Effect of limb muscle fatigue on perception of respiratory effort in healthy subjects

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Grippo A, Carrai R, Chiti L, Innocenti Bruni G, Scano G, Duranti R. Effect of limb muscle fatigue on perception of respiratory effort in healthy subjects, J Appl Physiol 109: 367–376, 2010. First published May 20, 2010; doi:10.1152/japplphysiol.00608.2009.—The role of nonrespiratory peripheral afferents in dyspnea perception has not been fully elucidated yet. Our hypothesis is that fatigue-induced activation of limb muscle metaboreceptors served by group IV fine afferent fibers may impact on respiratory effort perception. We studied 12 healthy subjects breathing against progressive inspiratory resistive loads (10, 18, 30, 40, and 90 cmH2O·l−1·s) before and after inducing low-frequency fatigue of quadriceps muscle by repeating sustained contractions at ≥80% of maximal voluntary contraction. Subjects also underwent a sham protocol while performing two loaded breathing runs without muscle fatigue in between. During the loaded breathing, while subjects mimicked the quiet breathing pattern using a visual feedback, ventilation, tidal volume, respiratory frequency, pleural pressure swings, arterial oxygen saturation, end-tidal partial pressure of CO2, and dyspnea by a Borg scale were recorded. Compared with prefatigue, limb muscle fatigue resulted in a higher increase in respiratory effort perception for any given ventilation, tidal volume, respiratory frequency, pleural pressure swings, end-tidal partial pressure of CO2, and arterial oxygen saturation. No difference between the two runs was observed with the sham protocol. The present data support the hypothesis that fatigue of limb muscles increases respiratory effort perception associated with loaded breathing, likely by the activation of limb muscle metaboreceptors.

respiratory breathing; peripheral muscle afferents; metaboreceptors

THE ROLE OF NEURAL AFFERENTS in the perception of dyspnea has been only partially disclosed (7, 8, 16, 36, 37, 48, 58). The available data concern principally the effects of stimulation of respiratory mechanical and/or chemical afferents, which play an important role in generating and modulating dyspnea through changes in either bulbary or cortical respiratory drive (7, 8, 17, 37).

Afferent activation of intercostal muscles by vibratory stimulation in humans does not affect dyspnea induced by hypercapnic stimulation, but reduces dyspnea without changes in motor output, when hypercapnic stimulation is associated with inspiratory load (19). Likewise, vibration does not affect the activity of phrenic or medullary neurons in the experimental animal (13, 32). These data suggest that cortical drive and dyspnea perception do not necessarily parallel.

The role of nonrespiratory peripheral afferents in dyspnea perception has not been fully elucidated yet. Recently, Gandevia (23) has underlined the importance of proprioceptive afferents in modulating dyspnea perception, observing that detection and grading of either limb or respiratory loads are served by the same mechanisms and that the relevant afferents project to the primary sensorimotor cortex. Fine afferents fibers from skeletal muscles (type III and IV) are strongly activated during fatiguing contractions (57), with accumulation of metabolites causing a prolonged activation of group IV afferent fibers (26, 42). Available data indicate that stimulation of group III and IV muscle afferent fibers causes an increase in bulbary inspiratory drive (28) and ventilation (18, 39, 52) and, therefore, in dyspnea (27, 36). Nonetheless, based on the observations of Gandevia, we wonder whether group III and IV nonrespiratory afferents may impact on dyspnea independently of increased ventilation. We reasoned that afferents subserving nonrespiratory sensations project to the same integrative sensorimotor brain areas (7, 11, 14, 22, 50, 51, 58), where respiratory afferents involved in the perception of dyspnea project. We, therefore, hypothesized that nonrespiratory afferents, triggered by fatigue-induced metaboreflex, may interact with respiratory afferents activated by mechanical constraints and contribute to perception of the sense of effort. To validate this hypothesis, we compared the sense of effort, evoked by breathing against progressive inspiratory resistive loads, with and without quadriceps muscle fatigue, in 12 healthy subjects. Some of the results of these studies have been previously reported in abstract form (24).

METHODS

Subjects

We studied 12 healthy, nonsmoking humans (5 men): age 30 ± 1 yr, height 172 ± 2 cm, and weight 70 ± 5 kg. Forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC) were 99 ± 3 and 104 ± 2% of predicted values (53), respectively. All subjects were experienced in physiological studies and in performing respiratory maneuvers. Written, informed consent was obtained after a description of the protocol, which was approved by the Firenze University Hospital Ethics Committee.

Measurements

Spirometry and measurement of pulmonary volumes. The FEV1 and the FVC were measured during maximal expiratory maneuvers by Vmax (SensorMedics, Yorba Linda, CA), according to American Thoracic Society/European Respiratory Society recommendations (43). The functional residual capacity was measured by washout nitrogen technique (Vmax SensorMedics, Yorba Linda, CA). The normal values for lung volumes were those proposed by the European Respiratory Society (53).

Esophageal and gastric pressures. Esophageal pressure (Pes) was measured (4) in seven subjects by a standard balloon-tipped catheter,
introduced via the nose and connected to a pressure transducer (± 100 cmH2O; Validyne, Northridge, CA; frequency response 0–1,000 Hz) and was used as an index of pleural pressure. The balloon was positioned in the midesophagus and contained 0.5 ml of air. Gastric pressure (Pg) was simultaneously measured (4) with a similar ballooncatheter system connected to a second differential pressure transducer. This balloon was positioned in the stomach 65–70 cm from balloon tip to nares and contained 1 ml of air. Transdiaphragmatic pressure (Pdi) was obtained by subtracting Pes from Pg. During tidal breathing from the Pes signal, we calculated the swings of pleural pressure (Pes,sw), as the difference between the pressure measured at end-expiration and peak value measured during inspiration.

Inspiratory muscle strength was assessed by measuring minimal (i.e., the greatest negative) inspiratory pleural pressure (Pes,sn) and maximal Pdi (Pdi,sn) at functional residual capacity during sniff maneuvers (4, 44); subjects had visual feedback of generated pressure and were repeatedly encouraged to try as hard as possible. The maneuvers were repeated until three measurements with <5% variability were recorded. The lowest Pes,sn and the highest Pdi,sn values obtained were used for analysis.

Ventilation variables. For ventilation measurements, patients breathed through a Fleisch no. 3 pneumotachometer connected to a flow transducer. Volume was obtained by electrical integration of the flow signal. From the spirogram, we derived the following: inspiratory time (Ti), expiratory time (Te), total time of the respiratory cycle (Ttot), and tidal volume (Vt). Mean inspiratory flow (Vt/Ti), duty cycle (Vt/Ttot), respiratory frequency (RF = 1/Ttot × 60), and minute ventilation (Ve = Vt × RF) were also calculated.

Inspiratory capacity. Inspiratory capacity (IC) measurements were collected as previously described (49). The correct execution of IC maneuvers was fully explained to the subjects and then performed until consistently reproducible efforts were made (i.e., within ±5%). Subjects were given a few breaths warming before an IC maneuver. The control IC was recorded as the mean of the two best reproducible efforts. During loaded breathing, IC maneuvers were performed during the last minute of each load. The change (decrease) in IC reflects the inverse change (increase) in end-expiratory lung volume (EELV), or the extent of dynamic hyperinflation (EELV – TLC – IC), on the assumption that total lung capacity (TLC) remains constant (50).

Arterial blood gases.Expired carbon dioxide (PETCO2) was sampled continuously from the mouth by an infrared carbon dioxide meter (Datex Normocap; Helsinki, Finland). Heart rate and arterial oxygen saturation (SaO2) were assessed with a pulse oxymeter (Masimo Rad 9) by means of a finger probe.

Pressure and flow signals were recorded onto an IBM-compatible personal computer by a 16-channel analog/digital board at 50-Hz sampling rate (National Instrument DAQCard 6024E).

Dyspnea. The perception of dyspnea was evaluated by asking the patients to score the intensity of the sensation by using a modified version of the CR-10 Borg scale, commonly used to rate the intensity of breathlessness (34). Before starting loaded breathing, subjects were familiarized with the 10-point Borg-category scale. The scale is a continuous vertical linear display associated with 10 verbal descriptors of the extent of the symptom, whose end points are anchored so that “0” represents no breathlessness and “10” represents maximum breathlessness. Specifically, we said to the subjects, “Please rate the respiratory effort sensation while breathing using the scale depicted on the sheet, where 0 indicates no effort at all and 10 the maximum tolerable level, which is the maximum effort you can imagine on the basis of your experience.” The subjects were instructed to indicate with a hand-controlled potentiometer how dyspneic they felt with reference to the category descriptor scale. Rating of effort was done by Italian descriptors, which are reported in the Table 1. Subjects were also instructed to describe the quality of dyspnea sensation (air hunger, effort sensation, inspiratory difficulty, and chest tightness) and to distinguish between dyspnea and any other uncomfortable sensation that they might perceive at the level of the fatiguing limb.

<table>
<thead>
<tr>
<th>Table 1. Modified Borg scale in Italian</th>
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<tr>
<td>Value</td>
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<tr>
<td>0</td>
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<tr>
<td>0.5</td>
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<tr>
<td>1</td>
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<td>1.5</td>
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<td>2</td>
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<td>5</td>
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<td>5.5</td>
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<td>7</td>
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<td>7.5</td>
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Quadriiceps muscle force. Force was measured via an inextensible ankle strap connected to a transducer (Digitalanzeiger mod. 9180) and carried to an amplifier. The signal was then recorded by a Medelec Synergy (version 8.2) machine (Oxford Instruments Medical Systems, Old Working, UK). The force transducer had a display located at chair side, showing the instantaneous force values expressed in Newton.

Femoral nerve stimulation was performed using a 90-mm circular coil powered by monophasic electromagnetic stimulators (Magstim 200; Magstim, Whitland, Dyfed, UK). The coil head was positioned high in the femoral triangle just lateral to the femoral artery; the best spot was determined with minor positional adjustments. This spot was marked, and the same position and coil were used for the remainder of the study.

Phrenic nerve stimulation. Cervical magnetic stimulation of the phrenic nerve was performed according to Similowski et al. (55), by using a 90-mm coil placed over C6-C7, with the coil current flowing clockwise or counterclockwise, depending on which produced the highest amplitude Pdi. For each determination, five successive stimuli were delivered at EELV at 100% stimulator maximal possible intensity; a mean value was obtained, including the three greater responses. The Pdi elicited by phrenic nerve stimulation was termed twitch Pdi (Pdi,tw). To be sure that diaphragm muscle fatigue was not present throughout the experimental session, Pdi,tw at the start of the test was compared with the value at the end of each loaded breathing test.

Procedure

A schematic representation of the procedure is illustrated in Fig. 1. The study was carried out in 3 nonconsecutive days for each subject.

First day. Subjects performed a routine spirometry, with the measurement of static and dynamic lung volumes.

Second and third day. In the second and third day, subjects were randomly assigned to either the fatigue or sham protocol.

Fatigue protocol. Subjects were seated in a relaxed position with the back inclined to 80° and the knee flexed at 90° on a chair modified to support the force transducer. Initially, we measured Pes,sn, Pdi,sn, and Pdi,tw. Then subjects breathed for at least 10 min through a mouthpiece and a Fleisch type 3 pneumotachometer attached to a Hans Rudolph valve, which separated the inspiratory from expiratory line. After 10 min of quiet breathing, subjects underwent the control test during which five progressive resistive loads (10, 18, 30, 40, and 90 cmH2O·l−1·s−1) were applied to the inspiratory line. Resistance was evaluated in the range of flows measured during the test (0.60–0.95 l/s). Inspiratory resistive load was obtained by interposing a disk with a variable number of holes in the inspiratory line. Loaded breathing lasted 25 min (5 per load), without any interval between successive loads. During loaded breathing, subjects had to mimic the same breathing timing used during quiet breathing by having a visual representation of their pattern displayed on a personal computer screen placed in front of them. After the end of loaded breathing, Pes,sn, Pdi,sn, and Pdi,tw were measured again. After a 30-min rest period, fatigue was induced in the quadriceps muscle of the right
thigh, according to the following procedure. The mean value of three short (5 s) maximal voluntary isometric contractions (MVC) was calculated for each subject. Successively, five magnetic stimulations of the femoral nerve at 100% stimulator output were administered to each subject, and the developed quadriceps twitch tension (TWQ) was measured. Then the subjects performed a fatiguing protocol (20) to induce low-frequency fatigue. Briefly, subjects sustained contractions of ≥80% of their mean MVC for as long as possible; at the point of task failure, they were allowed 15-s rest and then they resumed their contraction. This target force was reached and held by means of a visual feedback display located at chair side, showing the instantaneous force values. Standardized verbal encouragement was given. This was repeated until 50% of MVC could not be maintained for more than 5 s. At this time stimulations of femoral nerve were performed to measure TWQ. A TWQ degree more than 5 s. At this time stimulations of femoral nerve at 100% stimulator output were administered to one subject (no. 3 of Fig. 3), effort perception was not different in the two tests. No differences were observed between control and fatigue tests for Pes,sw, V˙E,V T, Rf, VT/TI,T I/Ttot, Ttot, TI, and IC (ANOVA for repeated measures, Table 3 and Fig. 6). During control test, subjects exhibited significant decreases in V˙E, V T, VT/TI and increases in VT/Ttot and PetCO2 (Table 2); T E decreased (P < 0.0001), while T I (P < 0.0001), Pes,sw (Fig. 2), and respiratory effort sensation (Fig. 3) increased. Changes in Rf, SaO2 (Table 2), Ttot, and IC were not significant.

Pes,sn and Pdi,sn were within the normal limits (44) at baseline and did not change after loaded breathing. Pdi, tw at baseline ranged from 25 to 49 cmH2O and showed a small but significant increase with loaded breathing (Fig. 4).

**Fatigue Protocol**

All subjects developed fatigue of the quadriceps muscle: a significant >60% mean reduction in strength from baseline lasted throughout the resistive breathing test (Fig. 5). Compared with control test, the perception of respiratory effort was significantly greater during fatigue test (Fig. 3). In one subject (no. 3 of Fig. 3), effort perception was not different in the two tests. No differences were observed between control and fatigue tests for Pes,sw, V˙E, V T, Rf, VT/TI, VT/Ttot, Ttot, T I, Te, SaO2, and IC (ANOVA for repeated measures, Figs. 2 and Table 2). Small, but significant, was the difference in PetCO2 between the two tests (Table 2). The ratio of Borg score to all measured respiratory variables was significantly greater (ANOVA for repeated measures) during the fatigue test (Fig. 6 and Table 3).

**Sham Protocol**

One subject did not volunteer to perform the control trial, so the data refer to 11 subjects.

No differences in any of the studied respiratory variables and in the time course of respiratory effort perception were found between control 1 and control 2 test (ANOVA for repeated measures, Figs. 2 and 3 and Table 2). Moreover, no differences were observed in the ratio of Borg score to each respiratory variable between control 1 and control 2 test (ANOVA for repeated measures, Table 3 and Fig. 6).

**DISCUSSION**

The novel finding of this study is that fatigue of limb muscles increases the perception of respiratory effort associated with loaded breathing.

Values are means ± SE. Significance of changes in variables during loaded breathing in basal conditions was evaluated by two-way analysis of the variance (ANOVA). To determine the significance of differences in variables between control and fatigue test, we used two-way ANOVA for repeated measures (2), with subjects and load as factors and control vs. fatigue as repeated measures. All statistical procedures were carried out using the PASW Statistics 18.0 (SPSS, Chicago, IL).

**RESULTS**

**Basal Resistive Breathing**

All subjects breathed for 5 min with 10, 18, 30, and 40 cmH2O·l−1·s resistance; breathing at 90 cmH2O·l−1·s was interrupted in most subjects because of the occurrence of unbearable respiratory effort perception.
Table 2. Respiratory variables recorded in the last minute of each respiratory load during both the fatigue and sham protocols

<table>
<thead>
<tr>
<th></th>
<th>Fatigue Protocol Load, cmH2O·1·s⁻¹</th>
<th>Sham Protocol Load, cmH2O·1·s⁻¹</th>
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<tr>
<td></td>
<td>10</td>
<td>18</td>
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<tr>
<td>$V_t$, l/min</td>
<td></td>
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<tr>
<td>Pre</td>
<td>12.0±0.8</td>
<td>12.8±0.7</td>
</tr>
<tr>
<td>Post</td>
<td>13.4±1.6</td>
<td>13.0±0.9</td>
</tr>
<tr>
<td>$V_t$, liter</td>
<td>0.80±0.1</td>
<td>0.88±0.1</td>
</tr>
<tr>
<td>Pre</td>
<td>0.99±0.1</td>
<td>0.88±0.1</td>
</tr>
<tr>
<td>Post</td>
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<td></td>
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<tr>
<td>$V_t/T_i$, l/s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>15.0±0.9</td>
<td>15.0±1.4</td>
</tr>
<tr>
<td>Post</td>
<td>14.2±2.1</td>
<td>15.0±0.8</td>
</tr>
<tr>
<td>$V_t/T_i$, liter/min</td>
<td>0.4±0.04</td>
<td>0.4±0.01</td>
</tr>
<tr>
<td>Pre</td>
<td>0.5±0.04</td>
<td>0.4±0.03</td>
</tr>
<tr>
<td>Post</td>
<td>0.5±0.01</td>
<td>0.5±0.02</td>
</tr>
<tr>
<td>$S_aO_2$, %</td>
<td></td>
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<tr>
<td>Pre</td>
<td>99.2±0.3</td>
<td>99.3±0.3</td>
</tr>
<tr>
<td>Post</td>
<td>99.1±0.4</td>
<td>99.1±0.5</td>
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<tr>
<td>$P_e\text{CO}_2$, Torr</td>
<td></td>
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<tr>
<td>Pre</td>
<td>39.1±1.2</td>
<td>39.2±1.4</td>
</tr>
<tr>
<td>Post</td>
<td>36.9±2.0</td>
<td>36.4±1.9</td>
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</table>

Values are mean±SE. $V_t$, minute ventilation; $V_t$, tidal volume; $R_f$, respiratory frequency; $T_i$, inspiratory time; $V_t/T_i$, mean inspiratory flow; $T_{tot}$, total time of respiratory cycle; $V_t/T_{tot}$, duty cycle; $S_aO_2$, arterial oxygen saturation; $P_e\text{CO}_2$, end-tidal partial pressure of carbon dioxide; Pre, control test during fatigue protocol and control test 1 during sham protocol; Post, fatigue test during fatigue protocol and control test 2 during sham protocol. *ANOVA, $P < 0.0001$. †ANOVA for repeated measures, Pre vs. Post, $P < 0.0001$.

Critique of Methods

Due to long-lasting, low-frequency fatigue, the control test always precedes the fatigue test, and this might introduce a bias, as a learning effect might influence the results. For this reason, we decided to introduce a sham protocol, where subjects underwent two loaded breathing tests without the induction of fatigue in between. The lack of any change in dyspnea...
Fig. 3. Individual Borg ratings (subject nos. in top left corner) recorded in the last minute of each respiratory load during either the fatiguing protocol or the sham protocol. Mean values are in the bottom panel. Subject 6 did not undergo sham test. ▲ and ●, control test and fatigue test, respectively, during fatigue protocol. □ and ○, control test 1 and control test 2, respectively, during sham protocol. au, Arbitrary units. *Comparison between control test and fatigue test during fatigue protocol (two-way ANOVA for repeated measures). ♭Comparison between control test 1 and control test 2 during sham protocol (two-way ANOVA for repeated measures).
perception during the sham protocol reasonably excluded a learning effect.

During resistive breathing, subjects had to mimic the same breathing pattern used during unloaded breathing. This choice was dictated by the need of standardizing the experiments as much as possible, eliminating the possible sources of dyspnea changes other than quadriceps fatigue. In particular, we considered that mimicking the same breathing pattern would prevent ventilation from changing greatly, so that the changes in dyspnea would largely be independent of changes in ventilation and/or breathing pattern.

The twitch torque obtained with magnetic stimulation is a validated method currently used in studies of muscle fatigue. However, the use of magnetic stimulation to assess fatigue of the respiratory and quadriceps muscles might not be adequate, as 100% stimulator output is not necessarily maximal, and individual input-output curves were not assessed. Moreover, because of decrease in axon excitability after voluntary contraction (29), the nerve might not be stimulated to the same degree before and after contraction. As to the quadriceps muscle, quadriceps fatigue was surely induced, as demonstrated by a drop in force to 50% MVC at the end of the fatiguing protocol. However, a sure demonstration that fatigue was present throughout the test is lacking, because, during resistive breathing, only quadriceps twitch but not MCV measurements were repeated. Nevertheless, the following has to be considered. 1) Force decreased similarly in all the subjects with no tendency to increase throughout the test (Fig. 5). 2) Low-frequency fatigue is long-lasting (up to 24 h), so that, after 30–40 min, quadriceps muscle was likely to be still fatigued. 3) After voluntary contraction, recovery time of axonal excitability lasts ~10 min, and the reduction of compound muscle action potential amplitude is maximal immediately after the cessation of the contraction and returns to the precontraction level in ~4 min (29). As a consequence, while we cannot exclude a contribution of reduction in axonal excitability to the reduction of quadriceps twitch at T1 (~10–15 min after the end of work), it appears very unlikely that a similar effect was still active when stimulating at T2, T3, and T4 (respectively, after 20, 30, and 35 min). 4) The possible underestimation of muscle fatigue does not alter the interpretation of the results. As to the diaphragm, the possible submaximal level of magnetic stimulation might have caused fatigue to be hidden. However, it is likely that the diaphragm did not fatigue, as it is relatively fatigue resistant (40), and both Pes,sn and Pdi,sn did not decrease in our subjects. Moreover, also for the diaphragm, there is the problem of the change in axon excitability after the strong respiratory muscle contractions occurring during the resistive breathing. This might explain the reduction in Pdi,tw (%basal) at some time points in some subjects in Fig. 4, but the progressive increase in Pdi,tw values observed during the test argues against a role of a reduction in axon excitability. Finally, a limitation of this study is that measurements of pleural pressure and Pg were accomplished only in 7 of the 12 subjects.

**Interpretation of Results**

The most important result of this study is that the fatigue of lower limb muscles is associated with an increase in perception of respiratory effort for any given resistive load. This conclusion is supported by both the increase in dyspnea after the induction of fatigue and the absence of any change in dyspnea perception with the sham protocol.

Dyspnea originates from interactions of multiple neurophysiological mechanisms and psychological factors (3, 48). We will discuss the possible role of some of these factors in determining the intensification in dyspnea induced by quadriceps fatigue.

**Respiratory effort.** Dyspnea originates from the increase in respiratory drive, which may be brainstem or cortical in origin, with the resultant sensation having the characteristics of air hunger or increased respiratory effort, respectively (7, 17). So a preliminary question is whether bulbar drive and air hunger have played an important role in the present study. We feel that they have not for the following reasons: 1) subjects reported a sensation of respiratory effort and denied when requested perception of air hunger; 2) subjects breathed through an
inspiratory load, a condition that implies the intervention of voluntary cortical drive; 3) activation of fine muscle afferents causes the increase of bulbar drive, but this is a short-lasting phenomenon (28), while, in our study, loaded breathing started 15–20 min after the end of quadriceps muscle work. Thus we feel that an increase in central motor command via corollary discharge is a plausible explanation for the perception of respiratory effort during loaded breathing (15, 48).

Increase in respiratory muscle effort, i.e., the ratio of the tidal pressure developed by the respiratory muscles to the maximum pressure-generating capacity of the muscles, is the main mechanism generating the perception of inspiratory effort (21, 27, 31, 47). However, the finding that fatigue resulted in an upward shift of change (H9004) in ratios of H9004Borg/H9004Pes, H9004Borg/H9004V˙E, and H9004Borg/H9004VT for any given inspiratory load (Fig. 6) argues against an increased drive as being a major reason for the greater Borg score.

Breathing against an inspiratory load may induce muscle fatigue (12, 33, 54), with a decrease in maximum pressure-generating capacity of the muscles, and increase in respiratory effort perception for a given pressure per breath (21, 27, 31, 47). However, the finding that fatigue resulted in an upward shift of change (Δ) in ratios of ΔBorg/ΔPes, ΔBorg/ΔV˙E, and ΔBorg/ΔVT for any given inspiratory load (Fig. 6) argues against an increased drive as being a major reason for the greater Borg score.

Breathing against an inspiratory load may induce muscle fatigue (12, 33, 54), with a decrease in maximum pressure-generating capacity of the muscles, and increase in respiratory effort perception for a given pressure per breath (21, 27, 31, 47). Because of the lack of any reduction in both Pdi,tw and maximal volitional inspiratory pressures during a sniff maneuver, neither central nor peripheral inspiratory muscle fatigue are deemed to have occurred in the present study. In turn, these findings suggest that central respiratory drive did not play a major role in the increase in dyspnea perception during the fatigue protocol. Pdi,tw slightly but significantly increased during the test (Fig. 4). A possible explanation for this phenomenon is twitch potentiation that occurs after vigorous inspiratory maneuvers (60). The long series of strong inspiratory efforts during loaded breathing may have induced the occurrence of a similar effect. However, although potentiation may have occurred after the first set of resistive breathing, it seems unlikely that there would be further potentiation that could explain the further increases in Pdi,tw at time points T2 and T3. Changes in lung volume, which can alter the size of the twitch evoked in the diaphragm during relaxation (41), might explain the increase in Pdi,tw. However, this does not seem to be the case in our subjects, in whom no change in EELV occurred, as demonstrated by the constancy of IC measurements throughout the test.

Ventilation and breathing pattern. Increase in ventilation reflects the increased motor command to the respiratory muscles. V˙E was not likely to play an important role in increasing dyspnea with quadriceps fatigue, as shown by the increased Borg rating for any given V˙E (see Fig. 6, middle), indicating that factors other than hyperventilation played a major role in the perception of dyspnea.

Time components of breathing pattern, Ti and Rf, independently contribute to respiratory difficulty during loaded breathing (21, 27, 31). This was not the case in the present study, where both Ti and Rf remained unchanged with fatigue. Our findings of unchanged IC during loaded breathing argue against the occurrence of hyperinflation as a factor contributing to increased respiratory difficulty during fatigue (8, 49).

Arterial blood gases. Both hypoxia and hypercapnia are able to generate or increase the dyspnea sensation per se (9, 10, 30, 37). Although we did not measure arterial PO2 during loaded breathing, the lack of changes in SaO2 throughout the tests does not indicate a significant reduction in arterial oxygenation.
not support the occurrence of hypoxemia as a possible cause of breathlessness. Likewise, the lower values of PETCO2 with fatigue and greater dyspnea at any given value of PETCO2 argue against arterial carbon dioxide affecting dyspnea perception.

**Neurological mechanisms.** The present results indicate that the increase in dyspnea perception observed during the fatiguing test was not associated with changes in respiratory variables, confirming the observation that the decrease in dyspnea sensation induced by vibratory stimulation was not accompanied by changes in respiratory output (19). So the question arises: how do we explain the increased dyspnea perception with fatigue? We postulate a neurological mechanism based on supraspinal contribution from fatigued resting muscles. Our hypothesis is supported by several data. On the one hand, evidence has been given that group III/IV muscle afferents transmit nociceptive and/or biochemical milieu-related inputs from working muscles to supraspinal areas of the central nervous system involved in the regulation of central motor drive (5, 38), as shown by the effects of epidural anesthesia (6). On the other hand, the afferent supraspinal projection of these nociceptive stimuli involves multiple ascending pathways feeding into subcortical and cortical structures, including thalamus, limbic system (insula and cingulated cortex), sensory motor cortex, and prefrontal cortex (1). It has been shown that anterior insula is activated not only during painful (i.e., eat, cold, etc.) and various aversive sensations (i.e., hunger, thirst, negative emotions) (58), but also during respiratory sensations, constituting a larger brain network underlying the perception of dyspnea, particularly in its affective and cognitive aspects (11, 14, 22, 51, 58, 59). Other structures, such as ventroposterior thalamus and sensory motor cortex, are also involved in the perception of both dyspnea and painful and aversive sensations, with a predominant function of processing their sensory aspects (58, 59). These integrative areas constitute a sort of dyspnea modulation network (50, 58) and represent a putative site for the setting up of the mechanisms controlling dyspnea perception. It is not unlikely, even if not yet demonstrated, that, at their level, a modulation of dyspnea by other afferent modalities may occur. Collectively these data and our results would support a role of peripheral muscle fatigue, through its effect on the central nervous system, in the observed changes in dyspnea perception.

As to the peripheral afferents likely involved in the increase in respiratory effort perception with muscle fatigue, we hypothesize a role for type III and/or IV afferents serving ergoreceptors that are excited by muscle fatigue (26, 42, 57). Two types of ergoreceptors have been described (25): mechanoreceptors, sensitive to mechanical distortion, and metaboreceptors, sensitive to chemical stimuli related to muscle work. In our study, metaboreceptors appear more likely to contribute for the following reasons: 1) the building up of metabolites due to fatiguing exercise is known to stimulate these receptors (26, 42); 2) mechanoreceptors tend to adapt rapidly to the stimuli, whereas metaboreceptors tend to increase their firing as fatigue develops (26, 42). Loaded breathing started 15–20 min after the end of quadriceps exercise, and there is no proof that metaboreceptors are still present in the muscle. In conclusion, a main
role of metaboreceptors in the observed responses may be reasonably hypothesized, even if it has not been demonstrated yet.

Our data do not allow us to draw any firm conclusion about the mechanisms involved in fatigue-induced increase in effort sensation. One possibility to be taken into account is that a nonspecific summation of different discomforts may occur. There are some experimental data supporting this hypothesis. Pain stimulation increases dyspnea sensation (46), while increased respiratory effort sensation induced by breathing against inspiratory resistance decreases nociceptive reflexes (45). These data suggest that uncomfortable sensations, such as pain and fatigue, associated with the activation of fine afferent fibers (Aδ and C, and III and IV groups) may interfere with dyspnea perception and vice versa. The existence of common cortical and subcortical areas integrating these different sensations (1, 11, 14, 22, 51, 58, 59) gives further support to this hypothesis.

In conclusion, we demonstrate that fatigue of limb muscles increases the perception of respiratory effort associated with loaded breathing, an effect likely mediated by the afferent outflow coming from fatigued muscles. These data suggest that nonrespiratory afferents may contribute to modulation of dyspnea associated with loaded breathing.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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