BMC Physiology

BioMed Central

Research article

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Endurance training of respiratory muscles improves cycling performance in fit young cyclists

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Published: 06 May 2004

BMC Physiology 2004, **4**:9

Received: 22 December 2003

Accepted: 06 May 2004

This article is available from: http://www.biomedcentral.com/1472-6793/4/9

Abstract

Background: Whether or not isolated endurance training of the respiratory muscles improves whole-body endurance exercise performance is controversial, with some studies reporting enhancements of 50 % or more, and others reporting no change. Twenty fit (VO_2 max 56.0 ml/kg/min), experienced cyclists were randomly assigned to three groups. The experimental group (n = 10) trained their respiratory muscles via 20, 45 min sessions of hyperpnea. The placebo group (n = 4) underwent "sham" training (20, 5 min sessions), and the control group (n = 6) did no training.

Results: After training, the experimental group increased their respiratory muscle endurance capacity by 12 %. Performance on a bicycle time trial test designed to last about 40 min improved by 4.7 % (9 of 10 subjects showed improvement). There were no test-re-test improvements in either respiratory muscle or bicycle exercise endurance performance in the placebo group, nor in the control group. After training, the experimental group had significantly higher ventilatory output and VO_2, and lower PCO_2, during constant work-rate exercise; the placebo and control groups did not show these changes. The perceived respiratory effort was unchanged in spite of the higher ventilation rate after training.

Conclusions: The results suggest that respiratory muscle endurance training improves cycling performance in fit, experienced cyclists. The relative hyperventilation with no change in respiratory effort sensations suggest that respiratory muscle training allows subjects to tolerate the higher exercise ventilatory response without more dyspnea. Whether or not this can explain the enhanced performance is unknown.

Background

Understanding the effects of respiratory muscle training on endurance exercise performance (e.g., large muscle mass dynamic exercise, such as running, cycling and rowing) is an important issue in human performance physiology because such supplemental training has the potential to improve performance even in endurance athletes [1]. This is also important from a clinical standpoint, because respiratory muscle training is often used as one component of the therapy plan in patients with obstructive lung diseases. Several recent studies in healthy subjects have shown that specific training of the respiratory muscles is associated with enhanced endurance exercise performance, at least when the exercise tests require the subjects to

Table 1: Characteristics of all subjects in the respiratory muscle endurance training (RMET), control [C] and placebo (P) groups.

Subject	Group	Sex	Age (y)	Height (cm)	Weight (Kg)	Years Cycling	FVC (ml)	$FEV_{1.0}$ (ml)	$FEV_{1.0}$/FVC (%)	MVV_{12} (L/min)
IP	RMET	F	30	157.5	54.4	8	3750	3100	83	109.5
RK	RMET	M	45	177.8	77.1	5	5683	4616	81	167.5
MB	RMET	M	27	175.3	71.7	4	5783	4900	85	196.7
DN	RMET	M	29	188.0	72.6	3	5483	4350	80	158.3
BH	RMET	M	35	177.8	84.8	7	4866	4100	84	189.1
KH	RMET	F	22	167.6	58.1	3	3550	3067	86	110.0
ZC	RMET	F	30	162.6	56.7	10	3325	3063	92	126.9
TH	RMET	M	32	167.6	68.0	10	5075	4025	79	175.7
NW	RMET	M	24	188.0	90.7	2	6250	4650	74	196.9
DB	RMET	M	29	167.6	67.1	6	4375	3775	86	164.5
DJ	C	M	43	175.3	73.9	4	5533	4283	77	157.5
CJ	C	F	21	162.6	61.2	4	4075	3475	85	136.2
TK	C	M	24	175.3	68.1	1	5800	4700	81	205.2
JS	C	M	31	172.7	74.4	10	5334	4467	84	212.8
SC	C	M	24	172.7	69.4	9	5767	4484	78	186.4
SL	C	M	35	180.3	83.9	2	6216	5033	81	205.9
TZ	P	M	22	182.9	82.1	5	5188	4338	84	149.7
PF	P	M	18	198.1	77.1	5	6187	5150	83	203.5
SE	P	M	20	170.2	68.0	2	5200	4050	78	198.8
JM	P	M	29	188.0	74.8	1	5450	4650	85	200.5
Mean ± SD			28.5 ± 7	175 ± 10	71.7 ± 9.6	5 ± 3	5144 ± 888	4213 ± 635	82.3 ± 4	173 ± 33

FVC, forced vital capacity; $FEV_{1.0}$/FVC, ratio of the forced expiratory volume in 1.0 second to the FVC; MVV_{12}, maximal voluntary ventilation, averaged over 12 sec.

work at about 70–80 % of their maximal capacity or less [2-8]. Others have examined the effects of respiratory muscle training on the ability to perform very intense endurance exercise (85–95 % of maximal capacity), and uniformly find no change in performance [9-12]. Thus, one might conclude that specific training of the respiratory muscles leads to enhanced endurance exercise performance as long as the intensity is below about 85 % of the individual's maximal capacity.

Nevertheless, it is noteworthy that all of the above-cited studies used "open ended", constant work rate exercise tests as the index of performance. These tests rely on subjective feelings of exhaustion as the end point, and are highly variable, with repeat testing in the same subjects differing by as much as 30 % [13,14]. Given that the variability of such tests far exceeds the expected change in exercise performance [13,14], several investigators have recently applied performance-oriented tests to examine the influence of respiratory muscle training on endurance exercise performance. These tests typically take the form of a "time trial" task where subjects complete a fixed quantity of work or a fixed distance as quickly as possible. The major advantage of these tasks is that they are highly reproducible (coefficient of variation of 1–4 %; [13,14], this study). Of five recent studies that used such tests to examine the influence of respiratory muscle training on exercise performance, three showed an approximately three percent improvement in performance [15-17], and two failed to find changes in performance that were significantly different than the changes observed in sham-training groups [13,18]. Importantly, the three studies that did report significantly enhanced time trial performance after training of the respiratory muscles [15-17] were carried out by the same group of investigators, and the subjects were trained for respiratory muscle strength, not endurance. Our examination of the literature on respiratory muscle training and exercise performance reveals that no single study has coupled respiratory muscle endurance training (RMET) with a time-trial exercise performance test. Accordingly, our major objective was to test the hypothesis that RMET will significantly improve both respiratory muscle endurance, and cycling exercise performance in fit, experienced cyclists.

The mechanism underlying the apparent enhancement in exercise performance following respiratory muscle training is unknown. Although many hypotheses have been advanced, a popular idea is that there is a lowering of ventilation (V_E) during exercise after respiratory muscle training that reduces respiratory muscle blood flow, in turn benefiting the exercising locomotor muscles with increased blood availability [13,19,20]. Alternatively, exercise related increases in V_E following respiratory muscle training may positively impact blood acid base balance and/or blood oxygen transport [21,22]. But data on the effects of respiratory muscle training on the ventilatory response to endurance exercise is equivocal, probably because it depends importantly on the type of exercise task used and on the details of the respiratory muscle

Table 2: VO_2 max and exercise performance before and after RMET. *, different than RMET group-pre-training value.**

	RMET-pre training	RMET-post training	Control/Placebo-pre training	Control/Placebo-post training
VO_2 max (ml/kg/min)	54.0 ± 4.7	55.5 ± 4.9	56.8 ± 3.9	55.9 ± 7
Peak work (watts)	340 ± 28	348 ± 26	376 ± 32	388 ± 33
Constant-work rate endurance test time (min)	37.5 ± 4.3	37.0 ± 5.2	43.4 ± 4.1	42.5 ± 4.6
Time trial time (min)	47.1 ± 5.5	44.9 ± 5.5 ***	42.9 ± 3.2	43.0 ± 3.9

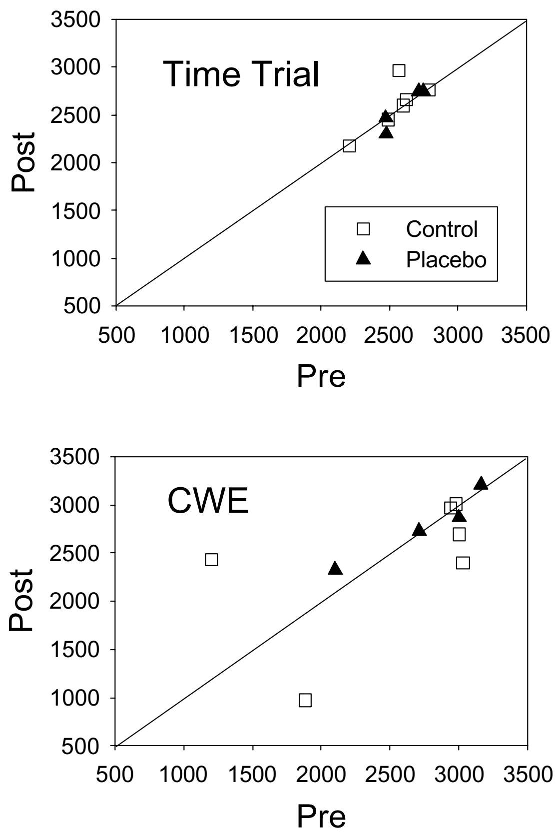

Figure 1
Test-re-test reproducibility in the control/placebo group subjects during the time trial test and the constant work-rate exercise (CWE) test, before (Pre) and after (Post) the intervention period. Control and placebo group subjects are depicted with different symbols. Identity lines drawn in both graphs. Note that the time trial test is significantly more reproducible than the CWE test, consistent with the findings of others [13,14]. See text for numerical analysis.

training protocol. For example, an examination of the results of fifteen studies that report exercise V_E before and after a period of respiratory muscle training reveals the following: eight studies report no change in V_E after training [4,7,10-13,15,18], two show a decrease [2,3], two show an increase [6,8] and three show a clear trend towards an increase [5,9,17]. These are clearly equivocal results, especially when one considers the wide variety of training regimens and performance tests that were used. Thus, a secondary goal was to test the hypothesis that endurance training of the respiratory muscles results in an increased ventilatory response to constant work-rate endurance exercise.

Results

Subjects

General characteristics of the subjects in all three groups are summarized in Table 1. Subjects in the three groups were similarly experienced cyclists, were of approximately the same age, had spirometry values that were within the normal range, and were predominately male. There were no significant differences in VO_2 max between the groups, either before or after training (Table 2).

Reproducibility of the exercise tests

The coefficient of variation for the time trial test was 3.94 % (Fig. 1, top panel), even though there were two subjects (one from the true control and one from the placebo training group) with vastly different values before and after the training period. If these two data points are not used, the coefficient of variation was only 0.78 %. In contrast, the coefficient of variation on the constant work-rate exercise test averaged 15.6 % (Fig. 1, bottom panel).

Respiratory muscle strength and endurance following RMET

As expected, respiratory muscle strength, as assessed by measuring maximal inspiratory and expiratory mouth pressures, was unchanged by RMET. For example, peak inspiratory mouth pressure averaged 83.5 ± 8.3 cmH_2O before training, and 93.9 ± 7.2 cmH_2O after training, and peak expiratory pressure was 91 ± 10 cmH_2O before and 88.5 ± 11 cmH_2O after training. For the Control/Placebo group inspiratory pressure averaged 86.5 ± 7.4 cmH_2O before training, and 88.8 ± 7 cmH_2O after training; corresponding values for expiratory pressure were 104 ± 8.4 cmH_2O before training, and 97 ± 9.3 cmH_2O at the end of

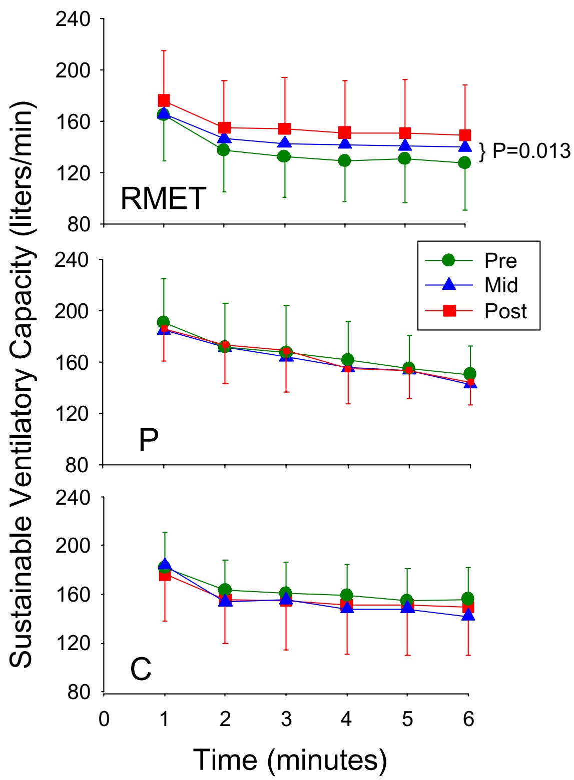

Figure 2
Sustainable ventilatory capacity before the training phase (Pre), after two weeks of training (Mid) and immediately after (Post) the training phase in the three subject groups. The RMET group had a higher sustainable ventilatory capacity after training (see text for detailed explanation of this test).

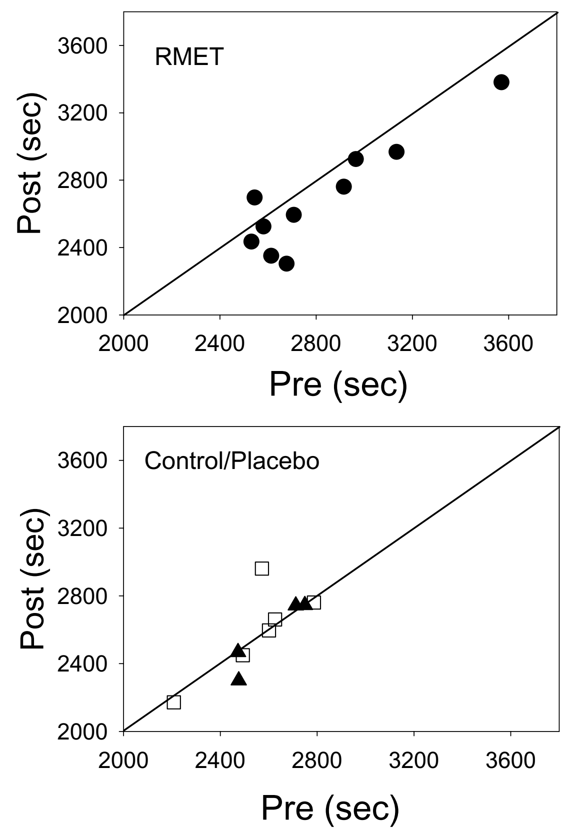

Figure 3
Identity plots comparing time trial performance before (Pre) and after (Post) the intervention period in the RMET group (top panel) and in the control (bottom panel, open squares) and placebo (bottom panel, filled triangles) groups. Note that all but one subject in the RMET group improved their performance time, and that only three subjects in the control and placebo groups improved (two controls, one placebo), and then only marginally.

the training period. The maximal mouth pressure values that we recorded during both inspiration and expiration are similar to those observed in other young, fit subjects [13,15,23], and none of the values changed significantly with training of the respiratory muscles, nor were there any differences between the subject groups. In contrast to strength training, the endurance capacity of the respiratory muscles (estimated as the sustainable ventilatory capacity) increased significantly after training in the RMET group, but not in any of the control or placebo group subjects (Fig. 2).

Exercise performance following RMET
We assessed cycling performance by using the time trial test, and the performance of all subjects before and after the intervention period is shown in Fig. 3. With one exception, all subjects that underwent RMET improved their performance time (top panel). The average performance time fell from 47.1 ± 5.5 min before RMET, to 44.9 ± 5.5 min after RMET (Table 2, $P < 0.05$), a reduction of 4.75 %. Performance time did not change significantly in the control/placebo group (Table 2 and Figure 3, bottom panel). The percent change in performance time in the

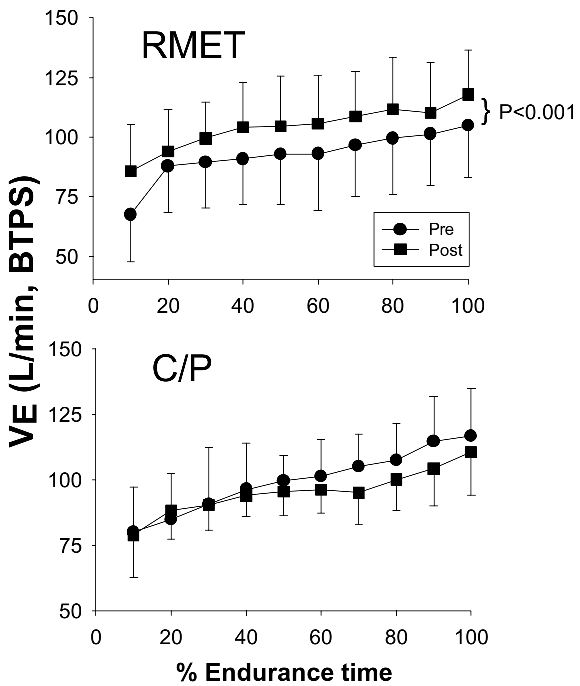

Figure 4
Changes in pulmonary ventilation rate (V_E) as a function of the % endurance time during the constant work-rate exercise test. The RMET group showed a significant increase in V_E after training; there were no significant pre-post differences in the control/placebo group. Bracket, $P < 0.01$, by ANOVA, for pre vs. post-training comparison.

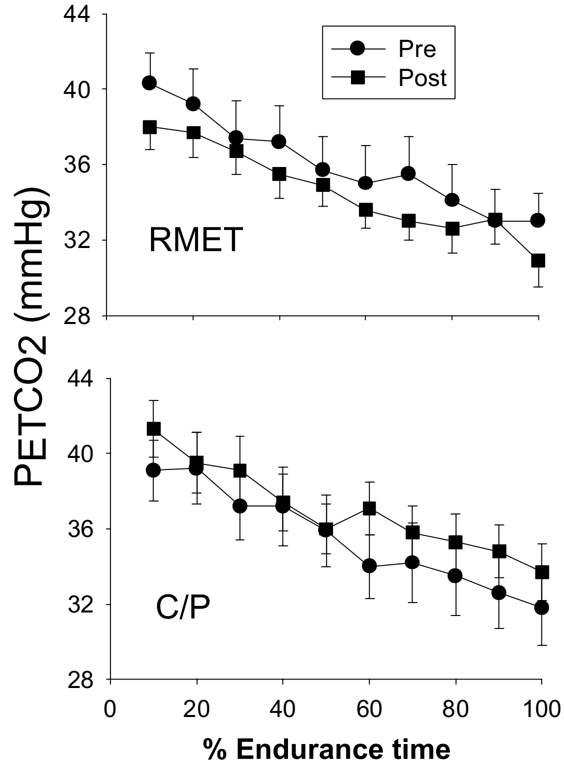

Figure 5
Changes in the partial pressure of end-tidal CO_2 ($P_{ET}CO_2$) as a function of the % endurance time during the constant work-rate exercise test, before and after training. There were no significant changes in either group, although the RMET group showed a trend towards a decrease after training ($P = 0.18$). Note that $P_{ET}CO_2$ did decline markedly as a function of time during exercise in both groups, both before and after training, consistent with the upward drift in ventilation.

RMET group (-4.75 ± 1.6 %) and in the control/placebo group (0.46 ± 1.8 %) revealed a statistically significant difference (P = 0.015, Kruskal-Wallis ANOVA on ranks). As shown in Table 2, there were no significant changes in constant work-rate endurance time before and after the training period in either group.

Cardiorespiratory responses and perceived exertion during constant work-rate exercise before and after RMET

We measured cardiorespiratory responses to constant work-rate exercise over the last 30 sec of each 10 % epoch of endurance time, before and after the intervention period. Pulmonary ventilation was higher after training in the RMET group and unchanged in the control/placebo group (Fig. 4). The increased V_E in the RMET group was explained by a significant rise in f (P = 0.013) with no change in V_T. The partial pressure of end-tidal CO_2 ($P_{ET}CO_2$) was not significantly different after the training period in either group (Fig 5). VO_2 increased significantly in the RMET group, and did not change in the control/placebo group (Fig. 6). Heart rate was the same before and after the training period throughout the exercise bout in both groups. For example, at the point of exhaustion in the RMET group, heart rate was 173 ± 2 beats/min before training and 172 ± 5.2 beats/min after training. For the control/placebo group, the corresponding values were 178 ± 3.6 beats/min before the training period, and 170 ± 8 beats/min after (P > 0.05 in both groups). Perceived exertion for both breathing and leg muscle effort rose monotonically and significantly (P < 0.001) over the course of the time trial test, but there were no significant

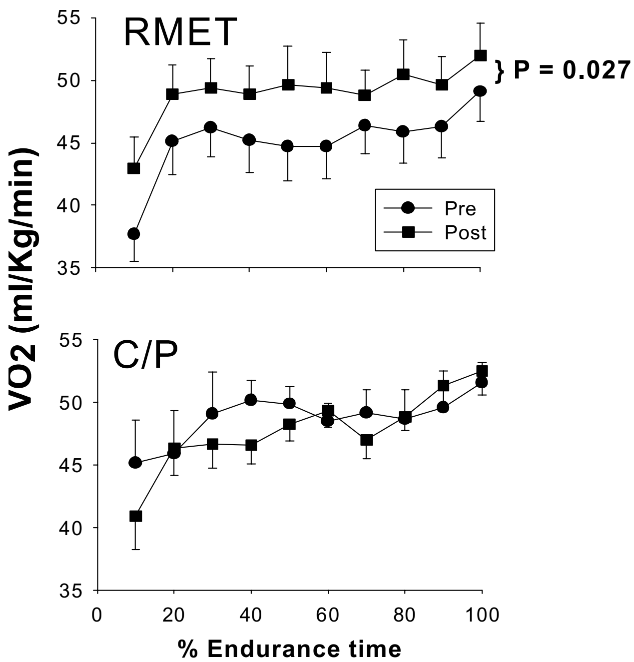

Figure 6
Changes in VO_2 as a function of the % endurance time during the constant work-rate exercise test. The RMET group showed an increase after training (Bracket, P < 0.027, by ANOVA, for pre vs. post-training comparison); no significant changes were observed in the control/placebo group.

differences after the intervention period in either the RMET or the control/placebo groups (data not shown).

Cardiorespiratory responses during the time trial tests, before and after RMET

An example of the changes in V_E and VO_2 during the time trial tests, before and after the intervention period, are given in figure 7 for a representative control and RMET subject. In all RMET subjects, V_E and VO_2 were higher in the post-RMET time trial test compared to the pre-RMET time trial test (Table 3). There were no consistent changes observed in the control/placebo group (Table 3). Note that the pre-training V_E was lower in the RMET group because the subjects in this group were significantly smaller, and three of the four female subjects were in this group (Table 1).

Correlation between the change in time trial performance and the change in V_E

The correlation between the change in V_E and the change in performance time on the time trial test for all subjects is shown in Fig. 8. The correlation coefficient was -0.522, and was statistically significant (P = 0.0183).

Discussion

Summary of major findings

The major, new finding of this study is that twenty sessions of respiratory muscle endurance training significantly increased both respiratory muscle endurance capacity and bicycle time trial performance in nine of ten fit, experienced cyclists and/or triathletes. We also showed that the improved performance was accompanied by an enhanced ventilatory response during constant work-rate exercise. We did not observe significant changes in either respiratory muscle endurance or time trial performance in a control group consisting of six subjects that did not train their respiratory muscles and four "placebo" subjects that trained their muscles at a low and ineffective intensity and duration.

Endurance training of the respiratory muscles

Subjects that underwent RMET showed a significant, 12 % increase in the sustainable ventilatory capacity, with no change in respiratory muscle strength, demonstrating that the training protocol specifically enhanced respiratory muscle endurance performance. In contrast, the control and placebo groups did not show significant changes in either sustainable ventilatory capacity or respiratory muscle strength. The sustainable ventilatory capacity test was initially used by Leith and Bradley [23], who showed a 15 % increase in sustainable ventilatory capacity in subjects that underwent a respiratory muscle training program similar to that used in the present study. Fairbarn et al [10] also used the sustainable ventilatory capacity as an index of respiratory muscle training, and showed a 12% increase after sixteen endurance training sessions. Thus, the present results are consistent with those obtained by other investigators that have used the same training protocol. In contrast, Sonetti et al. [13] failed to show a significant improvement in the sustainable ventilatory capacity following five weeks of respiratory muscle training in fit cyclists, although they used a slightly different method than ours.

Exercise performance after training of the respiratory muscles

Previous studies have examined the influence of respiratory muscle endurance or strength training on VO_2 max and found no significant improvement [9,10,18]. However, VO_2 max is unlikely to be limited by an inadequate ventilatory response [24], so these negative results are not surprising. More recent studies have examined the influence of RMET on exercise endurance performance, and have shown that specific training of the respiratory muscles is associated with enhanced endurance performance, at least when the exercise tests require the subjects to work

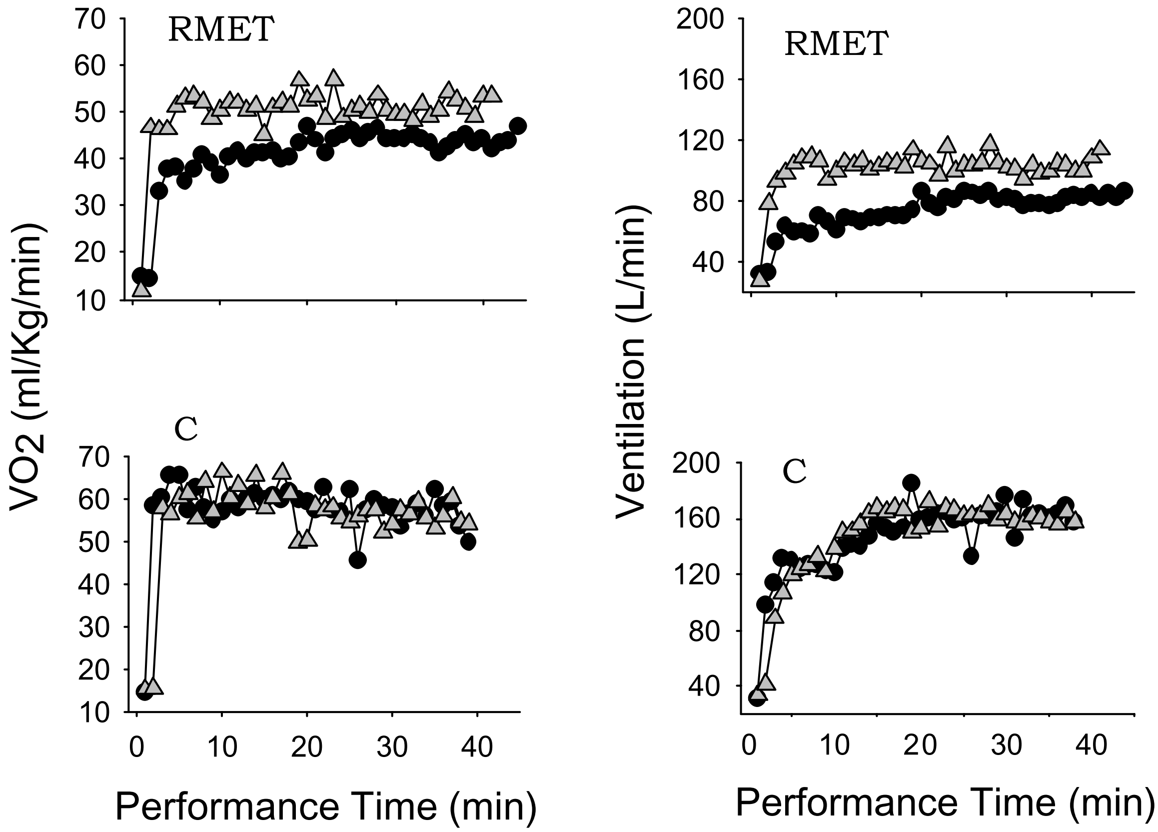

Figure 7
Changes in VO_2 (left-hand panels) and ventilation (V_E, right-hand panels) as a function of performance time during the time trial test, before and after either RMET (top two panels) or a period of no training (control group, C, bottom two panels), in two representative subjects. The *filled circles* represent data obtained before the period of training, and the *shaded triangles* are the post-training values. The subject in the RMET group showed an increase in V_E and VO_2 after training, mirroring the changes observed with constant work-rate exercise. The control subject showed no changes between the first and second test. See text for a more detailed explanation, and Table 3 for the average data.

Table 3: Ventilation and VO_2 data during the time trial test, before and after the intervention period in the control/placebo and RMET groups. The data represent the average of all data obtained from the fifth minute through the end of the test (see Fig. 7). Values are means ± SEM.

	RMET-pre training	RMET-post training	Control/Placebo-pre training	Control/Placebo-post training
V_E (L/min, BTPS)	80.2 ± 7.5	96 ± 9.5[*,a]	97.6 ± 5	98.2 ± 8
VO_2 (ml/kg/min)	42 ± 3.2	46.3 ± 4[*,b]	47 ± 0.3	51.1 ± 4

[*,a], different than before training, P = 0.018; [*,b], different than before training, P = 0.008.

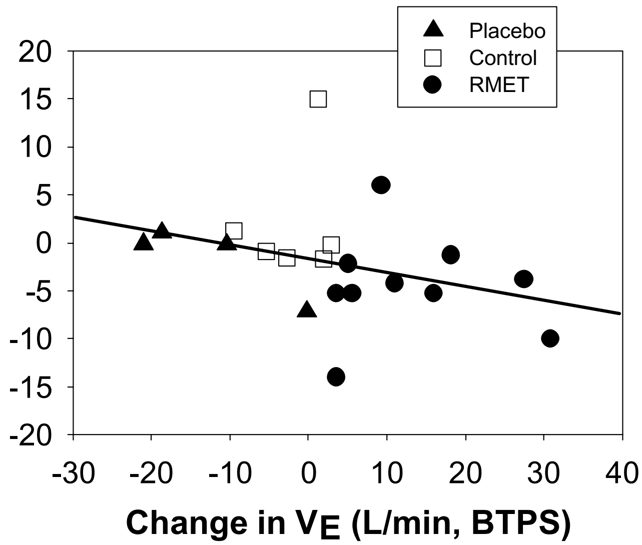

Figure 8
Correlation between the change in performance time and the change in V_E with RMET. Values were computed as the difference between the values obtained on the pre-training test and the post-training test in the RMET group, and in the control and placebo group subjects. The correlation was statistically significant ($r = -0.522$, $P = 0.0183$), suggesting that the subjects that breathed more during exercise also had better performance, as reflected as faster times (negative % change) on the time trial test.

at about 70–80 % of their maximal capacity or less [2-8]. Others have examined the effects of respiratory muscle training on the ability to perform very intense endurance exercise (85–95 % of maximal capacity), and uniformly find no change in performance [9-12]. However, in all of these studies the subjects completed an open-ended exercise test, where they were asked to perform constant work rate exercise until "exhaustion". The subjective end point of such tests results in high variability, with the coefficient of variation for repeat testing in the same subjects ranging from 15 % (this study, Fig. 1) to as high as 30 % [13,14]. In contrast, the fixed end-point performance test, or "time trial" that we used has a coefficient of variation of 3.89 %, which is similar to recent work reporting values ranging form 1 % [13] to 3.4 % [14]. Moreover, the time trial test is very similar to an actual bicycle race, wherein the goal is to cover the race distance, from starting line to finish line, in as short a time as possible.

Of five recent studies that used time trial tests to examine the influence of respiratory muscle training on exercise performance, three showed enhanced performance (on the order of 3 %) [15-17], and two failed to find changes in performance that were significantly different than the changes observed in sham-training groups [13,18]. However, the three studies that did report enhanced time trial performance after training of the respiratory muscles [15-17] were carried out by the same group; and more importantly, the respiratory muscles of their subjects were trained for strength, not endurance. Thus, the present study is the first to show that specific endurance training of the respiratory muscles leads to enhanced time trial performance in fit, young cyclists. Nevertheless, our findings are consistent with the results of recent studies that used strength rather than endurance training of the respiratory muscles [15-17], indicating that either method can lead to enhanced exercise performance.

The almost 5 % improvement in cycling performance in the trained subjects is remarkable considering that our subjects were fit and already close to the limit of their performance potential. Thus, a race that a cyclist could complete in 40 minutes before respiratory muscle training would be completed in just over 38 minutes with a 4.7 % improvement in performance. We are unaware of other training methods that result in similar performance increments in experienced bicycle racers. Whether or not more extended or more intense respiratory muscle training would lead to even larger improvements in exercise performance is uncertain, but the results of this study suggest that this should be addressed.

Cardiorespiratory responses to constant work-rate exercise after RMET

Comparison of cardiorespiratory responses to CWE before and after RMET was done to determine if any key cardiorespiratory variables changed in response to the training stimulus. We found that V_E and VO_2 were significantly higher in CWE after RMET. Our subjects kept their training regimen constant, with the exception of adding the RMET, as evidenced by regular evaluation of their daily exercise logs. Thus, changes in the ventilatory response to CWE after RMET may be attributed to alterations in the performance of the respiratory muscles.

Accordingly, we examined the results of fifteen studies that reported ventilatory responses to exercise before and after a period of respiratory muscle training. Of the ten studies that used a respiratory muscle endurance training protocol, two report that V_E during exercise was reduced after training [2,3], four show no change in V_E [4,7,10,13] two showed a significant increase [6,8] and two showed large average increases that apparently were not significant. For example, in the study by Morgan et al [9] V_E averaged 160 L/min during maximal exercise before training and 175 L/min after training, while McMahon et al [5] reported that sub maximal exercise V_E rose from 147 L/min before training to 156 L/min after training. Of the five studies that used a protocol designed to enhance respiratory muscle strength and power, four showed no change in exercise V_E after training [11,12,15,18], and one showed an increase that just failed to reach significance (P= 0.051, [17]. Our data demonstrate a consistently higher ventilatory response to both constant work rate exercise and a simulated time trial test after RMET. It is important to emphasize that the increase in V_E was observed in all ten subjects in the RMET group. Thus, our data are consistent with the results of previous studies, wherein the results of all but two of fifteen show either no change in V_E, a significant increase in V_E with RMET, or a clear trend towards an increase in V_E.

Correlation between increased V_E and improved exercise performance after RMET

Although we cannot explain the rise in V_E during constant work-rate exercise after RMET, we found a significant, inverse correlation between the change in V_E and the change in performance time in all 20 subjects, with the subjects that ventilated more after the training period having better performance. This is at odds with the data of Boutellier et al [2], who showed the opposite effect (see their Fig. 4). Given this qualitative difference, we interpolated all of the numbers from their figure 4 and re-ran the data analysis. Several interesting observations result: first, they studied 8 subjects, but ran the correlation between V_E and cycling performance time on only 7 because "minute ventilation in subject AH showed such an unrealistically large scatter during the endurance cycling test after respiratory training that the values had to be disregarded"; second, when we analyzed the data we found a similar correlation coefficient (0.75), with a P value of 0.051, but noticed that the data were dominated by a clear outlier that had a 156 % increase in cycling performance and a 35 % decline in V_E after training. When we removed this outlier from the analysis, the correlation coefficient fell to 0.45, and the P value rose to 0.364. Thus, the relation that they reported was dominated by the data from this one subject. In a more recent report from the same laboratory [6], 6 of 8 subjects that had higher exercise V_E after RMET had reduced cycling endurance performance, although statistics were not reported in that analysis. We cannot explain the reason behind the discrepant findings, but wish to emphasize that our data are consistent and statistically significant, and that more detailed studies of this issue are needed.

Would a higher V_E during exercise be expected to improve performance?

The increase in V_E observed during exercise after RMET was associated with a higher whole body VO_2, suggesting that the respiratory muscles consumed at least some of the extra oxygen to cover the requirements of the increased ventilatory response. This would appear to "steal" more blood from the exercising locomotor muscles (see [19] for discussion) and therefore predispose the subject to an earlier onset of fatigue [20]. However, our trained subjects performed better, with the performance enhancement associated with the increase in post-training V_E. This study was designed to examine the change in performance after RMET (see above), and because of this we avoided invasive measures that could have provided insight into mechanisms. For example, it is possible that the higher ventilatory response to exercise after RMET improved oxygen transport and/or acid base balance during exercise [25]. However, Stuessi et al [4] measured arterial blood gases during constant work-rate exercise before and after respiratory muscle training and found no differences in acid base or oxygen transport variables. However, they did not observe changes in ventilation with training, so blood gases and pH would not be expected to change significantly.

A possible explanation for our findings is that RMET allowed the subjects to incur a higher ventilatory load without any increase in dyspneic sensations. Although a higher ventilatory response during exercise might be expected to lead to greater feelings of dyspnea, we found no significant changes in sensations of dyspnea or leg effort after RMET. Thus, in spite of the higher V_E after RMET, the subject's perception of dyspnea (and leg fatigue) was unchanged. In other words, the subjects could tolerate a greater ventilatory load without an increase in dyspneic sensations after RMET, which may lead to enhanced exercise performance.

In conclusion, twenty sessions of rigorous endurance training of the respiratory muscles lead to significantly improved exercise performance (on average, 4.7 %) in fit, experienced cyclists and/or triathletes. The improved cycling performance after the training period was significantly correlated with an increase in the ventilatory response to exercise, without an increase in sensations of dyspnea; thus, the subjects could breathe more without increased feelings of breathlessness. The mechanism behind either the enhanced performance or the enhanced ventilatory response after respiratory muscle endurance training remains to be established.

Conclusions

The results suggest that respiratory muscle endurance training improves cycling performance in fit, experienced cyclists. The relative hyperventilation with no change in respiratory effort sensations suggest that respiratory muscle training allows subjects to tolerate the higher exercise ventilatory response without more dyspnea. Whether or not this can explain the enhanced performance is unknown.

Methods

Subjects

Twenty fit, experienced cyclists and/or triathletes agreed to participate in the study. Requirements for enrollment included that they: (a) be a cyclist or triathlete, (b) be involved in at least 3 hours of endurance training per week, (c) keep a constant level of training over an 8 week period and (d) be free of any past or present respiratory illnesses. Using a standard randomization procedure, we initially assigned 10 subjects (3 female, 7 male) to the RMET group and 6 to a control group (1 female, 5 male). We subsequently added 4 additional subjects (4 males) that did "placebo training" (see below). The Human Subjects Committee at the University of Arizona approved the study protocol, and all subjects provided written informed consent prior to participation.

Overview of Protocol

The study protocol was divided into three phases:

Pre-training

After an introduction to the laboratory and equipment, subjects completed standard lung function testing followed by a two week period consisting of: an incremental cycling test to exhaustion to determine the maximal oxygen uptake rate (VO_2 max); a constant work-rate cycling endurance test; a time trial cycling performance test; two-three sustainable ventilatory capacity (SVC) tests; and two maximal mouth pressure tests, to estimate respiratory muscle strength. The subjects had at least three opportunities to practice the lung function and sustainable ventilatory capacity and mouth pressure tests, and were familiarized with the cycle ergometer and were allowed to take practice rides and become familiar with the seat and the procedure for adjusting it to their level of comfort; these practice sessions occurred on a laboratory familiarization day that occurred 5 – 7 days prior to the onset of actual testing. At least one day separated all cycling sessions, and subjects were asked to record their dietary intake and to consume similar foods before the post-training cycling tests.

Training

Approximately 2–5 days after the pre-training phase, 14 subjects were placed into our training group (10 experimental, 4 placebo) and completed 20 sessions of RMET over a 4-week period, while 6 subjects acted as pure controls (see above) and did no RMET. After the second week of RMET, all 20 subjects completed a mouth pressure and a SVC test to assess training progress.

Post-training

In the post-training period, all subjects initiated testing within 2 days after the completion of training. In all but three subjects, testing was initiated within 1 day. The other three subjects finished their training fairly late in the evening because of work or school-related scheduling conflicts, so we gave them an additional day after the cessation of training. Once the post-training testing began, the following sequence was completed: on the first day, the subject completed either the time trial test or the SVC test, with the order randomized within and between groups; on day two they completed either the SVC or the time trial, depending upon which test they did on day one; on day three they completed simple spirometry tests (with the exception of MVV, which was not done after training) and either the constant work-rate exercise test or the VO_2 max test, with randomization within and between groups; on day four they completed either the constant work-rate exercise test or the VO_2 max test, depending upon which test they did on day three; and on day five they did the maximal inspiratory and expiratory mouth pressure tests. In this way, exercise tests and SVC or mouth pressure tests were not done on the same day. And more importantly, the SVC and time trial tests, our most important outcome measures, were done within the first 2–2.5 days after the end of training.

Lung Function tests

Forced Vital Capacity (FVC) and Maximal Voluntary Ventilation in 12 seconds (MVV_{12}) were measured on a spirometer (model 827, Ohio Medical, Toledo, OH, USA). Forced Expiratory Volume in 1 second (FEV_1) was obtained from the FVC tracing to calculate the FEV_1/FVC ratio. For the MVV_{12}, subjects were instructed to "move as much air as possible" and were given feedback from the investigator until a maximal level of ventilation was obtained. For both tests, the best two out of three trials were averaged, and expressed under BTPS conditions as described previously [26].

Sustainable Ventilatory Capacity (SVC)

To assess the endurance of the respiratory muscles, we used a SVC test similar to that used by Leith and Bradly [23]. A breathing mask and head cap (Hans Rudolph, Kansas City, MO, USA) were secured to the subject, and to prevent air leakage, the mask was sealed to the bridge of the nose with dental impression material (Exaflex, GC America, Alsip, Ill, USA). A large 2-way valve (Hans Rudolph 3700) was attached to the front of the mask. The subjects inspired air from a Douglass bag that was partially filled with warm water to prevent throat dryness during the test. A mixture of 10% CO_2, 21% O_2 (nitrogen balance) from a separate gas tank was fed into the Douglass bag via flexible tubing for maintenance of end-tidal CO_2 ($ETCO_2$) throughout the sustained period of hyperpnea. Respiratory tubing connected the bag to the inspired side of the breathing valve and was interrupted by a pneumotachometer (model 3813, Hans Rudolph) connected to a differential pressure transducer (Validyne MP45-871, Irvine, CA, USA) to measure inspired flow. The flow signal was split and sent to a polygraph integrator (model 7P10F, Grass, Quincy, MA) and to an analog-to-digital converter (model 1401 plus, Cambridge Electronic Design, London, UK). The integrated flow signal was sent to a chart recorder (model TA11, Gould, Courtaboeuf, France), and the raw flow signal was recorded digitally (Spike II software, Cambridge Electronic Design) for later analysis. To ensure the linearity and accuracy of the system, a 3-liter calibration syringe (Hans Rudolph) was used to calibrate the integrated flow signal before each test, while the raw flow signal was calibrated over a range extending from 0–220 L/min with a Matheson Rotameter. The expired side of the breathing valve was open to the room. A small sample of expired air was collected from each expired breath and sent to a rapidly responding CO_2 analyzer (model CD-3A, Ametek, Pittsburgh, PA, USA). The CO_2 signal was sent to the chart recorder such that the investigator could continuously monitor end-tidal CO_2. A one-two minute period of room air breathing was necessary prior to each test to find the resting end-tidal CO_2 level. Once established, this level of end-tidal CO_2 was maintained during all breathing maneuvers by addition of variable amounts of the air/CO_2 mixture to the Douglass bag.

A three-minute warm up period (at approximately 80% MVV) preceded all tests. Before the test began, the subjects were reminded to breathe maximally ("like the MVV") for "at least 10 minutes". Although measurements of V_E were made each minute over the first six minutes of the test (see Fig. 3), the extra few minutes of hyperpnea allowed us to insure that a clear ventilatory asymptote was reached in all subjects. The first test was considered a practice test and the subject was given immediate feedback on the results. At least two more tests followed on separate days (see below). In order for a test to be included in the final analysis, V_E during the first 30 breaths of the test had to be greater than or equal to the subjects MVV to ensure that the effort was maximal. In addition, V_E had to decrease monotonically over the course of the test. Further analysis of each test consisted of finding the average ventilation over 30 breaths occurring at the beginning of each minute of the first six-minutes of the test (Fig. 3). The average V_E during the plateau phase (over the last 2–3 minutes of the test) was taken as the SVC. The SVC from the two tests had to be within 10% of each other before the training phase commenced, and this was considered a baseline measurement to which all subsequent SVC tests were compared.

Maximal oxygen uptake (VO_2 max)

Inspiratory and expiratory airflow rates were measured breath-by-breath with a turbine flowmeter attached to a facemask that allowed the subjects to breathe orally or nasally (Vacumed, Torrance, CA, USA). This configuration is designed for very low dynamic flow resistance, which we calculated as 0.25 cmH_2O/L/sec at a flow of 6 L/sec. A small bore tube connected to a vacuum pump continuously sampled gases in the mask at a flow rate of 200 ml/min, and the fractional concentrations of CO_2 ($F_{ET}CO_2$) and O_2 ($F_{ET}O_2$) were measured with Vacumed analyzers (Vista mini-CPX Silver Edition, VacuMed). End-tidal CO_2 was monitored with a rapidly responding CO_2 analyzer (model CD-3A, Ametek) that sampled gas via a second port in the facemask. Gas analyzers were calibrated with precision gas and the flow meter was calibrated with a precision 3.0 L syringe (W.E. Collins, Inc., Braintree, MA). Heart rate (HR) was monitored and recorded on-line with a HR interface unit (VacuMed).

Subjects performed a progressive intensity exercise test to exhaustion on an electronically braked cycle ergometer (Excalibur, Lode, Stockholm, Sweden). The first 15 minutes of the test consisted of sitting motionless for 5 minutes (to collect resting data), followed by a 10-minute warm-up period. Upon completion of the warm-up, the workload was set at 50 W and increased to 100 W after 1 minute, and then by 30 W (25 W for women) every minute until a pedal rate of at least 60 revolutions per minute could no longer be maintained. A test was considered maximal if two of the following three criteria were reached: the respiratory exchange ratio (RER) reached 1.10, HR reached the age-predicted maximum, or VO_2 plateaued or decreased with an increase in workload.

Constant work-rate cycling endurance

To compare ventilatory and metabolic parameters at the same work-rate before and after RMET, subjects performed a constant work-rate exercise test to the point of volitional exhaustion. The work-rate for this test was set at approximately 80 % VO_2 max. Subjects warmed up for 10 minutes at the wattage of their choice, and then the wattage was ramped up to the target work-rate within 2 minutes. Endurance time was the elapsed time between reaching the target work-rate and when the subject could no longer maintain a pedal rate of 60 revolutions per minute or higher, as described previously [26]. Parameters measured and displayed every six breaths included V_E (BTPS), tidal volume (V_T) (BTPS), respiratory rate (f), HR, VO_2, carbon dioxide production (VCO_2), RER, and $ETCO_2$.

Time trial cycling performance

To test cycling performance while minimizing the variability observed in open-ended cycling endurance tests

([13,14], and see Fig. 1), subjects performed a cycling task meant to simulate a time trial. When set in linear mode, the Lode ergometer becomes pedal rate dependent, such that as pedal speed increases, work rate also increases according to the following formula:

$$W = L \cdot (RPM)^2$$

Where L is a constant linear factor and RPM is the pedaling rate in revolutions per minute. The linear factor L was chosen so that each subjects preferred pedaling rate (observed during constant workload exercise) would cause them to work at the same wattage that elicited approximately 80 % VO_2 max. Then, the target amount of work each subject had to complete was calculated based on the following formula:

$$\text{Target amount of work (J)} = 0.80 \cdot \text{Wmax} \cdot 2700 \text{ (seconds)}$$

Where Wmax was the maximal workload achieved during the incremental cycling test and 2700 seconds (45 minutes) was chosen to simulate a 40-kilometer time trial. The subject was instructed to complete the required work as quickly as possible. The subject viewed the required work on a computer monitor, and as work was done the number decreased accordingly, with the test ending when the required work was completed (i.e., a reading of zero on the computer monitor). The subject was unaware of the elapsed time, the work-rate, and the HR. Subjects were instructed to warm up for 10 minutes at the wattage of their choice before the test began. Performance time was the time elapsed between the onset of the time trial and attainment of the target (i.e., work-rate = 0).

Maximal mouth pressure

To determine the strength of the respiratory musculature, maximal inspiratory and expiratory mouth pressures were measured, as described in detail previously [26]. Maximal mouth pressure was measured from a port in a mouthpiece that was connected via a small length of tubing to a pressure transducer and bridge amplifier (Coulbourn Instruments, Allentown, PA, USA). The mouthpiece contained a valve that could be manually opened and closed by the subject. There was a small leak in the valve to equalize pressure and to prevent buccal pouching. With nose clips attached and the valve open, the subject was instructed to either inhale (to total lung capacity) or exhale (to residual volume) maximally, and then to quickly close the valve and exhale or inhale in concert with a cadence produced by an investigator. The cadence was one that we have used previously when measuring upper airway or expiratory muscle force [26,27], and is described as follows: "up, 1, 2, 3, hold, hold, relax". In this way the subjects had to reach their maximum pressure gradually, over 3–4 sec, and then hold it for another two seconds. Our previous work has demonstrated that this method is highly reproducible and well understood by the subjects. We then measured the average pressure over a window encompassing 1.5–3 sec into the maneuver as our peak mouth pressure. The pressure signal was sent to the chart recorder and displayed to the subject via the monitor of a digital oscilloscope. Three inspiratory and 3 expiratory maneuvers were done, and the best two out of three efforts were averaged.

Rating of perceived exertion

Approximately every 2–3 min throughout the time trial and constant work-rate exercise tests, the subjects were asked to estimate their respiratory and leg effort using a visual analogue scale as described previously [26]. The scale ranged from 0–20, and the subjects simply pointed to the appropriate number when asked.

Respiratory muscle endurance training

Fourteen subjects completed twenty sessions of RMET over a 4-week period, a protocol that has been used successfully by others [2,9,10]. For the experimental subjects, each training session lasted 30 minutes. All subjects wore a mask (see above) with a two-way non-re-breathing valve from which the diaphragms were removed. A rubber cork was placed on the expired side of the valve such that the subject both inspired and expired from the inspiratory side of the valve. Respiratory tubing connected the inspired side of the valve to a pneumotachometer and pressure transducer (Validyne MP45) to measure airflow. The integrated flow signal was displayed on an oscilloscope monitor placed directly in front of the subject. As in the SVC test, a small sample of each expired breath was analyzed and sent to the chart recorder to monitor end-tidal CO_2. Tubing of various lengths could be attached to the distal side of the pneumotachometer to add sufficient dead space to maintain end-tidal CO_2 at the resting level.

Each day, a ventilatory target was set and the subject had to maintain the target for the duration of the training session. The target V_T was set on the oscilloscope screen. Breathing frequency was set with a metronome such that the subject inspired and expired to the sound of a tone. For the experimental group, we used an initial target that matched the subject's maximal V_T and f achieved during the incremental cycling test (94.8–134.5 L/min). Tidal volume or f was increased every one-two days for the first two weeks to continuously challenge the respiratory musculature. This was semi-quantified by keeping subjects at a level of ventilation that induced a value of 18–19 on the 20 point respiratory effort scale (see above).

For the second two weeks, V_T reached an upper limit while f continued to increase. Subjects were instructed to consider the target V_T and f as a goal rather than a limit. Many times, the subjects voluntarily increased V_T and f in order to increase the intensity of RMET. To further enhance the training, subjects were asked to perform a 30–60 second MVV three-four times during most training sessions. The placebo group followed the same protocol as the experimental group, except that each training session lasted only 5 minutes, the target ventilation rate was set at 65% of maximal V_T and breathing f, and was never increased. Subjects in the control group did no respiratory muscle training at any time. To avoid biasing the training effort, we did not verbally encourage either the training or placebo group during the RMET sessions.

Statistical analysis

Control and placebo groups

Two-tailed t-tests revealed that differences in sustainable ventilatory capacity, exercise performance and exercise V_E between the control group and the placebo training group on both the time trial and constant work-rate exercise tests (see below) were not significantly different. In spite of the small sample size, all t-tests passed the criteria for equal variance and normal distribution. Accordingly, the control and placebo subjects were combined into one group for statistical comparisons with the RMET group. For clarity and completeness, the subjects in the control and placebo groups have been differentiated graphically for all variables that relate to ventilatory muscle or exercise performance.

Reproducibility of exercise tests

We estimated the coefficient of variation of the time trial and constant work-rate exercise tests using the pre and post-training data from the 6 control and 4 placebo training subjects. We used the "method error" measurement as described previously [27]:

$$CV = SD / \sqrt{2} / (\text{mean Test 1} + \text{mean Test 2}) / 2 \times 100$$

Where CV = the coefficient of variation, SD = the standard deviation of the difference between the pre-training (Test 1) and post-training (Test 2) scores.

Mouth pressure, sustainable ventilatory capacity and exercise performance

We used two-way repeated measures ANOVA, with group (control/placebo or RMET) and time of measurement (pre, mid, post) as factors. When the F value was significant at the P = 0.05 level or less, post-hoc analysis of all pair wise contrasts was completed with the Student Neumann Keuls procedure. The time trial data were not normally distributed, so those data were analyzed with the non-parametric Kruskall-Wallis one-way ANOVA on ranks, again using P = 0.05 or less as the cutoff for statistical significance.

Cardiorespiratory variables and rating of perceived exertion measured during the constant work rate exercise test

We analyzed the pre-and posttest and group differences for V_E, VO_2 and end-tidal CO_2 during the constant work-rate exercise test with a two-way repeated measures ANOVA, with group (control/placebo or RMET) and percent endurance time as the factors. For each subject, the endurance time was computed and then divided into 10, 10 % epochs. V_E, VO_2, end-tidal CO_2 and values for leg and breathing effort at the end of each epoch were computed for each subject, and used in the analysis. The post-hoc testing procedure was as described above.

Measurement of V_E and VO_2 during the time trial test, before and after the training period

Six-breath averages of all gas exchange variables were computed throughout the time trial test, and the average of 10 breaths at the end of each minute of exercise was calculated for each subject, and then averaged across all subjects (see Fig. 8). To compare the overall response in the RMET and control/placebo groups, all individual subject data from the fifth minute through the end of the test were averaged, resulting in a single, steady state value for V_E and VO_2, before and after RMET or the control/placebo period. The data were then analyzed with a paired t-test, using P = 0.05 or less as the criterion for a significant difference.

Correlation between the change in time trial performance and the change in V_E

Because the RMET group had significantly better time trial performance and significantly higher post-training V_E compared with the control/placebo group, we examined the relation between these two variables. Because the data were not normally distributed, we used the non-parametric Spearman Rank Order correlation for analysis. All group average data are presented together with the SEM throughout the text and in all figures.

Authors' contributions

PH helped to design the studies, recruited subjects, conducted the training and exercise experiments, and assisted with data analysis. AS helped to train the subjects, assisted with the exercise studies, managed all of the data, and played the lead role in initial data analysis. RFF conceived of the study, participated in its design and coordination, and conducted all statistical and graphical analyses. All authors read and approved the final manuscript.

Acknowledgments

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