Increased fatigue resistance of respiratory muscles during exercise after respiratory muscle endurance training

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Increased fatigue resistance of respiratory muscles during exercise after respiratory muscle endurance training. Am J Physiol Regul Integr Comp Physiol 292: R1246–R1253, 2007. First published October 26, 2006; doi:10.1152/ajpregu.00409.2006.—Respiratory muscle fatigue develops during exhaustive exercise and can limit exercise performance. Respiratory muscle training, in turn, can increase exercise performance. We investigated whether respiratory muscle endurance training (RMT) reduces exercise-induced inspiratory and expiratory muscle fatigue. Twenty-one healthy, male volunteers performed twenty 30-min sessions of either normocapnic hyperpnoea (n = 13) or sham training (CON, n = 8) over 4–5 wk. Before and after training, subjects performed a constant-load cycling test at 85% maximal power output to exhaustion (PREEXH, POSTEXH). A further post-training test was stopped at the pretraining duration (POSTISO) i.e., isotime. Before and after cycling, transdiaphragmatic pressure was measured during cervical magnetic stimulation to assess diaphragm contractility, and gastric pressure was measured during thoracic magnetic stimulation to assess abdominal muscle contractility. Overall, RMT did not reduce respiratory muscle fatigue. However, in subjects who developed >10% of diaphragm or abdominal muscle fatigue in PREEXH, fatigue was significantly reduced after RMT in POSTISO (inspiratory: −17 ± 6% vs. −9 ± 10%, P = 0.038, n = 9; abdominal: −19 ± 10% vs. −11 ± 11%, P = 0.038, n = 9), while sham training had no significant effect. Similarly, cycling endurance in POSTEXH did not improve after RMT (P = 0.071), while a significant improvement was seen in the subgroup with >10% of diaphragm fatigue after PREEXH (P = 0.017), but not in the sham training group (P = 0.674). However, changes in cycling endurance did not correlate with changes in respiratory muscle fatigue. In conclusion, RMT decreased the development of respiratory muscle fatigue during intensive exercise, but this change did not seem to improve cycling endurance.

INSPIRATORY (17, 24) as well as expiratory (46), muscle fatigue can develop during exhaustive high-intensity exercise in healthy subjects, and respiratory muscle fatigue has been shown to decrease exercise performance (22, 27). Numerous studies have tested the ability of specific respiratory muscle training to improve respiratory muscle strength, endurance, and exercise performance (29, 39). Although the ergogenic effect of respiratory muscle training remains controversial (42, 50), several recent, well-controlled studies showed that threshold inspiratory muscle training (11, 38, 49), as well as voluntary normocapnic hyperpnoea training (16, 26, 31, 44) can improve exercise performance in healthy subjects.

The physiological mechanisms, however, by which respiratory muscle training improves whole body exercise performance remain unclear. Respiratory muscle training has been shown to have no influence on either cardiovascular response (26) or gas exchange (44) during exercise. Some studies reported a decrease in dyspnea (37, 45, 49) and blood lactate concentration (30, 37, 43) during exercise following respiratory muscle training. Two studies suggest a reduction in the development of inspiratory muscle fatigue during exercise after an inspiratory muscle training phase (38, 49). These authors assessed inspiratory muscle fatigue by using maximal voluntary inspiratory maneuvers before and after exhaustive exercise. However, when maximal voluntary maneuvers are used to assess respiratory muscle fatigue, it remains unclear to what extent poor coordination, submaximal effort (7), and central or peripheral fatigue (47) account for the change in pressure production after exercise.

To avoid the above confounding factors, “artificial” (electric or magnetic) nerve stimulation can be used to assess muscle contractility before and after exercise. Magnetic stimulation has been shown to be a sensitive effort-independent measurement for detecting inspiratory (20, 41), as well as expiratory (19, 46) muscle fatigue. Therefore, we used this technique to investigate the effect of respiratory muscle endurance training (RMT) on the development of inspiratory and expiratory muscle fatigue during exercise. To train inspiratory and expiratory muscles by simulating exercise hyperpnoea, we used normocapnic hyperpnoea for RMT. We hypothesized that RMT would delay the development of inspiratory and expiratory muscle fatigue as assessed by magnetic stimulation after intensive, constant-load exercise and that this would translate into improved exercise performance.

METHODS

Subjects

Twenty-one moderately trained, nonsmoking, male subjects (18–44 yr old) with normal lung function were studied, having given their written informed consent. Subjects were randomly assigned to two groups: RMT group (n = 13; performing RMT in the form of normocapnic hyperpnoea) and CON group (n = 8; the control group performing sham training). RMT group characteristics were (means ± SD): age, 31 ± 6 yr; height, 179 ± 8 cm; weight, 73 ± 8 kg; peak oxygen consumption (VO2peak), 55.6 ± 4.6 ml/min·kg−1; and maximal power (Wmax), 340 ± 44 W. CON group characteristics were: age, 31 ± 5 yr; height, 179 ± 4 cm; weight, 71 ± 10 kg; VO2peak, 55.6 ± 6.6 ml/min·kg−1; and Wmax, 336 ± 41 W. Weekly training
amounted to 6 ± 3 h/wk in RMT-subjects and 6 ± 2 h/wk in the CON group. Subjects were involved in endurance sports and performed mainly low-to-moderate intensity running, cycling, or swimming two to five times per week. The above variables were similar for both groups (P > 0.05). Subjects were requested to keep their individual training constant (same duration, intensity, and type of training) for at least 2 wk prior to, and throughout the course of, the study. All training details, including respiratory training (see below), were entered in a diary that was regularly checked by the experimenters to ensure subject compliance. Subjects refrained from physical exercise on the 2 days prior to the tests, refrained from drinking caffeinated beverages on test days, and were required to sleep for at least 7 h the night before the tests and to have their last meal at least 2 h prior to the tests. On each exercise test day, subjects loaded their carbohydrate stores by drinking 0.5 liters of an isotonic beverage (Isostar Long Energy; Wander, Bern, Switzerland) 2 and 4 h prior to the experiment (or the evening before, if the test took place within 2–4 h of waking). The study was approved by the local ethics committee and was performed according to the Declaration of Helsinki.

Measurements

Lung function and exercise tests. Resting lung function [vital capacity (VC), forced expiratory volume in 1 s, peak expiratory flow, and maximal voluntary ventilation in 15 s (MVV15)] assessed according to the guidelines of the American Thoracic Society (2) was measured with an ergospirometric device by using a calibrated turbine for volume measurement (Oxycon Alpha Plus; Jaeger, Höchberg, Germany). Ventilatory variables, oxygen consumption, and CO2 production during exercise were measured with the same device by using paramagnetic (O2) and infrared absorption (CO2) gas sensors for gas analysis. Exercise tests were performed on an electromagnetically braked bicycle ergometer (Ergometrics 800S; Ergoline, Bitz, Germany).

Blood lactate concentration ([La]) was determined in whole blood by using an enzymatic approach (ESAT 6661; Eppendorf, Hamburg, Germany).

Breathlessness, respiratory exertion, and leg exertion were assessed by means of a visual analog scale (VAS) consisting of three horizontal lines split post hoc into values between 0 and 10. They were labeled with the qualities of breathlessness, respiratory exertion, and leg exertion and ranged from none on the left side to maximum on the right side. Subjects were interviewed extensively prior to the tests on their experiences and understanding of different respiratory sensations. Subsequently, we discussed which sensations meant breathlessness (Atemnot, i.e., the sensation of “not getting enough air”) and which meant respiratory exertion (Atmungsanstrengung, i.e., “how difficult it is to breathe”). This ensured that subjects could distinguish between breathlessness and respiratory effort (14, 21).

Magnetic stimulation. Cervical and thoracic magnetic stimulations were performed by using a circular 90-mm coil powered by a 200 Hz stimulator (MagStim, Whitland, UK). Esophageal (Pes) and gastric (Pgs) vascular pressures were measured by conventional balloon catheters [length, 130 cm, diameter, 1 mm]; balloons (length, 10 cm, diameter 1 cm) contained 1 ml (esophagus) and 2 ml (stomach) of air, respectively] (32), connected separately to differential pressure transducers (model DP45-30; Validyne, Northridge, CA). Transdiaphragmatic pressure (Pdi) was obtained by online subtraction of Pes from Pgs. Two Pneumotrace bands (ADInstruments, Castle Hill, Australia) were used during magnetic stimulation to evaluate changes in thoracoabdominal configuration (40). One band was placed around the lower border of the pectoralis major and the other around the abdomen at the level of the umbilicus. Pressure and Pneumotrace analog signals were A/D converted (MacLab, ADInstruments) and recorded simultaneously on a computer (Chart Software version 4.0; ADInstruments).

Cervical magnetic stimulation of the phrenic nerves was performed while subjects were seated comfortably in a chair with the center of the coil positioned at the seventh cervical vertebra (40). Thoracic stimulation of the nerve roots innervating the abdominal muscles was performed while subjects lay prone on a bed with the center of the coil positioned at the intervertebral level T10 (19). Subjects’ position on the chair and the coil position were marked and checked continuously throughout the experiment. To avoid the confounding effect of potentiation (19, 23), subjects performed three 5-s maximal inspiratory efforts from functional residual capacity (for cervical stimulation) or three 5-s maximal expiratory efforts from total lung capacity (for thoracic stimulation) against a closed airflow prior to a series of nine stimulations at 100% of the stimulator output. After three and six stimulations, another 5-s maximal voluntary contraction followed. All stimuli were delivered at functional residual capacity after a normal expiration, with the airway occluded. As pressure recording was triggered by the magnetic stimulator, we were unfortunately not able to measure maximal inspiratory and expiratory muscle pressures during these 5-s maximal maneuvers. To ensure the same lung volume at all times, therefore and after each exercise, the experimenter checked on an oscilloscope (Tektronix, Beaverton, OR) that Pes ranged between 0 and −5 cmH2O for all subjects and that the level was similar for each subject immediately before each stimulation. Recordings that showed changes in Pes and/or thoracoabdominal configuration were rejected post hoc. Thus, for data analysis, the average amplitude (baseline to peak) of four to nine twitches (at each stimulation site) was calculated. To check for supramaximal stimulation before and after each exercise test, additional twitches were performed with 70, 90, 94, and 98% of the maximal stimulator output (6 twitches at each stimulator intensity) in 10 subjects during cervical stimulation (RMT, n = 6; CON, n = 4) and in 11 subjects during thoracic stimulation (RMT, n = 7; CON, n = 4).

Electromyogram. During magnetic stimulation, the M-waves of the diaphragm (cervical stimulation, n = 10) and of the rectus abdominis (thoracic stimulation, n = 11) were measured transcutaneously with skin-taped silver cup electrodes (1 cm diameter) to check for supramaximality of the magnetic stimulation. For recordings of the costal diaphragm, the two electrodes were placed on the anterior axillary line at the level of the eighth intercostal space (48). For recordings of the rectus abdominis, the two electrodes were placed 2 cm superior and 2–4 cm lateral of the umbilicus (10). Distance between the two electrodes was < 2 cm. Electrode placement was optimized for amplitude of the raw EMG signal in response to magnetic stimulations. All signals were amplified and band-pass filtered (bandwidth 10 Hz to 5 kHz; Neuropack Sigma, Nihon Kohden, Tokyo, Japan). Peak-to-peak amplitudes of the M-waves were measured for every twitch and averaged over six twitches. EMG analog signals were A/D converted (MacLab; ADInstruments) and recorded simultaneously with pressure signals on a computer (Chart Software version 4.0; ADInstruments).

Respiratory Endurance and Sham Training

Both the RMT group and the CON group performed 20 training sessions of 30 min duration with 1 day of rest between 2 days of consecutive training (duration of the training period: 4–5 wk). Training was performed at home, except every fifth training session was supervised in the laboratory. During every training session, heart rate was recorded using a Polar Vantage heart rate monitor (Polar Electro, Kempele, Finland), and training settings were recorded in a diary to verify compliance. The RMT group performed voluntary normocapnic hyperpnoea at a given tidal volume (VT) and breathing frequency (fR; paced by a metronome) with a duty cycle of 0.5. Subjects used a device developed in our laboratory allowing partial CO2 rebreathing. The device is fully described elsewhere (31). The size of the rebreathing bag was set at 50–60% VC, and minute ventilation (VE) of the first training
session was set to 60% MVV15. Subjects were instructed to increase \( f_R \) after 25 min of training, if they felt they would not be exhausted by 30 min. If they felt they could not sustain the target for 30 min, they were to decrease \( f_R \). In the former case, subjects were instructed to increase \( f_R \) for the next training session by 1–2 breaths/min; in the latter case, they should perform another session at the same \( f_R \) until they were able to sustain this frequency for 30 min. During supervised training sessions in the laboratory, the training device was connected to the metabolic cart to control training technique and to ensure normocapnia was maintained. If subjects felt uncomfortable with the setting of \( V_T \) and \( f_R \) at the \( V_T \) they had reached, \( V_T \) was changed and \( f_R \) was adjusted accordingly.

The CON group performed a sham training using an incentive spirometer (Voldyne 5000R; Sherwood Medical, St. Louis, MO). Subjects were instructed to perform one inspiration with \( V_T \) set to 70% VC every 30 s during 30 min (i.e., 60 maneuvers paced by a metronome). Between maneuvers, subjects breathed normally out of the spirometer. Subjects were told that this device trained respiratory coordination and alveolar recruitment, potentially allowing better performance.

Experimental Protocol

Subjects reported to the laboratory at least once prior to testing to familiarize themselves with the procedures and apparatus, as well as to adjust the individual training device. This familiarization session was critical to avoid any learning effects. After familiarization, subjects underwent three test sessions before the training phase started (pretraining tests), and four test sessions after the training phase (posttraining tests), all sessions being at least 2 days apart and performed at the same time of the day.

Pretraining tests. The first experimental session consisted of baseline spirometric measurements that were followed after a 30-min break by a respiratory endurance test (RETpre). The RET was performed with the RMT device connected to the metabolic cart. Subjects were requested to sustain a given \( V_E \) with a predetermined \( V_T \) and \( f_R \) (duty cycle 0.5) for as long as possible, i.e., until subjective exhaustion or until the subject could not sustain either \( V_T \) or \( f_R \) any longer, despite three consecutive “warnings” by the experimenter. During familiarization, a level of ventilation was chosen such that the subject could sustain the target \( V_T \) for a minimum of 6 min but no longer than 15 min (normally 70% MVV15). If the subject was unable to reach 6 min, the test was performed with a smaller target \( V_T \) (−5% MVV15), if the subject reached 15 min, the test was performed at +5% MVV15, both after a break of at least 2 days. The mean target \( V_E \) of the RET was 69 ± 7% of MVV15. Every 2 min during the test, subjects rated their perception of breathlessness and respiratory exertion using a VAS, and a 20-µl capillary blood sample was drawn from an earlobe for blood lactate analysis.

In the second experimental session, an incremental cycling test was performed to determine \( V_{O2peak} \) and \( W_{max} \). After 5 min of breathing at rest while seated on the bicycle with noseclip and mouthpiece in place, subjects started cycling at 100 W for 2 min. Subsequently, the load was increased by 30 W every 2 min until voluntary exhaustion. \( V_{O2peak} \) was defined as the highest 15-s average. The workload corresponding to the last stage sustained for at least 1.5 min was defined as \( W_{max} \). Subjects chose their preferred pedaling cadence (between 70 and 100 rpm) at the beginning of the incremental test, which was then maintained during this and subsequent cycling tests.

During the third experimental session, subjects performed a constant-load cycling endurance test (CETpre, Fig. 1). After 5 min of breathing at rest while seated on the bicycle with noseclip and mouthpiece in place, subjects cycled for 2 min at 40% \( W_{max} \) and 2 min at 60% \( W_{max} \), after which the workload was held constant at 85% \( W_{max} \) until the subject was exhausted or unable to sustain the individual target pedaling cadence anymore. Ventilation and gas exchange were recorded breath by breath. Every 2 min, subjects rated their perception of breathlessness, respiratory exertion, and leg exertion using the VAS, and 20 µl of capillary blood was drawn from an earlobe for blood lactate analysis. Twitch pressure measurements were performed before exercise, at the time of exhaustion, and after 30 and 60 min of recovery, as described above. These test sessions were followed by the 4–5 wk RMT, or sham training, phase.

Posttraining tests. The first posttraining experimental session (2–4 days after the last training session) consisted of baseline spirometric measurements that were followed, after a 30-min break, by a RETpost with the same target \( V_T \), \( V_E \), and \( f_R \) as during RETpre. The test was performed to exhaustion or was stopped by the experimenter after a duration of 40 min.

The second and third posttraining experimental sessions (performed at least 2 days after RETpost) consisted of two constant-load cycling endurance tests performed in randomized order with at least 2 days in between (Fig. 1). Both tests were performed at the same intensity as CETpre, but one test was stopped by the experimenter at the CETpre time of exhaustion, i.e., at isotime (CETpost,iso), while the other test was performed to exhaustion or until the individual target pedaling cadence was no longer sustainable (CETpost,exh). Prior to all cycling tests, subjects were told they had to cycle to exhaustion, while
no feedback regarding cycling duration was provided at anytime. To avoid experimenter bias, subjects were not encouraged during the tests.

In the fourth experimental session (at least 2 days after the second CETpost), the incremental cycling test was performed as described above.

Data Analysis

To assess the development of respiratory muscle fatigue during exercise, absolute values of twitch pressures before and after exercise were compared. To evaluate changes in the amount of fatigue between pre- and posttraining tests, the before-to-after decrease in twitch pressures (ΔPtw,di and ΔPtw,ga) were compared.

Ventilation, gas exchange, [La], and subjective measures were compared by calculating average values during the constant-load phase at 85% Wmax and during the last 15 s of CETpre. For CETpost,exh, two representative values were calculated: 1) the average corresponding to the CETpre duration at 85% Wmax and 2) the 15-s average corresponding to the last 15 s of CETpre. Five RMT and four CON subjects did not quite reach CETpre duration for both CETpost,iso and CETpost,exh; the longer of both tests was used for comparisons at isotime. In this case, constant-load and isotime averages were calculated accordingly, taking times of CETpost,exh for reference. For isotime-comparisons of variables that were assessed every 2 min (subjective measures and [La]), the value closer to isotime was chosen. For [La], increases from resting value (= [La] during test − [La] at rest) were calculated. For comparisons between RETpre and RETpost, subjective measures and [La] were averaged over the duration of the shorter of both tests.

Within-group comparisons from pre- to posttraining were performed by using the Wilcoxon Sign Rank Test. Changes in twitch pressures during each test session were assessed with a Friedman analysis of variance in combination with the Wilcoxon test with Bonferroni correction for post hoc analysis. Between-group comparisons of pre/post changes were performed using the Mann-Whitney U-test. The Spearman rank correlation coefficient was used to analyze relationships between selected variables. All statistical analyses were performed using standard statistical software (Statview version 5.0; SAS Institute, Cary, NC). All results are presented as mean ± SD and P < 0.05 was considered to be statistically significant.

RESULTS

Training compliance was excellent as all subjects completed 20 training sessions. Average heart rate was 99 ± 17 beats/min during RMT and 68 ± 6 beats/min during CON. In addition, subjects kept their individual training program constant during the 2 wk prior to, as well as throughout, the course of the study, as confirmed by the personal training diaries.

Spirometry and RET

Spirometry. After training, no significant changes were observed in lung function for either group except that MVV15 tended to increase post-RMT (P = 0.075; between groups: P = 0.060) (Table 1).

RET. Respiratory endurance significantly increased post-RMT, while no change was observed post-CON. Additionally, [La], breathlessness, and respiratory exertion decreased significantly in the RMT group, but these changes did not differ significantly between groups ([La], P = 0.877; breathlessness, P = 0.880; respiratory exertion, P = 0.174) (Table 1).

Table 1. Lung function, and respiratory sensations, blood lactate concentration, and duration of the respiratory endurance test before and after respiratory muscle training or sham training

<table>
<thead>
<tr>
<th></th>
<th>Pretraining</th>
<th>Posttraining</th>
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<tbody>
<tr>
<td>FVC, liters</td>
<td>RMT 5.55 (0.71)</td>
<td>CON 5.64 (0.78)</td>
</tr>
<tr>
<td>FEV1/FVC, %</td>
<td>RMT 85.4 (5.6)</td>
<td>CON 82.6 (8.7)</td>
</tr>
<tr>
<td>PEF, log^-1</td>
<td>RMT 11.59 (1.63)</td>
<td>CON 10.68 (0.83)</td>
</tr>
<tr>
<td>MVV1, l.min^-1</td>
<td>RMT 208.6 (23.9)</td>
<td>CON 203.4 (16.7)</td>
</tr>
<tr>
<td>BR, points</td>
<td>RMT 3.5 (2.6)</td>
<td>CON 2.0 (2.5)</td>
</tr>
<tr>
<td>RE, points</td>
<td>RMT 6.6 (1.9)</td>
<td>CON 5.6 (1.6)</td>
</tr>
<tr>
<td>[La], mmol.l^-1</td>
<td>RMT 1.6 (0.6)</td>
<td>CON 1.7 (0.8)</td>
</tr>
<tr>
<td>Respiratory endurance, min</td>
<td>RMT 9.88 (3.87)</td>
<td>CON 10.96 (3.64)</td>
</tr>
</tbody>
</table>

Values are means (SD). FVC, forced vital capacity; FEV1, forced expiratory volume in 1 s; PEF, peak expiratory flow; MVV1, maximal voluntary ventilation in 15 s; BR, breathlessness; RE, respiratory exertion; LE, leg exertion; [La], increase in blood lactate concentration from resting value. BR, RE, and [La] are calculated for isotime durations before and after the (sham) training phase (see Methods). RMT, respiratory muscle training group (n = 13); CON, sham training group (n = 8).*Significantly different from pretraining (P < 0.05).

Cycling Tests

Incremental cycling test. Wmax and VO2peak did not change from pre- to posttraining in either the RMT group or the CON group (results not shown).

Cycling endurance test. Cycling time at 85% Wmax did not change significantly from CETpre to CETpost,exh either in the RMT group (15.66 ± 3.72 vs. 17.26 ± 4.05 min, P = 0.071) or the CON group (15.36 ± 3.19 vs. 15.76 ± 2.81 min, P = 0.674). Changes were similar between the groups (P = 0.469). While 8 of 13 RMT subjects increased cycling time, exactly half of the CON subjects did so.

Twitch pressures before and after CET. Supramaximality of magnetic stimulation was confirmed by stimulating with increasing power output. Twitch Ptw,di (Ptw,di) during cervical stimulation and twitch Ptw,ga (Ptw,ga) during thoracic stimulation, as well as M-wave amplitudes of the diaphragm and the rectus abdominis, reached maximal levels at submaximal outputs of the stimulator (25) at all times, before and after exercise, as well as pre- and posttraining.

Before-exercise Ptw,di and Ptw,ga were similar between CETpre, CETpost,iso and CETpost,exh, both in the RMT group (Ptw,di = 36.9 ± 6.8 vs. 35.7 ± 6.8 vs. 37.6 ± 7.6 cmH2O, P = 0.416; Ptw,ga = 44.3 ± 9.2 vs. 41.6 ± 7.2 vs. 43.0 ± 9.8 cmH2O, P = 0.233) and in the CON group (Ptw,di = 41.0 ± 8.1 vs. 42.1 ± 5.6 vs. 42.2 ± 5.9 cmH2O, P = 0.881; Ptw,ga = 42.9 ± 6.8 vs. 50.9 ± 8.5 vs. 47.7 ± 7.4 cmH2O, P = 0.067).

After-exercise Ptw,di and Ptw,ga were significantly reduced in pre- as well as posttraining in both groups (all P < 0.01). Results of CETpre and CETpost,iso are presented in Fig. 2.

Posttraining, diaphragm and abdominal muscle fatigue, i.e., ΔPtw,di and ΔPtw,ga, did not differ significantly between CETpre and CETpost,iso in both groups (Fig. 2). However, when ana-

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lyzing those subjects separately that showed a physiologically relevant amount (25) of diaphragm or abdominal muscle fatigue in CETpre, i.e., >10% ΔPdi,tw (RMT, n = 9; CON, n = 6) and >10% ΔPga,tw (RMT, n = 9; CON, n = 4), a significant reduction in ΔPdi,tw and ΔPga,tw was detected in the RMT but not in the CON group after CETpost,iso (Table 2). There was, however, no difference between groups (ΔPdi,tw P = 0.556; ΔPga,tw P = 0.758).

The separate analysis of changes in cycling duration in subgroups with >10% ΔPdi,tw revealed a significant increase in CETpost,exh compared with CETpre after RMT (+15 ± 15% P = 0.017) but not after CON (+2 ± 20% P = 0.917); the difference between groups, however, did not reach the level of significance (P = 0.157). In subgroups with >10% ΔPga,tw, cycling duration did not change (RMT: +5 ± 14%, P = 0.374; CON: +8 ± 23%, P = 0.465; between groups: P = 0.758).

At the point of exhaustion of CETpost,exh, ΔPdi,tw (RMT: −12 ± 9%, CON: −17 ± 13%) and ΔPga,tw (RMT: −10 ± 10%, CON: −10 ± 5%) did not differ significantly from CETpre for all subjects, as well as the subgroups with >10% ΔPdi,tw (RMT: −13 ± 11%, CON: −23 ± 12%) and >10% ΔPga,tw (RMT: −12 ± 11%, CON: −15 ± 6%).

Table 2. Exercise-induced diaphragm and abdominal muscle fatigue (twitch pressures) before cycle endurance test (CETpre) and after (CETpost,iso) RMT or sham training (CON) in subjects with >10% pretraining reduction in twitch pressures after CETpre

<table>
<thead>
<tr>
<th>Group</th>
<th>CETpre</th>
<th>CETpost,iso</th>
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<tr>
<td></td>
<td>ΔPdi,tw</td>
<td></td>
</tr>
<tr>
<td>RMT</td>
<td>9</td>
<td>−17 (6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>−29 (7)</td>
</tr>
<tr>
<td>CON</td>
<td>6</td>
<td>−19 (10)</td>
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<tr>
<td></td>
<td>4</td>
<td>−18 (4)</td>
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</tbody>
</table>

Values are means (SD) in percent. CETpre, pretraining cycle endurance test; CETpost,iso, posttraining cycle endurance test stopped at CETpre time of exhaustion; ΔPdi,tw, %decrease in transdiaphragmatic twitch pressure from before to immediately after exercise; ΔPga,tw, %decrease in gastric twitch pressure from before to immediately after exercise. *Significantly different from CETpre (P < 0.05).

DISCUSSION

This study investigated the effect of respiratory muscle training on exercise-induced inspiratory, as well as expiratory muscle fatigue. At identical exercise times, the development of diaphragm and abdominal muscle fatigue was significantly reduced post-RMT but not post-CON in those subgroups of subjects showing a significant amount of respiratory muscle fatigue (>10% decrease in twitch pressure) pre-RMT. In the subgroup with a significant amount of diaphragm fatigue in CETpre, cycling endurance increased
significantly post-RMT but not post-CON. In the RMT group as a whole, however, cycling endurance did not increase posttraining, possibly due to side effects of RMT, as discussed below. Cycling endurance did not change post-CON.

Table 3. Ventilation, gas exchange, respiratory and leg sensations, and blood lactate concentration during CET_{pre} and CET_{post,exh} RMT or CON

<table>
<thead>
<tr>
<th></th>
<th>CET_{pre}</th>
<th>CET_{post,exh}</th>
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<tbody>
<tr>
<td>( V_E, \text{ l} \cdot \text{min}^{-1} )</td>
<td>RMT 109.6 (20.2)</td>
<td>113.2 (19.9)</td>
</tr>
<tr>
<td></td>
<td>CON 111.2 (22.7)</td>
<td>105.1 (18.0)</td>
</tr>
<tr>
<td>( V_T, \text{ liters} )</td>
<td>RMT 3.2 (0.4)</td>
<td>3.3 (0.4)</td>
</tr>
<tr>
<td></td>
<td>CON 3.1 (0.5)</td>
<td>3.1 (0.4)</td>
</tr>
<tr>
<td>( f_R, \text{ min}^{-1} )</td>
<td>RMT 34.7 (7.6)</td>
<td>34.5 (6.8)</td>
</tr>
<tr>
<td></td>
<td>CON 37.3 (10.6)</td>
<td>34.5 (7.4)</td>
</tr>
<tr>
<td>( V_{CO_2}, \text{ ml} \cdot \text{min}^{-1} )</td>
<td>RMT 3644 (489)</td>
<td>3603 (427)</td>
</tr>
<tr>
<td></td>
<td>CON 3690 (507)</td>
<td>3684 (563)</td>
</tr>
<tr>
<td>( PETCO_2, \text{ mmHg} )</td>
<td>RMT 3924 (509)</td>
<td>4013 (464)</td>
</tr>
<tr>
<td></td>
<td>CON 3943 (462)</td>
<td>3832 (337)</td>
</tr>
<tr>
<td>( BR, \text{ points} )</td>
<td>RMT 3.6 (2.6)</td>
<td>2.2 (2.8)*</td>
</tr>
<tr>
<td></td>
<td>CON 3.2 (3.3)</td>
<td>3.1 (3.5)</td>
</tr>
<tr>
<td>( RE, \text{ points} )</td>
<td>RMT 6.7 (1.4)</td>
<td>5.1 (2.2)*</td>
</tr>
<tr>
<td></td>
<td>CON 6.1 (1.9)</td>
<td>5.1 (2.9)</td>
</tr>
<tr>
<td>( LE, \text{ points} )</td>
<td>RMT 7.9 (1.1)</td>
<td>7.1 (1.4)</td>
</tr>
<tr>
<td></td>
<td>CON 7.9 (0.6)</td>
<td>7.2 (2.1)</td>
</tr>
<tr>
<td>[La], mmol-l^{-1}</td>
<td>RMT 7.3 (1.0)</td>
<td>7.2 (1.4)</td>
</tr>
<tr>
<td></td>
<td>CON 7.9 (2.2)</td>
<td>7.7 (1.7)</td>
</tr>
</tbody>
</table>

Values are means (SD) of average values corresponding to the duration of the shorter test; see METHODS. \( V_E \), minute ventilation; \( V_T \), tidal volume; \( f_R \), breathing frequency; \( V_{CO_2} \), oxygen consumption; \( PETCO_2 \), end-tidal \( CO_2 \) partial pressure; \( LE \), leg exertion. *Significantly different from CET_{pre} (\( P < 0.05 \)).

Fig. 3. Correlations between the pre- and post-RMT change in cycling endurance (\( \Delta \)cycling duration of CET_{pre} vs. CET_{post,exh}) and the pre- and post-RMT changes in minute ventilation (\( \Delta V_E \); A), respiratory frequency (\( \Delta f_R \); B), end-tidal \( CO_2 \) partial pressure (\( \Delta PETCO_2 \); C), and blood lactate concentration (\( \Delta [La] \); D) compared at isotime of CET_{pre} and CET_{post,exh} (pre- and posttraining changes are given as a percentage of pretraining values).

Exercise-Induced Respiratory Muscle Fatigue

Thanks to the assessment of inspiratory, as well as expiratory, muscle fatigue after one single test in each subject, we were able to observe that prior to training, abdominal muscles

Critique of Methods

**Supramaximal magnetic stimulation.** When measuring \( P_{di,tw} \) and \( P_{ga,tw} \), it is essential that stimulation is supramaximal to interpret differences in twitch pressures before and after exercise as being caused by muscle fatigue (3). Due to the large amount of stimulations needed when testing twitch pressures during increasing stimulator output, the time associated with this procedure, and the ensuing discomfort for the subjects, supramaximal stimulation was confirmed only for one stimulation site per subject, i.e., 10 subjects were tested during cervical stimulation and the other 11 subjects during thoracic stimulation. Although supramaximal stimulation was tested only for one stimulation site per subject, previous results from our laboratory using the same method (36, 46), as well as confirmation of supramaximal stimulation on the sites tested, suggest that most, if not all, stimulations were supramaximal.

**Twitch potentiation.** To avoid the confounding effect of twitch potentiation following contraction of skeletal muscle (3), subjects performed 5-s maximal inspiratory or expiratory efforts before twitch pressures were assessed, allowing full potentiation as previously shown (23, 51). Importantly, the present study exclusively assessed the effect of RMT on exercise-induced changes in single twitch response to cervical and thoracic nerve stimulation, i.e., low-frequency peripheral fatigue of the diaphragm and abdominal muscles (9, 33). Thus, no conclusions can be drawn on the potential effect of RMT on high frequency or central fatigue or on fatigue of rib cage muscles.
of healthy humans fatigue similarly to the diaphragm as a result of intensive, constant-load, exhaustive exercise. The level of diaphragm and abdominal muscle fatigue was similar to previous studies using electrical or magnetic stimulation of phrenic or thoracic nerves (17, 24, 46). Such a reduction in twitch pressure suggests impaired excitation-contraction coupling, possibly due to altered Ca$^{2+}$ exchange (9, 33).

Post-RMT, however, there was no significant reduction in respiratory muscle contractile fatigue at pretraining exercise durations. There may be several reasons: 1) RMT was not efficient enough to have an effect on the development of respiratory muscle fatigue during intensive exercise, or 2) some subjects did not develop significant respiratory muscle fatigue pretraining, and thus fatigability of these muscles could not be improved. Since respiratory muscles are involved not only in breathing but also in postural functions, it may be argued that RMT did not completely prevent fatigue of the respiratory muscles associated with their postural role, despite a large effect on respiratory muscle endurance. Although we cannot rule this out, the degree of involvement of respiratory muscles in postural work during cycling may not be critical (1).

To investigate the second point, we applied the criteria used initially by Mador et al. (25), i.e., we analyzed the subgroup of subjects with a $>10\%$ decrease in twitch pressure after exercise. Indeed, subjects in these subgroups showed a significant exercise-induced reduction in $P_{di,tw}$ and $P_{ga,tw}$ post-RMT, but not post-CON. Hence, these results suggest that normocapnic hyperpnoea training delays the development of both diaphragm and abdominal muscle contractile fatigue during whole body exercise. However, the postraining changes in exercise-induced fatigue were not significantly different between RMT and CON groups, nor between their subgroups with a relevant amount of fatigue, potentially because inspiratory and expiratory muscle fatigue also tended to be reduced post-CON. The small sample size, and thus increased possibility of type II error with subgroup analysis, may have resulted in the lack of significance in the CON group. Hence, we cannot exclude an effect of CON, e.g., improvement of respiratory muscle coordination and subsequent efficiency, which may have been sufficient to increase fatigue resistance of respiratory muscles. This is supported by the fact that CON subjects significantly changed their breathing pattern when approaching exhaustion, breathing deeper and slower, similar to their sham training.

The comparison of decreases in $P_{di,exh}$ and $P_{ga,exh}$ at the point of exhaustion revealed no significant difference between CETpre and CETpost,exh, even in the subgroup of subjects with increased cycling duration. This suggests that respiratory muscle fatigue from exhaustive endurance exercise is similar pre- and posttraining, despite increased cycling time and therefore increased overall respiratory work. This supports a positive effect of RMT on the development of respiratory muscle fatigue.

**Exercise Performance Post-RMT**

In the present study, cycling endurance significantly increased post-RMT only in the subgroup of subjects with a relevant amount of diaphragm fatigue pre-RMT, while in previous studies (4, 5, 26, 31, 43, 44), RMT resulted in significantly increased cycling endurance in the entire training group. Despite a reduction in diaphragm fatigue and improved cycling endurance, changes in respiratory muscle fatigue after RMT did not correlate with changes in cycling endurance in this subgroup with relevant diaphragm fatigue pre-RMT. Thus, other mechanisms may contribute to the changes in exercise endurance post-RMT. For example, pre-to-post-RMT changes in cycling endurance correlated with changes in ventilation and $[La]$, i.e., all but one subject who hyperventilated ($n = 5$) had a reduced cycling endurance post-RMT, while all subjects with reduced ventilation ($n = 7$) cycled for a longer time. Whether a change in $[La]$ might have caused the change in ventilatory drive or vice versa remains unclear.

It is, in fact, not the first time that respiratory drive increased during exercise after repetitive normocapnic hyperpnoea (4, 18, 35). In two studies, endurance performance was impaired when subjects breathed more (4, 18), suggesting that the change in exercise ventilation post-RMT is a critical factor determining cycling endurance. Several experimental results indeed emphasize the plasticity of the exercise ventilatory response (15, 28, 35). Hence, long-term modification of the respiratory drive induced by repeated voluntary normocapnic hyperpnoea might be responsible for the relative hyperventilation during cycling post-RMT in some subjects. An increase in respiratory work as observed in some subjects during exercise after RMT can, in turn, reduce leg blood flow and cycling performance, while a reduction in respiratory work has the opposite effect (8, 12, 13). Increased respiratory muscle work and/or compromised leg blood flow could have favored anaerobic metabolism in respiratory and/or leg muscles. However, respiratory alkalosis per se due to hyperventilation ($n = 5$) could also have increased $[La]$ during exercise (6).

Finally, we discuss the effect of changes in subjective perceptions, e.g., breathlessness, respiratory or leg exertion, that could potentially affect exercise performance (34). In the present study, although breathlessness as well as respiratory exertion were reduced after RMT, these improvements were not per se related to improvements in cycling duration. However, perception of leg exertion did not change after RMT, neither in the entire group nor in subgroups where respiratory muscle fatigue was reduced. Thus, an effect as described by Dempsey et al. (8) (with less respiratory muscle fatigue, leg blood flow is less compromised, and as a consequence, leg exertion is reduced and exercise performance improved) could not be confirmed in the present study. Hence, respiratory muscle work, rather than respiratory muscle fatigue or changes in sensations, seem to be an important determinant of changes in exercise performance after RMT.

In summary, the present results showed that RMT can reduce the development of inspiratory and expiratory muscle contractile fatigue, as well as the perception of adverse respiratory sensations during exhaustive, constant-load exercise. These changes, however, did not directly translate into an improvement in exercise performance. Changes in exercise ventilatory drive may affect performance more directly.

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