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ORIGINAL ARTICLE

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Genioglossal electromyogram during maintained contraction in normal humans

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Abstract Knowledge of the fatigability of the genioglossus muscle is of interest because this muscle prevents pharyngeal collapse, especially during sleep. In the present investigation, signs of fatigue in the genioglossus muscle were studied by measuring the tongue endurance using a force transducer and electromyographic (EMG) activity of the genioglossus muscle in eight nonapnoeic men. Mean absolute EMG values and spectrum analysis were calculated at three levels of submaximal effort. Median frequency and the force:mean absolute EMG value ratio were independent of force level ($F=0.37$, $P=0.93$; $F=0.35$, $P=0.94$, respectively) but dependent on effort duration ($F=52$, $P<0.0001$; $F=16$, $P<0.0001$). Force:mean absolute EMG value and logarithmic median frequency decreased linearly with respect to time and were similar at the three force levels when time was expressed as a percentage of total test time ($F=0.37$, $P=0.93$). The decrease in median frequency was ascribable to a larger increase in low- than in high-frequency components, as shown by the significant decrease in the high-frequency:low-frequency ratio ($F=27$, $P<0.0001$) with time. The method of investigation used in this study allowed detection of the behaviour of the tongue during fatigue and, therefore, should be useful in disorders where mechanical failure of the tongue is suspected, such as the sleep apnoea syndrome or in neuromuscular disorders.

Keywords Tongue · Genioglossus muscle · Fatigue · Electromyography · Sleep apnoea syndrome

Introduction

Muscle fatigue is defined as an exercise-induced reduction in the maximal capacity to generate force output (Vollestad 1997) and can be evaluated mechanically during voluntarily maintained submaximal contractions. Endurance time, or time to exhaustion, can be quantified using various techniques. Surface electromyography (EMG) is often recommended as an indirect tool for studying muscle fatigue. During a maintained submaximal effort, mean absolute EMG value increases gradually (Lind and Petrofsky 1979) and EMG median frequency shifts to lower values (Vollestad 1997). This shift has a linear (Krivickas et al. 1998) or logarithmic (Bellemare and Grassino 1982) relationship with respect to time and precedes mechanical failure. Thus, the EMG can be considered useful in the detection of muscle fatigue.

Scardella et al. (1993) studied the mechanical characteristics of the human tongue during intermittent submaximal efforts, using the EMG of the genioglossus muscle to assess fatigue. The median frequency of the EMG of the genioglossus muscle decreased when mechanical failure occurred, i.e. when the subject was not able to maintain 90% of the previously generated force. However, this study evaluated the dynamic pattern of EMG data only when exhaustion occurred. Knowledge of this pattern could perhaps be used to predict exhaustion before its occurrence. An ability to predict exhaustion should be useful in conditions such as the sleep apnoea syndrome or in cases of neuromuscular disease.

The aim of this study was to investigate the mechanical characteristics of the tongue and to determine

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relationships among parameters determined from analysis of the EMG spectrum during maintained isometric contraction of the tongue at various levels of submaximal force. We focused on changes with respect to time in the power spectrum of the EMG of the tongue throughout fatiguing contraction, and we determined whether EMG analysis was effective in detecting early fatigue of the tongue muscle, before the occurrence of mechanical failure.

Methods

Subjects

Eight male subjects aged 25–60 years were studied. Exclusion criteria were factors having potential effects on genioglossal function: chronic snoring and sleep apnoea ascertained from the medical histories and from overnight polygraph monitoring of respiration and oxygen saturation, loose teeth, malignant or inflammatory disease of the oral cavity or pharynx, neuromuscular disease, excessive consumption of tobacco or alcohol, use of medications having neuromuscular effects, thyroid dysfunction, and severe nasal obstruction.

The eight men had a mean (SD)age of 38.8 (6.8) years. Their mean body mass index and neck circumference were 24.6 (2.1) $kg{\cdot}m^2$ and 39.4 (0.8) cm, respectively. All but one were intermittent snorers. The mean respiratory disturbance index was 6.1 (2.5) $events{\cdot}h^{-1}$ and the mean apnoea index was 0.7 (0.8) $events{\cdot}h^{-1}$.

The study was approved by the appropriate institutional review board (Ambroise Paré Teaching Hospital, Boulogne-Billancourt, France), and informed consent was obtained from each subject prior to inclusion in the study.

Genioglossal force and EMG

The genioglossus muscle is composed of three fascicles (anterior, middle and inferior; Rouvière and Delmas 1997) whose proximal ends are inserted on the superior genial tubercle of the mandible. The anterior and middle fascicles lie directly under the mucosa of the anterior floor of the mouth. The middle fascicle is adjacent and lateral to the anterior fascicle, whose distal ends are in the mid-portion and tip of the tongue, respectively (Rouvière and Delmas 1997). Contraction of the genioglossus muscle moves the tongue forward and upward. Accordingly, the device used to investigate the mechanical characteristics and EMG of the tongue was a U-shaped plastic plate to which two EMG electrodes and a lingual force transducer were attached (Fig. 1). High-viscosity dental impression material (Optosil P and Activator paste Universal Optosil – Xantropen, Dormagen, Germany) was used to secure the mouthpiece to the lower teeth. The dental impression material, while still soft, was spread over the floor of the mouth to ensure optimal contact of the mouthpiece with the upper surface of the genioglossus muscle. The dental impression material was allowed to solidify and any excess material was trimmed away. Two Teflon-coated stainless steel wires (diameter 0.33 mm) were incorporated into the mouthpiece to enable the recording of surface EMG signals (A-M Systems, Everett, Wash.). The last 15 mm of the electrodes were uncoated (diameter 0.25 mm). They were placed parallel to the genioglossus fibres and in contact with them, approximately 10 mm apart, as previously described by Doble et al. (1985). The tips of the wires were buried in the impression material. The lingual force transducer (Neuro Logic, Inc., Lawrence, Kan.) was attached to the upper aspect of the U-shaped plastic plate and used to measure the direct compression force applied by the tip of the tongue to an active, 5 mm-wide contact arm. The contact arm carried a slight indentation to indicate where to place the tongue and was connected to a strain gauge located outside the mouth.

The response signal was linear from 0.49 to 9.81 N (model 205, Biocommunication Electronics, LLC, Madison, Wis.).

The stainless steel wires and the strain gauge were connected to amplifiers. The signals were A-D converted and sampled at 2,000 Hz. They were recorded in a microcomputer using an analogue-digital system (MP100, Biopac System, Santa Barbara, Calif.) for subsequent analysis (Fig. 1).

Experiment protocol

Standard weights were used to calibrate the transducer system before each measurement session.

All measurements were made early in the afternoon. The subject was seated in a comfortable chair. The examiners made a careful visual check of the position of the head, neck, and back of the subject. To provide visual feedback, the force generated by the tongue was displayed on a computer screen placed in front of the subject. The position of the contact arm in the mouth was adjusted to maximize tongue force.

The mean maximal force of the tongue was then determined by asking the subject to push with the tip of the tongue as hard as

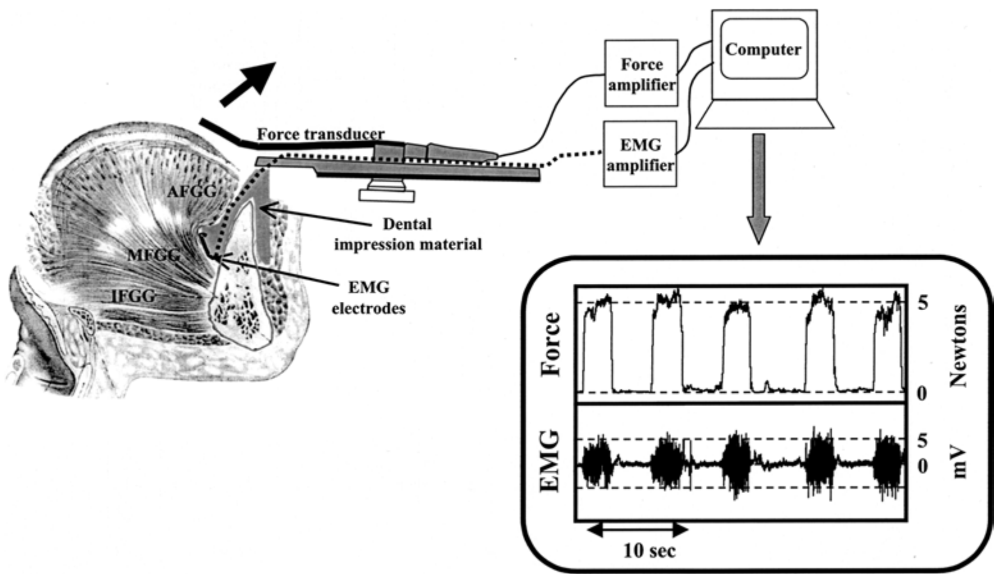

Fig. 1. Recording apparatus and representative data from a subject during five maximal forces. *EMG* Electromyogram, *AFGG* anterior fascicle of the genioglossus muscle, *MEGG* middle fascicle of the genioglossus muscle, *FGG* inferior fascicle of the genioglossus muscle

possible against the force transducer for at least 2 s. This was repeated five times, at 5 s intervals. The mean force of the three strongest pushes was calculated. The subject was then asked to perform one set of tests at each of three force levels, 30%, 60%, and 80% of his mean maximal force. The trials were performed in random order. The mean maximal force was re-evaluated before each test. The subject rested for 15 min after each test.

The target force level was shown to the subject on the computer screen as a solid black line. The subject was asked to push in an isometric and constant fashion, in order to match the target. Two examiners verbally encouraged the subject to maintain the contraction as long as possible.

Data analysis

Data analysis began when the force provided by the subject was equal to or just above the target force for 4 s. Data analysis ended at the occurrence of tongue fatigue, which was defined as an instant force level of less than 80% of the target force maintained for longer than 4 s. Because we wanted to be sure that fatigue occurred, we used a larger decrease to define tongue fatigue than in previous studies (Viitasalo and Komi 1977; Beliveau et al. 1991; Mannion et al. 1998). The power spectrum of the EMG was analysed.

The median frequency of the EMG was obtained by processing the raw digitized data with a Hanning window and a band-pass filter between 10 and 350 Hz, applying a fast Fourier transform to the data, and calculating the median frequency.

The procedure for quantifying EMG activity and calculating the mean absolute EMG value was as follows. The signal was filtered, full-wave rectified, and smoothed using a low-pass 200 ms filter. Three band-pass filters were used with a Hanning window. The first filter was set between 10 Hz and 350 Hz, providing an overall mean value of the absolute EMG and the overall activity of the genioglossus muscle. The other two filters had band-passes ranging from 10 to 49.5 Hz for the low-frequency electrical component and from 150 to 350 Hz for the high-frequency component. All consecutive values of the low- and high-frequency electrical components and the mean absolute EMG value during each test were normalized using the corresponding reference value measured during a 4 s period that started 8 s after the beginning of the test at 30% of the subject's mean maximal force.

The median frequency, mean absolute EMG value, low-frequency component, and high-frequency component were measured at the start of the test and after 25%, 50%, 75%, and 100% of the total test time (i.e. of the endurance time).

A shift in median frequency and a decrease in the ratio of the high- over the low-frequency components of the EMG were used as indicators of impending fatigue. The validity of these indicators has been established for other muscles (Lindstrom et al. 1977; Bellemare and Grassino 1982; Bendahan et al. 1996).

Statistical analysis

Results are reported as mean (SD). Comparisons of several variables were made using two-way analysis of variance with force level and percentage test duration as the categorical variables. Where appropriate, the post-hoc Student-Newman-Keuls test was used for pairwise comparisons. Pearson correlation analysis was used to evaluate relationships between parameters. Those P values which were less than 0.05 were considered significant.

Results

Mean maximal force

Mean maximal force for all subjects before the three sets of trials was 5.44 (1.52) N. No significant differences were found among mean maximal forces measured before each test and between tests ($F=2.7$, $P=0.1$). In addition, mean maximal force was not influenced by the order of the tests.

Endurance time

Endurance time, or time to mechanical failure, was significantly longer ($F=39$, $P<0.0001$) at lower force levels: mean endurance time was 34.8 (19.8) s at 80%, 50.3 (28.3) s at 60%, and 164.5 (60.5) s at 30% of the mean maximal force.

Median frequency

Mean median frequencies at the beginning of the test were 116.4 (12.5) Hz at 80%, 122.0 (11.0) Hz at 60%, and 122.7 (13.0) Hz at 30% of the mean maximal force. Although each patient reached the target force, median frequency at the start of the test did not differ significantly among the three force levels, and median frequency was independent from the output generated. For each subject and for each test, median frequency was higher at the beginning of the test than at the occurrence of mechanical failure. Mean median frequency decreased significantly with respect to time ($F=52$, $P<0.0001$). As shown in panel A of Fig. 2, median frequency decreased linearly with respect to time when the y-axis was given a logarithmic scale. There were no significant differences in mean median frequency between the three tests at each percentage of total test time (25%, 50%, 75%, and 100% of total test time) ($F=0.37$, $P=0.93$).

The time-course of median frequency was identical during the three tests when the duration of effort was normalized for the level of the test force.

Total power spectrum of the EMG and electromechanical dissociation

The absolute value of the EMG increased significantly both with the level of force ($P<0.001$) and with respect to time ($F=16$, $P<0.0001$). In contrast, as shown in panel B of Fig. 2, the quotient of force divided by the absolute EMG value was independent of the level of force ($F=0.35$, $P=0.94$) but decreased significantly with respect to time ($F=10$, $P<0.0001$).

The mean value of the absolute EMG increased significantly ($F=16$, $P<0.0001$) with respect to time. As compared to baseline values, the mean absolute EMG was greater when mechanical failure occurred in all subjects during the 60% test but in only six of the eight subjects during the 30% and 80% tests. In Fig. 2 the time-course of the quotient of force divided by mean absolute EMG is shown. During none of the three tests were significant differences in the quotient found at each percentage of the total test times (25%, 50%, 75%, and 100% of total test time) ($F=0.35$, $P=0.94$).

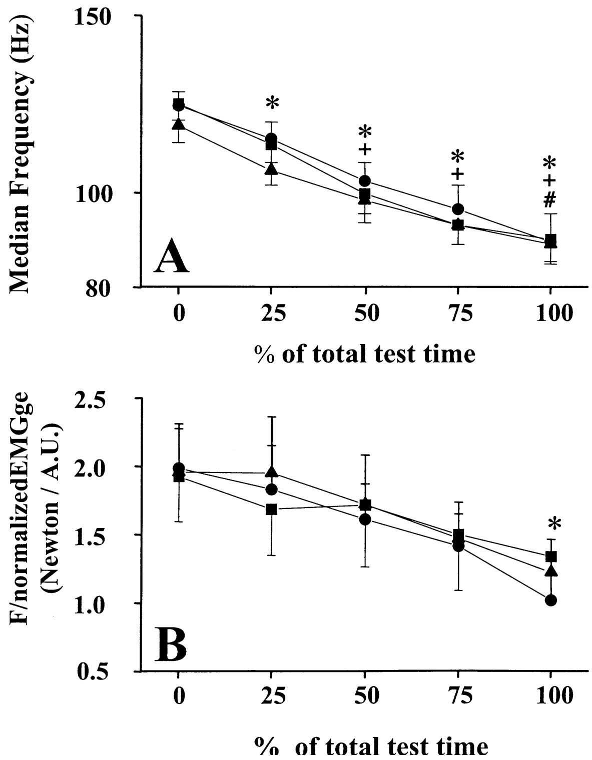

Fig. 2. Changes with respect to time in median frequency **A** and in the quotient of force divided by mean absolute EMG **B** at the three force levels tested (*squares* 30% of mean maximal force, *circles* 60%, and *triangles* 80%). The measurement times are expressed as percentages of the test duration at each level of force. Mean values of the absolute genioglossus electromyogram (*EMG*ge) were normalized as the quotient of the mean value of the absolute EMG during each test divided by the mean absolute EMG over a 4 s period starting 8 s after the beginning of the 30% test. The *bars* indicate 1 standard deviation. *,+,#Significantly lower compared with 0%, 25% and 50% of total test time respectively ($P<0.05$)

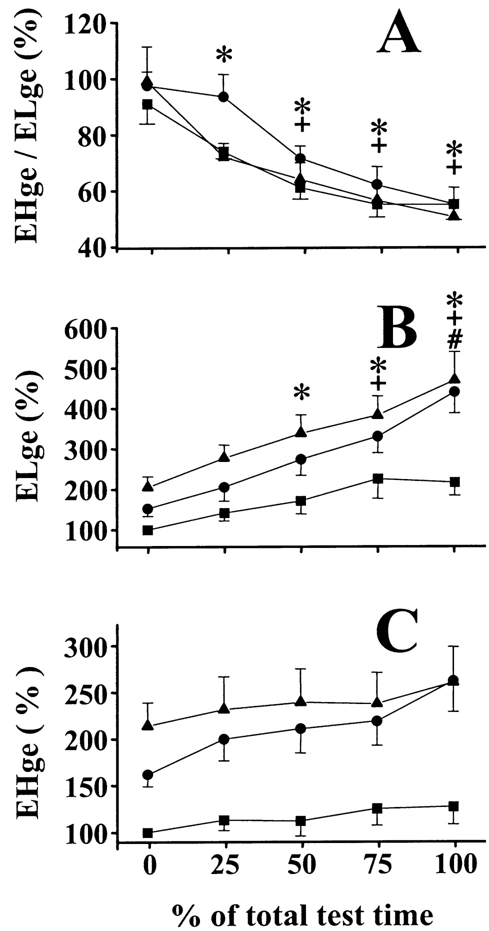

Fig. 3. Changes with respect to time in the ratio of high-frequency band-pass to low-frequency band-pass **A**, in the low-frequency band-pass (10–49.5 Hz) **B**, and in the high-frequency band-pass (150 to 350 Hz) **C** components of the mean absolute EMG at the three levels of force tested (*squares* 30% of mean maximal force, *circles* 60%, and *triangles* 80%). All consecutive values of the low- and high-frequency signal components during each test were normalized using the corresponding reference value measured over a 4 s period starting 8 s after the beginning of the 30% test. The measurement times are expressed as percentages of the total test time for each force level. The *bars* indicate 1 standard deviation. *,+,#Significantly lower for **A** and higher for **B** and **C** compared with 0%, 25%, and 50% of total test time, respectively ($P<0.05$). *EHge* High frequency electrical component of the genioglossus muscle, *ELge* low frequency electrical component of the genioglossus muscle

Ratio of high- to low-frequency components

The ratio of the high-frequency to low-frequency component decreased significantly ($F=27$, $P<0.0001$) with respect to time. This decrease was significant for each of the intervals studied (Fig. 3). No significant differences were found between the three tests ($F=0.72$, $P=0.67$). Both the low-frequency component and the high-frequency component increased significantly with respect to time ($F=33$, $P<0.0001$ and $F=8$, $P<0.0001$, respectively). The increase in the low-frequency component and the decrease in the ratio of the high- to low-frequency component were correlated to the decrease in median frequency ($r=-0{,}574$, $P<0.0001$; and $r=0.753$, $P<0.0001$ respectively).

Population

None of the clinical data (age, body mass index, neck circumference, respiratory disturbance index, apnoea index) were correlated with the mean maximal force, total test time, median frequency, power density EMG, or high- to low-frequency ratio.

Discussion

The main finding from this study was that median frequency and the quotient force/mean absolute EMG were independent of the force generated. Both variables were dependent on endurance time. The logarithmic median frequency and the quotient force/mean absolute EMG decreased linearly with respect to time and the decrease in the median frequency of the EMG occurred before mechanical failure. When time was expressed as a percentage of the endurance time, these two variables were similar among the various submaximal levels of force studied. However, endurance time was shorter at higher levels of force.

Before discussing the implications of these findings, we will address several methodological issues.

Methodological issues

Tongue force

The tongue is comprised of 17 muscles, whose effects are determined by their attachment sites. Little is known about synergies and antagonisms among these tongue muscles or between them and the neighbouring extralingual muscles. Therefore, the global physiological actions of the tongue are difficult to interpret. Many muscles seem to contribute to tongue protrusion (Napadow et al. 1999). Thus, the forces measured in the present study would reflect the activity of a number of groups of tongue muscles. However, the genioglossus muscle has been determined to be the main tongue protruder (Launois and Whitelaw 1990; Horner 1996). It is reasonable to assume that the genioglossus muscle was activated when the subjects protruded their tongues during the tests; therefore, our data reflect genioglossus muscle function in our experiment setup. The force of tongue protrusion has been studied in humans using a variety of devices ranging from the custom-designed miniature Flatline Load Cell force transducer to balloons and metallic lingual force transducers (Dworkin et al. 1980; Dworkin and Aronson 1986; Scardella et al. 1993; Sha et al. 2000). Several parameters related to the subject and to the position of the measuring system must be standardized. Because tongue force varies with age and between men and women, we studied only subjects who were of the same sex and within a given age range (Mortimore et al. 1999). Tongue force also varies with tongue position at rest, as shown by Sha et al. (2000).

Before we began the tests, we positioned the tongue force transducer in a manner that kept the subject comfortable and allowed the production of the greatest force possible. The contraction that produced the force was considered isometric, although two factors may have influenced maintenance of the tongue in an unvarying position. One was lack of rigidity of the contact arm. However, when we attached a 1,000 g weight to the end of this 2 mm-thick metal rod, we found an arm displacement of less than 5 mm. Because none of the subjects could achieve a force of 9.81 N, we considered that the arm was rigid. The second factor was a possible difficulty in maintaining the tip of the tongue in the appropriate position. Subjects with large tongues described slight displacements outside the indentation at the tip of the contact arm, particularly during the 80% tests. These displacements were immediately corrected, however, because the subjects returned the tongue to the indentation in the contact arm.

The lingual transducer used to measure tongue force in our study was similar to that used by Scardella et al. (1993). Mean maximal forces in our study were 5.44 (1.52) N, compared to 12.43 (1.22) N in the study by Scardella et al. (1993). This difference may be ascribable to interindividual variability in tongue force and to the way in which the subjects pushed on the lingual transducer. In the present study, the subjects were instructed to push with the tip of their tongue because this movement is produced almost entirely by the genioglossus muscle. Pushing with the mid-portion of the tongue, as in the study by Scardella et al. (1993), calls several muscles into play and may, therefore, generate greater force. Similarly, Sha et al. (2000), as well as Mortimore et al. (1999), found mean forces greater than 24.50 N. The higher values found in these studies (Mortimore et al. 1999) may be due to the precontractile muscle length, tongue position, and direction required to generate force against the arm transducers, whose shape was very different from that of the device used in the present study. In the studies by Mortimore et al. (1999) and by Sha et al. (2000), the transducers were placed right in front of the tongue and the subjects were asked to push forward only. In contrast, we asked the subjects to push forward and upward. In addition, in the two previous studies (Mortimore et al. 1999; Sha et al. 2000), the device was maintained not only by the lower but also by the upper jaw, and the masticatory muscles were probably activated. Thus, the mandible was anchored more firmly, which probably enabled the tongue to generate a greater force.

Genioglossus muscle EMG

The anatomic position of the genioglossus muscle allows intraoral surface EMG recording of the activity of this muscle. In our set-up, the two electrodes placed on the floor of the mouth were about 10 mm apart. In theory, the genioglossus muscle is the only muscle in this area. In addition, the myohyoid muscle, which lies immediately lateral to the genioglossus muscle, is not known to produce tongue protrusion.

Tongue protrusion was associated with a change in the EMG signal that confirmed the contribution of the genioglossus muscle to the movement. Doble et al. (1985) showed that surface EMG compared favourably with needle EMG. Although the electrodes were placed in the same manner in all the subjects studied, interindividual variability in the width of the anterior floor of the mouth, height of the inferior dental arch, and position of the lingual frenulum resulted in some variability in the distance between the two electrodes. A larger distance has been shown to result in less recording of higher-frequency components (Lynn et al. 1978). Thus, in some of our subjects, the lower-frequency components may have been artificially increased. However, the

EMG characteristics were identical in all the subjects. Therefore, it is reasonable to assume that the differences observed were not due to the nature of our set-up.

Tongue force and genioglossus muscle EMG

An orderly motor unit recruitment has been reported to occur during gradually increasing static contraction (Thomas et al. 1987), with type I fibres being recruited first, then type IIA, and finally type IIB. This may be the main explanation for the reported increase in median frequency (and mean power frequency) as force level increases in various muscles including the rectus femoris (Nagata et al. 1983), biceps brachii (Nagata et al. 1983; Moritani and Muro. 1987), and knee extensors (Komi and Viitasalo 1976). In contrast, we found that median frequency was independent of the level of force when the latter was made to vary over a broad range before, during, and at exhaustion caused by an isometric submaximal contraction. A similar independence of the median frequency from the level of force has been reported for handgrip muscles (Petrofsky and Lind 1980a, b), elbow flexors (above 30% of maximal voluntary contraction, Hagberg and Ericson 1982), and shoulder muscles (Gerdle et al. 1988). The very limited EMG and histological data in the literature suggest that the median frequency response to changes in the level of force may be dictated by the distribution of fibre types in the muscle (Komi and Tesch 1979; Gerdle et al. 1991; Pincivero et al. 2001), with a greater percentage and/or surface area of type II fibres being associated with an increase in median frequency as force level increases (Roy et al. 1986; Gerdle et al. 1991; Kupa et al. 1995).

In our study, the quotient of force divided by mean absolute EMG was also independent of the level of force across a broad range, before, during and at exhaustion. Some experimental studies have found a linear relationship between mean absolute EMG and force (DeVries 1968; Moritani and Muro 1987), whereas others have found a curvilinear relationship, with an exponential increase of mean absolute EMG as force increases (Zuniga and Simons 1969; Bigland-Ritchie 1981). These discrepancies may be largely ascribable to differences in the size and position of the electrodes. However, the changes in the EMG quantification-force relationship as force increases probably also reflect differences in the properties of the various muscle fibre types (I, IIA, IIB, Brooke and Kaiser 1970). Two histological studies (Series et al. 1996; Carrera et al. 1999) investigated fibre type distribution in the genioglossus muscle of patients who did or did not suffer from sleep apnoea. In the control subjects, Carrera et al. (1999) found a predominance of type I fibres (61%, compared to 39% of type II fibres), whereas Series et al. (1996) found a predominance of type II fibres (67%, compared to 33% of type I fibres). This discrepancy may be related to the fact that the controls in the study by Series et al. (1996), but not those in the study by Carrera et al. (1999) were chronic snorers, a population whose respiratory physiology differs from that of nonsnorers. In snorers, the genioglossus muscle is subjected to greater stress, which influences its activity (Patrick et al. 1982; Skatrud and Dempsey 1985; Leiter et al. 1992; Horner 1996) and can ultimately induce histological changes (Salmons and Henriksson 1981; Carrera et al. 1999). Our subjects were not chronic snorers and may, consequently, have shared with the controls in the study by Carrera et al. (1999) a predominance of type I fibres. This would explain the absence of any augmentation of median frequency with increasing levels of force. However, this hypothesis needs confirmation.

We found that relationships between the percentage of endurance time and the median frequency or the quotient of force divided by mean absolute EMG were similar at all the force levels studied. However, endurance time decreased when the level of tongue force increased. These results suggested that the velocity of change in EMG parameters such as median frequency and the force/mean absolute EMG may be dependent on endurance time. Median frequency and force/mean absolute EMG were significantly reduced at 25% of endurance time, indicating electromechanical dissociation. These early changes followed the same pattern at all the levels of submaximal effort studied. This phenomenon has been extensively described for other muscles (Bigland-Ritchie et al. 1981; Woods et al. 1987; Jammes and Balzamo 1992) and has been shown to indicate local muscle fatigue (Lindstrom et al. 1970; Bellemare and Grassino 1982; Bendahan et al. 1996).

Clinical implications

These findings on the genioglossus muscle may prove useful for predicting variations in EMG during maintained submaximal effort and the occurrence of tongue mechanical failure.

In patients suffering from obstructive sleep apnoea syndrome, genioglossus muscle activity is increased during wakefulness and sleep (Suratt et al. 1988; Mezzanotte et al. 1992). This chronic hyperactivity may lead to changes in muscle histology (Salmons and Henrikson 1981) and in the mechanical and EMG properties of the genioglossus muscle. In keeping with this possibility, Carrera et al. (1999) reported that the type II fibre content was increased in genioglossus muscle sampled from sleep apnoea patients. Series et al. (1996) found a predominance of type IIA fibres. Thus, patients with obstructive sleep apnoea syndrome may have decreased tongue strength and endurance, compared to normal individuals. The variations in EMG median frequency may be less marked than normal because of a greater proportion of intermediate type IIA fibres (Pincivero et al. 2001). If mechanical and EMG differences are found, they may be correlated with the number of abnormal respiratory events and/or to the site of obstruction at the level of the tongue base, since these factors govern the intensity and number of tongue contractions needed to relieve pharyngeal obstruction.

Conclusion

We found that, in normal subjects, both median EMG frequency and the quotient of force divided by mean absolute EMG obtained at the beginning of tongue contraction and at exhaustion were independent of the level of force. In addition, the logarithmic value of median frequency and force/mean absolute EMG decreased linearly with respect to time, before the occurrence of mechanical failure.

Knowledge of this pattern allows the detection of fatigue before the occurrence of mechanical failure and suggests that the time to exhaustion at any submaximal force can be predicted from the median EMG frequency at exhaustion for a single submaximal force test.

This quick, simple, and noninvasive method could be useful for comparing tongue performance in various populations, such as patients with sleep apnoea syndrome and patients with neuromuscular disorders. We suggest that the present data can be used as a reference for further studies.

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