surgery III class patients opened their mouth significantly less than the control group, whereas post-surgery patients regained the normal range of motion. In particular, this difference was completely due to the significant reduced caudal component, which was in turn partially explained by a rather limited sagittal angle (tab. 14, figure 34).

Overall, however, mouth opening was symmetric in each group, being the horizontal and coronal angles not significantly different from 0° (t-test, p > 0.05).

-		R_CRP caudal	L_CRP caudal	R_CRP ventral	L_CRP ventral
	Measure	component [mm]	component [mm]	component [mm]	component [mm]
Control (n = 19)	Mean	9.3	8.8	13.1	12.0
	SD	3.9	2.7	6.7	5.7
Pre (n = 10)	Mean	4.3	4.6	6.5	7.3
	SD	3.5	3.5	5.2	5.9
Post (n = 9)	Mean	5.7	5.7	9.6	10.9
	SD	3.8	3.1	2.4	3.9
K-W-test	p-value	0.006	0.004	0.041	0.175
Post hoc U-test	Ctrl vs Pre	0.003	0.004	0.016	
	Pre vs Post	0.165	0.327	0.165	
	Ctrl vs Post	0.052	0.016	0.248	

Table 15. Condylar linea	ar displacements at MMO.
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R, right; L, left.

In the pre-surgery group both condyles moved downward significantly less than in healthy subjects, and this limitation was not recovered in post-treatment subjects. Also, III class patients, on average, had a right condyle that moved forward significantly less than the control group, with only a partial improvement of the performance in post-surgery patients; a similar behaviour was observed for the left condyle, even if it was not statistically significant (tab. 15).

Subsequently, the global path of the condyle resulted significantly reduced in pre-surgery patients, and the treatment did not help in reaching normal gliding condylar movements (fig. 35).



Figure 35. Condylar path lengths described during mouth opening and closing in the three groups, mean±1SD. Right and left condyle paths are averaged. K-W-test: p = 0.002. Significant post hoc U-test p-values are displayed for opening/closing.

During mouth opening and closing, standardized as a percentage of MMO distance, the relative contribution of condylar rotation and translation was significantly different between healthy subjects and untreated patients during the middle part of opening and the last part of closing. These discrepancies resulted only moderately modified by surgery (fig. 36), while the overall difference resulted unchanged (tab. 16).



Figure 36. Gliding component of the mandibular movement in opening and closing as a percentage of the total mandibular displacement. *Ctrl vs Pre: p < 0.017 (Pre vs Post: p > 0.017, Ctrl vs Post: p > 0.017).

		Opening Translation	Closing Translation
	Measure	component [%]	component [%]
Control (n - 10)	Mean	28.3	28.7
Control (II – 19)	SD	4.4	5.9
Pre (n = 10)	Mean	22.5	22.4
	SD	5.9	6.3
Post (n = 9)	Mean	23.4	23.4
	SD	3.4	3.8
K-W-test	p-value	0.003	0.014
	Ctrl vs Pre	0.005	0.015
Post hoc U-test	Pre vs Post	0.623	0.120
	Ctrl vs Post	0.007	0.022

Table 16. Global translation contribution on mouth opening and closing.

DISCUSSION

Literature showed that one of the most common short and medium-term complications during function recovery after orthognathic surgery is MMO reduction. Different causes have been considered, such as the duration of the post-surgery intermaxillary fixation, surgical trauma, changes in soft-tissue and muscular traction, amount of mandibular setback and surgeon's experience. A minimum of 6 months has been deemed necessary for recovery from passivity and minor muscular trauma (Song et al., 1997).

In the past decades, the clinical measure of MMO was one of the most important indices of a good post-surgery recovery. Usually, the MMO was directly measured with a ruler or a gauge in the patient's mouth (Zimmer et al., 1992; Nagamine et al., 1993; Ueki et al., 2008; Yazdani et al., 2010) or recorded by means of kinesiography or axiography (Zarrinkelk et al., 1995, 1996). However, standard clinical outcomes or the use of diagnostic instruments without an ad hoc biomechanical model cannot be sensitive enough to detect oral dysfunction, like the change in the percentage contribution of translation and rotation of condylar movements. The widely use of the lower interincisor path alone for TMJ kinematic assessment is inadequate (Mapelli et al., 2009); in the current study a three-dimensional optoelectronic motion analyzer was used together with ad-hoc biomechanical model (see Materials and Methods for details) to detect all the 6 spatial degrees of freedom of mandibular kinematics in patients with skeletal Class III malocclusion.

Ten individuals affected by Class III dentoskeletal deformity, and who had been received no treatment yet, showed a strongly limited condylar translation in almost all steps of mouth opening and closing. When compared to the normal values (as detailed in Study I), their condylar path was almost halved, being so both its ventral and caudal displacements. Together with a limited amount of sagittal opening angle, this latter restriction determined a remarkable lower distance of maximum mouth opening, which was mainly characterized by an important reduction of its caudal component.

Nine patients with the same dentoskeletal dysfunction who were recorded, on average, 20 months after having received orthognathic surgery (together with proper pre- and post-orthodontic treatments), showed no notable gain in condylar movement, during mouth opening. Even if a slight increment in condylar advancement was found (whereas the downward one was negligible), overall, condylar rototranslation did not change after surgery. This may be explained because, at the same time, the amount of mandibular rotation increased, up to physiologic values. In patients who had received orthognathic surgery, also Sforza et al. (2008) measured a lower percentage of condylar translation than in healthy subjects, even not statistically significant.

Anyway, the increased amount of the sagittal angle at MMO determined, in turn, the recovery of the normal caudal component of maximum mouth opening and consequently the normal MMO linear displacement at the interincisor point.

Furthermore, both mouth opening symmetry, and laterotrusions and protrusion ranges of motion did not differ significantly from what was found in healthy subjects, either in pre-surgery or post-surgery patients. In particular, the normal amount of condylar displacement during protrusion should not appear astonishing, since it was quite the same as in mouth opening. Indeed, in healthy subjects, condylar paths during jaw protrusion are usually shorter than during mouth opening (Study I).

Overall, then, the present investigation only partially upholds previous findings (Throckmorton et al., 1995; Sforza et al., 2010b) reporting functional recovery referable to orthognathic surgery. In fact, kinematic parameters which were not dissimilar between the post-surgery patients and the control group (protrusion and laterotrusion ranges of motion, symmetric border movements) were also similar to the corresponding data of the untreated III class patients. On the other side, what actually resulted enhanced in post-surgery patients with respect to pre-surgery ones, as MMO, came out together with other unrecovered dysfunction, as condylar hypomobility. Only longitudinal investigations may help understanding the full amount of impairment given by this characteristic. However, condylar hypomobility has already been hypothesized in post-surgery Class III patients: it could be explained by modifications in condylar position related to the

glenoid fossa (Kim et al., 2010) or remodelling of the mandibular condyle after surgical setback (Gill et al., 2008). Also anatomical and functional alterations in the muscular control must be considered: Katsumata et al. (2004) suggested that the masseter muscle may undergo reversible atrophy after mandibular setback osteotomy and there may be alterations in the other masticatory muscles and temporomandibular ligaments. Notwithstanding, the present findings suggest that condylar hypomobility might be an intrinsic feature of the temporomandibular joint in skeletal Class III.

The current study has two main drawbacks: both pre- and post-surgery groups were independent to each other and were both composed of a limited number of patients. For this reason, a new project has just begun with the purpose of recording a larger number of patients, with longitudinal assessments during the subsequent phases of healing.

GENERAL CONCLUSIONS

Current clinical assessments and medical treatments are increasingly evidence-based, relying on a widespread diffusion of diagnostic tools and treatment protocols that should make scientificbased options available to the largest number of health professionals.

Indeed, the quantitative and accurate evaluation of masticatory muscle activity and jaw movement is mandatory for a better understanding of the normal function and dysfunction of the stomatognathic apparatus, and should assist conventional clinical assessment.

In an effort to make diagnosis as objective as possible, in the current thesis, a non-invasive and short-lasting protocol integrating EMG signals and kinematic data has been proposed, in order to develop multifactorial estimations of TMJ functioning. In particular, masticatory function has been objectively determined, assessing bite stability, mandibular border movements and chewing performance, in both clinically healthy and pathologic individuals.

The outcomes suggest that the proposed method could be a useful tool to evaluate the neuromuscular coordination during the performance of static and dynamic masticatory activities, and to detect functionally altered stomatognathic conditions. Diagnosis of alterations of the stomatognathic apparatus, and assessment of the effects of therapy, would both profit from this quantitative approach, thus reducing the discordance among several clinical examinations.

However, the findings of the present investigation are obviously strictly inherent to the standardized protocol, and cannot be directly extended to natural, habitual function.

Future investigations will focus on the following main developments:

- to expand the protocol to more natural examined conditions;
- to identify the interrelationship between the findings of clinical examination with the quantitative results provided by the current protocol;
- to propose new diagnostic/predictive indices, after testing their reliability, in order to increase the precision of the clinical diagnosis and the choice of treatment.
LIST OF ABBREVIATIONS

- 2D: bi-dimensional
- 3D: three-dimensional
- ANCOVA: analysis of covariance
- ANOVA: analysis of variance
- ASIM: asymmetry index
- ATTIV: activity index
- BSSO: bilateral sagittal split mandibular osteotomy
- CCD: charge coupled device
- CLENCH: maximum voluntary contraction without cotton rolls
- COT: maximum voluntary contraction on cotton rolls
- CRP: condylar reference point
- CT: computed tomography
- Ctrl: control group
- DDR: disc displacement with reduction
- EMG: electromyography
- FARC: Functional Anatomy Research Centre
- ICP: intercuspal position
- IMPACT: integrated area of the standardized EMG potential over time
- IP: inter-incisor point
- K-W: Kruskal Wallis
- LED: light emitting diode
- Mk: marker
- MMO: maximum mouth opening
- MRI: magnetic resonance imaging
- MVC: standardized maximum voluntary contraction
- NS: not significant
- POC: percentage overlapping coefficient
- POST: after surgery group
- PRE: before surgery group
- r: mandibular radius

- RDC: research diagnostic criteria
- RMS: root mean square
- s: circumference arc of the interincisor point
- SD: standard deviation
- sEMG: surface electromyography
- SMI: symmetric mastication index
- TMD: temporomandibular disorder
- TMJ: temporomandibular joint
- TORQUE: torque coefficient
- TORS: torsion coefficient
- TVC: television camera
- w: mandibular width

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WEBSITES

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