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Pulmonary and respiratory muscle function in response to 10 marathons in 10 days

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Running Head: Respiratory responses to ultramarathon stage-racing

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ABSTRACT

Purpose: Marathon and ultramarathon provoke respiratory muscle fatigue and pulmonary dysfunction; nevertheless, it is unknown how the respiratory system responds to multiple, consecutive days of endurance exercise. **Methods:** Nine trained individuals (6 male) contested 10 marathons in 10 consecutive days. Maximum static inspiratory and expiratory mouth-pressures (MIP and MEP), pulmonary function (spirometry), perceptual ratings of respiratory muscle soreness (Visual Analogue Scale), breathlessness (dyspnea, modified Borg CR10 scale), and symptoms of Upper-Respiratory Tract Infection (URTI), were assessed before and after marathons on day 1, 4, 7 and 10. **Results:** Group mean time for 10 marathons was 276±35 min. Relative to pre-challenge baseline (159±32 cmH_2O), MEP was reduced after day 1 (136±31 cmH_2O, p=0.017), day 7 (138±42 cmH_2O, p=0.035), and day 10 (130±41 cmH_2O, p=0.008). There was no change in pre-marathon MEP across days 1, 4, 7, or 10 (p>0.05). Pre-marathon forced vital capacity was significantly diminished at day 4 (4.74±1.09 vs. 4.56±1.09 L, p=0.035), remaining below baseline at day 7 (p=0.045) and day 10 (p=0.015). There were no changes in FEV_1, FEV_1/FVC, PEF, MIP, or respiratory perceptions during the course of the challenge (p>0.05). In the 15-d post-challenge period, 5/9 (56%) runners reported symptoms of URTI, relative to 1/9 (11%) pre-challenge. **Conclusions:** Single-stage marathon provokes acute expiratory muscle fatigue which may have implications for health and/or performance, but ten consecutive days of marathon running does not elicit cumulative (chronic) changes in respiratory function or perceptions of dyspnea. These data allude to the robustness of the healthy respiratory system.

Key words: Ultramarathon, endurance, lung function, fatigue.

ABBREVIATIONS

FVC	forced vital capacity
FEV_1	forced expiratory volume in 1 second
PIF	peak inspiratory flow
PEF	peak expiratory flow
MVV	maximum voluntary ventilation
MIP	maximum inspiratory mouth-pressure
MEP	maximum expiratory mouth-pressure
URTI	upper-respiratory tract infection
VAS	visual analogue scale
SD	standard deviation
CV	coefficient of variation
SEM	standard error of measurement
CI	confidence interval
ICC	intraclass correlation
ANOVA	analysis of variance

INTRODUCTION

Respiratory muscle fatigue is a phenomenon whereby the inspiratory and/or expiratory musculature exhibit a transient reduction in force-generating capacity, relative to baseline values (Romer and Polkey. 2008). Respiratory muscle fatigue has been assessed objectively following high-intensity, exhaustive cycling and running, manifesting in a 15 - 30% pre-to-post-exercise reduction in transdiaphragmatic or gastric twitch-pressure in response to nerve stimulation (Johnson et al. 1993; Taylor et al. 2006). When respiratory muscle fatigue has been assessed indirectly using maximum volitional mouth-pressure manoeuvres, similar pre-to-post-exercise reductions were observed following rowing and swimming time-trials (Lomax and McConnell. 2003; Volianitis et al. 2001). Using a proportional assist ventilator to offload the respiratory muscles during exercise, Babcock et al. (2002) found that the workload endured by the diaphragm was a critical determinant of exercise-induced diaphragmatic fatigue. Moreover, using objective nerve stimulation techniques, we recently observed expiratory, but not inspiratory, muscle fatigue following maximal upper-body exercise (Tiller et al. 2017). Given that the exercise trial induced only a modest ventilatory demand, the data support the notion that high minute ventilations are a prerequisite for diaphragm fatigue, where-as the expiratory muscles may be less fatigue-resistant. Respiratory muscle fatigue is thought to be underpinned by peripheral, rather than central, mechanisms (Jones. 1996; Wuthrich et al. 2015), and contractile function typically returns to baseline within 1 - 2 h of exercise.

There is a growing body of work pertaining to respiratory muscle function following endurance and ultra-endurance running. Reductions in maximum inspiratory mouth-pressure in the region of ~15% have been observed immediately following single-stage marathon (Chevrolet et al. 1993; Ross et al. 2008), although no evidence of expiratory muscle fatigue was reported. Evidence of post-marathon decreases in respiratory muscle endurance (~27%) have been noted, when assessed via time-to-exhaustion (Tlim) during sustained inspiratory pressure (Ker and Schultz. 1996), with similar observations made following 24 h of treadmill running when respiratory muscle endurance was assessed via maximum voluntary ventilation in 12 s (MVV_{12}) (Warren et al. 1989). The only study to use magnetic nerve-stimulation to assess respiratory muscle fatigue following ultramarathon (defined

as a race that exceeds the traditional marathon distance of 42.2 km; Millet and Millet. 2012) observed a reduction in mouth twitch-pressure of ~19% immediately following a 110 km mountain race (Wuthrich et al. 2015); such a response is indicative of low-frequency inspiratory muscle fatigue.

Notwithstanding the implications of respiratory muscle fatigue, marathon and ultramarathon are also thought to negatively impact on pulmonary function. The first study to investigate this phenomenon measured lung capacity in the first 22 finishers of the 1923 Boston Marathon, noting that post-race values were significantly reduced by 0.8 L (17%) (Gordon et al. 1924). More recently, (Ross et al. 2008) reported an acute decrease in peak inspiratory flow (PIF; 6.3 to 4.9 $L \cdot s^{-1}$) and forced vital capacity (FVC; 5.73 to 5.46 L) immediately following a marathon, but parameters had recovered within 24 h. Races of extreme duration (330 km mountain ultramarathon) also elicited reductions in peak inspiratory and expiratory flow, as well as forced expiratory volume in 1 second (FEV_1) (Vernillo et al. 2015). Given the positive correlation between pulmonary function and marathon performance (Salinero et al. 2016), and the negative correlation between the pre-to-post exercise reduction in MVV_{12} and ultramarathon finish time (Vernillo et al. 2015), it is reasonable to suppose that a pulmonary dysfunction might negatively impact on exercise performance.

Despite the available literature on the respiratory responses to single-stage endurance running, an important, as of yet undetermined, component of pulmonary and respiratory muscle function is the impact of chronic endurance exercise that is performed on multiple, consecutive days. Multi-stage endurance running presents an excellent model with which to study the limits of human physiological function. Data on the respiratory responses to stage-racing would offer a novel insight into the robustness or fallibility of the human respiratory system in responding to repeated exercise stimuli. Furthermore, such data might influence endurance running training strategies, in addition to the best practice of medics overseeing these events.

Accordingly, this study assessed respiratory muscle and pulmonary function in a group of endurance runners who contested a pre-determined ultra-endurance exercise challenge comprising 10 marathons in 10 consecutive days. It was hypothesised that: i) there would be an acute (within-day) reduction in respiratory muscle and pulmonary function following any given marathon; and ii) there would be a chronic (between-day) reduction in baseline parameters as the challenge progressed.

MATERIALS AND METHODS

Participants

Eleven recreationally-active endurance runners (8 male, 3 female), volunteered to participate in data-collection protocols. Two participants withdrew from the study due to injury at day six and eight, respectively; therefore, statistical data are presented for n = 9 (6 male, 3 female) (mean ± S.D. age = 48.6 ± 9.4 y; mass = 74.7 ± 14.2 kg; stature = 174.1 ± 10.8 cm). Participants had been training for 10 ± 4 y (range = 5 - 14 y), ran 47 ± 16 miles (7.7 ± 2.8 h) per week, and exhibited a group mean season's best marathon time of 217 ± 22 min (3 h 37 min ± 22 min). Participants were free from known cardiorespiratory diseases, with the exception of one participant who had previously been treated for asthma (FEV_1/FVC, 0.65 [77% predicted]). There were three ex-smokers in the group, all with > 4 y smoking cessation (mean = 9.0 ± 8.7 y). Procedures were approved by the institution Research Ethics Committee, and performed in accordance with the 1964 Declaration of Helsinki. Prior to data collection, participants were issued with a *Participant Information Document*, completed a pre-test medical questionnaire, and provided written, informed consent.

Experimental Overview

Participants contested 10 marathons in 10 consecutive days on courses of varying terrain (The Great Barrow Challenge '10-in-10'; Suffolk Academy, Suffolk, UK). The marathons began from the same location at 08:00 each day, affording participants consistent recovery time between races. Mean temperature and humidity throughout the challenge was 22.2 ± 1.5 ◦C and 69 ± 4%, respectively. Assessments of respiratory muscle strength, pulmonary function, and perceptual responses were made before and within 10 min of finishing marathons on day 1, 4, 7 and 10. Prior to testing, participants were familiarised with the respiratory manoeuvres, aided by demonstrations and tutorial videos.

Respiratory Measures

Maximum Inspiratory and Expiratory Mouth-Pressure: Maximum static inspiratory mouth-pressure (MIP, from residual volume) and maximum static expiratory mouth-pressure (MEP, from total lung

capacity) were assessed as a simple, convenient, and non-invasive index of respiratory muscle strength (Evans and Whitelaw. 2009). The merits and limitations of volitional manoeuvres for assessing respiratory muscle function are discussed later (see *Technical Considerations*). Manoeuvres were performed using a handheld device (MicroRPM; CareFusion, Hampshire, UK), attached to a phlanged mouthpiece with a 1-mm leak to prevent glottic closure during the MIP manoeuvre and to reduce the use of buccal muscles during the MEP manoeuvre (American Thoracic Society/European Respiratory Society. 2002). Participants were seated, and given verbal encouragement to maintain a maximal effort for ~2 - 3 s, with the largest of three values within 5% variability recorded (Wen et al. 1997).

Spirometry: Pulmonary volumes, capacities, and flows were assessed via spirometry, whereby participants performed between three and eight FVC manoeuvres into a two-way disposable mouthpiece connected to a portable pneumotachograph (Alpha Touch; Vitalograph Ltd., Buckingham, England), with the nose occluded. Participants were seated, and verbal encouragement was given to ensure consistent efforts. Spirometry was performed in accordance with ATS/ERS guidelines (Miller et al. 2005).

Within- and Between-Day Reliability of Respiratory Measures

Six healthy participants, independent from the main study, were recruited in order to quantify the reliability of maximum static mouth-maximum manoeuvres and spirometry. Within-day reliability was determined by comparing baseline measurements to those made after ~4 h passive rest, and between-day reliability was determined by re-assessing participants three days later. Tests were performed following similar coaching and instructions to that used with the main-study participants. Moreover, reliability data were collected under the same time-constraints, following a similar schedule, and with identical apparatus to that applied in the field. Data on the reliability of maximum static mouth-pressure manoeuvres and spirometry are shown in Table 1. There were no systematic differences in measurements ($p > 0.05$), and the between-occasion reliability was excellent (all CV < 5%; low SEM; all ICC > 0.94).

Perceptual Measures

Symptoms of Upper-Respiratory Tract Infection (URTI): Following each bout of respiratory assessment, participants were presented with four questions pertaining to symptoms commonly associated with URTI, and asked to rate the severity of their symptoms by marking a line on a series of 100 mm visual analogue scales. The questions posed were: 1) *Since waking this morning, have you experienced any coughing?* (Anchored by "completely free of cough" and "worst cough I can imagine"); 2) *Since waking this morning, have you experienced any wheezing?* (Anchored by "completely free of wheeze" and "worst wheeze I can imagine"); 3) *Since waking this morning, have you experienced any chest-tightness?* (Anchored by "completely free of chest-tightness" and "worst chest-tightness I can imagine"); *4) Since waking this morning, have you experienced any mucus secretions?* (Anchored by "completely free of mucus" and "worst mucus I can imagine"). Following the final marathon, symptoms were monitored for a 15-d period using a daily online symptom log. An individual was considered symptomatic of an URTI if ≥ 2 symptoms were present for at least 2-d in a 3-d period (Robson-Ansley et al. 2012). As a control, participants were asked to report on the prevalence of symptoms in another member of their household (adult, non-runner) using an identical questionnaire. Prior to testing, participants completed the Allergy Questionnaire for athletes (AQUA), with a score of ≥ 5 positively predicting allergy with a correlation coefficient of 0.94 (Bonini et al. 2009).

Respiratory Muscle Soreness: In an effort to quantify the degree of respiratory muscle damage, participants were asked to rate their perceived intensity of respiratory muscle soreness by marking a line on a 100 mm Visual Analogue Scale (VAS) - anchored by "no pain" and "unbearable pain", respectively - and to indicate the location of any muscle soreness by shading areas on a body diagram (Mathur et al. 2010). These measures of respiratory muscle soreness were made immediately following each set of MIP (MIP_{VAS}) and MEP (MEP_{VAS}) manoeuvres.

Dyspnea: Following baseline respiratory assessment, participants were asked to rate *the intensity of their breathing discomfort* since waking, by circling a number on the modified Borg CR10 Scale (Mahler and Horowitz. 1994). Following post-race assessment, participants were asked the same question in relation to the sensations experienced throughout the preceding marathon.

Data Analysis

Descriptive and inferential statistics were calculated using SPSS 24 for Windows (IBM; Chicago, IL). Reliability of respiratory measures was assessed using coefficient of variation (CV), standard error of measurement (SEM), and intra-class correlation coefficients (ICC; mean of trials one & two vs. trial three). Two main comparisons were made on mouth-pressure, pulmonary function, and perceptual data: i) pre-challenge baseline to post-marathon values on day 1, 4, 7 and 10 (acute response); ii) pre-challenge baseline to pre-marathon baseline values on day 4, 7 and 10 (chronic response). Respiratory and perceptual responses were assessed for differences using repeated-measures ANOVA (eight time-points; pre-to-post day 1, 4, 7, and 10) and Fisher's LSD post-hoc comparisons. The assumption of equal variance was assessed via Mauchly's test of Sphericity and, if violated ($p < 0.05$), a Greenhouse-Geisser correction applied. Effect size (Cohen's d) was calculated to estimate the magnitude of the difference between group means, with $d = 0.2$, 0.5, and 0.8 reflecting small, medium, and large effect size, respectively (Cohen. 1977). Alpha level was set at $p < 0.05$, and data were presented as mean ± S.D., unless stated.

RESULTS

Participants

Individual and group mean marathon times throughout the challenge are illustrated in Figure 1. Group mean time across all 10 marathons was 276 ± 35 min (4 h 36 min ± 35 min), with a mean range of 221 (3 h 41 min) to 319 min (5 h 19 min). Fifty six percent (5/9) runners exhibited a positive AQUA score (≥ 5) for allergic diseases. The single asthmatic participant exhibited responses consistent with the group-mean.

Respiratory Responses

Maximum Inspiratory and Expiratory Mouth Pressure: Group mean MIP and MEP responses are illustrated in Figure 2. Relative to pre-challenge baseline, MEP was reduced after day 1 (-14 ± 14%, p = 0.017, d = 0.73), day 7 (-14 ± 18%, p = 0.035, d = 0.56) and day 10 (-19 ± 18%, p = 0.008, d = 0.79), with a non-significant reduction after day 4 (-9 ± 18%, p = 0.111, d = 0.52). There was no change in pre-marathon (baseline) MEP across days 1, 4, 7, or 10 ($p > 0.05$). Relative to pre-challenge baseline, there were slight reductions in post-marathon MIP, but with no significant changes in the group mean at any time point.

Spirometry: Group mean FVC, FEV_1, and PEF, are illustrated in Figure 3. Relative to pre-challenge baseline, there were no differences in post-marathon FVC on day 1, 4, 7 or 10 ($p > 0.05$), but there was a significant reduction in pre-marathon (baseline) FVC at day 4 (p = 0.035, d = 0.17), which remained below baseline at day 7 (p = 0.045, d = 0.17) and day 10 (p = 0.015, d = 0.19). When assessing FEV_1, relative to pre-challenge baseline, there were no differences in post-marathon values on day 1, 4, 7, or 10, and no significant reduction in pre-marathon (baseline) FEV_1 across days 1, 4, 7, or 10 ($p > 0.05$). There were significant pre-to-post marathon increases in FEV_1 on day 1 (p = 0.012, d = 0.51), day 7 (p = 0.039, d = 0.90), and day 10 (p = 0.038, d = 0.40). Relative to pre-challenge baseline, there were no significant changes in group mean PEF at any time point. When assessing the FEV_1/FVC ratio, relative to pre-challenge baseline (0.70 ± 0.07), values had increased after day 1

(0.74 ± 0.06, p = 0.047, d = 0.61) and day 7 (0.74 ± 0.05, p = 0.015, d = 0.66), but there were no differences in pre-marathon (baseline) FEV_1/FVC at day 1, 4, 7 or 10 ($p > 0.05$).

Perceptual Responses

Group mean symptoms of URTI, perceptions of respiratory muscle soreness, and perceptions of dyspnea, are summarised in Table 2. The four symptoms of Upper Respiratory Tract Infection (URTI) (i.e., cough, wheeze, chest-tightness, mucus secretions) were assessed independently, with no significant changes in group mean values at any time point ($p > 0.05$). In the 15-d post-challenge period, 56% (5/9) runners reported symptoms of URTI (i.e., cough, watery eyes, blocked or runny nose, sneezing, sore throat), relative to 11% (1/9) pre-challenge, and 11% (1/9) of non-running controls. Respiratory muscle soreness was assessed following MIP and MEP manoeuvres before marathons on day 1, 4, 7 and 10. Relative to pre-challenge baseline, there were no significant changes in group mean values, for either MIP or MEP, at any time point ($p > 0.05$). Dyspnea (subjective ratings of *the intensity of breathing discomfort*) was first compared among the pre-marathon (baseline) scores, and then among the post-marathon scores, with no significant changes in group mean values at any time point ($p > 0.05$).

DISCUSSION

This study assessed respiratory muscle and pulmonary function in a group of endurance runners who contested 10 marathons in 10 consecutive days. The principal findings were: i) there was evidence of acute pre-to-post-marathon expiratory muscle fatigue as demonstrated by reductions in maximum static expiratory mouth-pressure, but no cumulative (chronic) changes in baseline respiratory muscle strength; ii) despite a fall in baseline forced vital capacity at day 4, other indices of pulmonary function were maintained; iii) changes in respiratory function were not associated with changes in perceptual responses during the challenge, although 56% of runners exhibited symptoms of URTI within 15-d of the final marathon. These novel data speak to the robustness of the healthy respiratory system to maintain baseline pulmonary and respiratory muscle function during multiple, consecutive days of endurance exercise.

Technical Considerations

There are certain technical considerations that should predicate a discussion of our findings. First, maximum static pressure manoeuvres are considered a global measure of respiratory muscle strength (Polkey et al. 1995). The techniques are widely used in the assessment of respiratory muscle fatigue (44% of 77 studies; Janssens et al. 2013), and the manoeuvres show strong test/re-test reliability (Dimitriadis et al. 2011). These techniques are non-invasive, easily applied in the field, and can be reported alongside well-established normative data. Nevertheless, a common limitation is that manoeuvres are volitional, dependent on participant motivation, and might be subject to a practice effect. To increase the likelihood that maximal efforts were achieved, we followed standard guidelines by recording a minimum of three manoeuvres within 5% variability (American Thoracic Society/European Respiratory Society. 2002; Wen et al. 1997). Participants were familiarised with respiratory manoeuvres prior to data-collection, and our reliability data show strong between-occasion reliability (Table 1), congruent with previously-reported test/re-test reliability coefficients for these techniques (Dimitriadis et al. 2011). Moreover, the finding that MEP was acutely diminished following a given marathon while maximum indices of pulmonary function (e.g., PEF) were well

maintained, suggests a mechanism that was independent of motivation and/or a practice effect. Although objective measures (i.e., nerve-stimulation) are preferable in the assessment of respiratory muscle fatigue, the invasive nature of such protocols, coupled with the ecological nature of our experimental design, made nerve stimulation inappropriate for this study.

Second, in order to evaluate the carry-over effects of the previous day's marathon, we would have preferred to have collected additional data before each of the 10 marathons. Respiratory and perceptual assessments can be time-consuming, and it was not logistically feasible to take daily measurements from our cohort. Our measures, therefore, strike a balance between obtaining sufficient data to address our research questions, while not overly inconveniencing our participants. Should respiratory muscle strength have not recovered following an overnight rest, we reasoned that function would have steadily fallen on subsequent days, manifesting in lower baseline values. Accordingly, it was deemed appropriate to test baseline function at four time points throughout the challenge. Finally, it is likely that our participants implemented pacing strategies which allowed them to exhibit consistent marathon times throughout the 10-day challenge (Fig. 1). This would preclude any concerns that participants did not sufficiently recover between marathons; accordingly, a general whole-body fatigue and/or insufficient recovery are less likely to have influenced our data.

Respiratory Muscle Fatigue

Throughout the challenge, the magnitude of the post-marathon fall in maximum expiratory muscle strength ranged from 15 – 20%, and is in accordance with earlier reports of diminished respiratory muscle strength following single-stage marathon (Chevrolet et al. 1993; Loke et al. 1982; Ross et al. 2008), and ultramarathon (Wuthrich et al. 2015). Nevertheless, this is the first study to assess these parameters in response to multiple, consecutive days of endurance exercise. Respiratory muscle fatigue is defined as *a condition in which there is a loss in the capacity for developing force and/or velocity of a muscle, resulting from muscle activity under load, and which is reversible with rest* (NHLBI 1990). Moreover, respiratory muscle fatigue is considered to be detectable if the measured reduction in pressure-generating capacity (relative to baseline) is two- to threefold the typical pressure variation (Guenette et al. 2010). The mean decrease in MEP was at least fivefold greater than the CV,

and at any given point of measurement, between 5 and 7 participants exhibited post-race decreases in MEP >10% (i.e., >threefold the CV). Based on these criteria, our strong reliability coefficients (Table 1), and the observation of a moderate-to-large effect size with respect to acute reductions in MEP (0.56 – 0.79), we are confident that our participants exhibited a fatigue that was underpinned by a physiological mechanism. The acute post-marathon fall in expiratory muscle strength is indicative of low-frequency fatigue, which is underpinned by two potential mechanisms: reduced Ca^{2+} release from the sarcoplasmic reticulum and/or damaged sarcomeres caused by overextension of muscle fibres (Jones. 1996). Given the time-course for the recovery of expiratory muscle strength (i.e., there was no systematic decay in pre-marathon values), we suppose that the transient post-marathon fatigue was due to reduced Ca^{2+} availability in the sarcolemma, rather than damaged sarcomeres, although neither were assessed directly. Furthermore, perceptions of respiratory muscle soreness following MIP and MEP manoeuvres did not rise above baseline at any time-point (Table 2) and we can, therefore, discount any cumulative mechanical contribution to fatigue. These observations support the notion that respiratory muscle contractility generally recovers within a few hours of exercise (for review, see (Romer and Polkey. 2008).

The abdominal muscles have an important role in regulating the ventilatory response to exercise (Abraham et al. 2002); however, it is unlikely that the post-race decreases in expiratory muscle strength were exclusively the result of high ventilation rates. The group mean marathon time over the 10-day challenge was ~20% slower than the season's best single-stage marathon, and individual performance times throughout the challenge were relatively consistent (Figure 1). It is likely, therefore, that participants implemented strategies of self-regulation (Barkley. 2001) to prioritise performance on consecutive days over any individual day, and work rate was tempered as a result. This notion of *preservation* is reflected in the modest ratings of post-marathon dyspnea (Borg CR10 scale; 2.0 ± 0.3), which are lower than that reported elsewhere during single-stage marathon (Borg 6 - 20 scale; 12 [Ross et al. 2008]). Expiratory muscle fatigue was more likely attributable to the additional non-ventilatory functions that these muscles assume during exercise (e.g., forced expiration and postural support [Hodges et al. 2005]), which render them more susceptible to fatigue during relatively low ventilation ultra-endurance activities.

By contrast, although we observed small decreases in post-marathon inspiratory muscle strength relative to baseline (Figure 2), the extent of the absolute reduction did not reach statistical significance. The magnitude and prevalence of diaphragmatic fatigue is significantly correlated with the ventilatory demands of exercise (Babcock et al. 2002; Johnson et al. 1993), and it may simply be that the multi-day challenge did not impose a sufficient ventilatory stimulus to significantly fatigue the inspiratory muscles. The diaphragm also has a postural role, but this is only coordinated with its respiratory functions during transient, intermittent disturbances to trunk stability (e.g., brief arm movements) (Hodges and Gandevia. 2000). Indeed, when venilation is mediated by humoral factors (e.g., during sustained exercise), postural drive to the phrenic motoneurons is withdrawn, and respiratory input is prioritised (Hodges, Heijnen et al. 2001). A diminished postural drive to the diaphragm, coupled with a modest ventilatory demand, might explain the lack of inspiratory muscle fatigue noted in this study.

Pulmonary Function

Relative to pre-challenge baseline, there was a fall in FVC at day 4, which remained below baseline for the remainder of the event (Figure 3). It was first suspected that these baseline reductions in FVC may have been due, at least in part, to modest (non-significant) reductions in expiratory muscle strength; however, others report no change in pulmonary function when the expiratory muscles are pre-fatigued via expiratory threshold loading (Haverkamp et al. 2001). As such, a more likely explanation for the observed pulmonary dysfunction is a modest degree of lower-airway obstruction, which manifested in a fall in the baseline FEV_1/FVC ratio at day 7 (0.65 ± 0.08) and at day 10 (0.68 ± 0.08). Upper-airway obstruction can be discounted, since this is typically characterised by discordance between FEV_1 and PEF (Miller et al. 1990), and the baseline ratio of these parameters was maintained throughout the challenge (day 1 = 6.9 ± 1.2; day 4 = 6.8 ± 1.2; day 7 = 6.3 ± 2.1; day 10 = 6.7 ± 1.4). Despite these observations, lower airway obstruction as a causative factor in reduced lung function is difficult to assert because others have observed post-race reductions in pulmonary function both with (Maron et al. 1979) and without (Vernillo et al. 2015) the presence of airway obstruction. Additional lung volume data collected via whole-body plethysmography, in addition to measures of airway

resistance, would further elucidate the mechanisms underpinning our observations. Worthy of note is that we also observed acute pre-to-post marathon increases in FEV_1 (Figure 3), which was likely attributable to exercise-induced bronchodilation (Freedman. 1991).

Upper-respiratory tract infection (URTI)

Finally, in the 15-d post-challenge period, 56% (5/9) runners reported symptoms of Upper-Respiratory Tract Infection (URTI) (i.e., cough, watery eyes, blocked or runny nose, sneezing, sore throat), relative to 11% (1/9) pre-challenge, and 11% (1/9) of non-running controls. Symptoms of URTI are a common complaint among endurance runners; for example, there are reports of URTI in 47% of 208 runners who completed a single-stage marathon, relative to 19% of non-running controls (Robson-Ansley et al. 2012). Moreover, URTI occurred in 33% of runners who completed a 56 km single-stage race, relative to 15% of non-running controls (Peters and Bateman. 1983). It has been postulated that symptoms of URTI are the manifestation of an allergic or pro-inflammatory response, coupled with a transient suppression of cellular immune functions; although, neither were assessed in the present study. Worthy of note, is that 56% (5/9) runners exhibited a positive AQUA outcome, suggesting the presence of allergy, which is consistent with 60% prevalence in elite marathoners, whose reported symptoms were predominantly related to the upper-respiratory tract (Teixeira et al. 2014). Consequently, both single-stage and multi-stage endurance competition appear sufficient to cause symptoms of URTI and, in light of the present findings, the development of URTI appears to be mechanistically unrelated to changes in pulmonary function.

Implications for Health and Endurance Performance

There may be several means by which our findings might impact on health and/or endurance performance. First, the respiratory muscles have a critical role in maintaining torso stabilisation during exercise (Celli et al. 1988). The major expiratory muscles contract to increase intra-abdominal pressure which, in turn, increases stiffness and stability of the lumbar spine (Hodges, Cresswell et al. 2001; Hodges et al. 2005). This likely helps to protect spinal structures during periods of postural disturbance. As a consequence, exercise that induces expiratory muscle fatigue might place the runner

at a greater risk of injury, and render them less able to sustain the rigours of competition. Moreover, given that the limb-locomotor muscles exhibit substantial neuromuscular fatigue following prolonged running (Millet and Lepers. 2004), it is plausible that a simultaneous respiratory and locomotor muscle fatigue may further increase the risk of fall and/or injury when traversing challenging terrain. Accordingly, we propose that marathon and ultramarathon runners investigate strategies that attenuate the degree of expiratory muscle fatigue that manifests during competition.

Second, respiratory muscle fatigue results in reflex effects of breathing on vascular function (Dempsey et al. 2008). This metaboreflex causes sympathoexcitation and vasoconstriction of exercising limb vasculature, thereby eliciting a fall in limb blood flow and vascular conductance (Harms et al. 1998). Diminished blood flow to working muscles would be expected to accelerate locomotor muscle fatigue. Indeed, a fatigue-induced reduction in respiratory muscle work capacity has been modelled to significantly predict ultramarathon performance (Vernillo et al. 2015), although further studies are needed to investigate the presence of a metaboreflex in response to ultra-endurance exercise.

Third, it is possible that the development of respiratory dysfunction might impact on endurance performance. In a sample of 110 marathon runners (Salinero et al. 2016), there existed a significant negative correlation between indices of pulmonary function and marathon finish time; i.e., faster marathon runners exhibited better metrics of lung function (FVC = r = -0.41, p < 0.001; FEV_1 = r = -0.40, p < 0.001; PEF = r = -0.50, p = 0.005). Moreover, in an earlier study, (Warren et al. 1989) assessed the predictive power of lung function on ultramarathon performance by testing runners every 3 h throughout a 24 h footrace. The authors reported a significant reduction in MVV_{12} after 24 h, and modelled the variance in MVV_{12} to predict 39% of the variance in running speed. Although the mechanisms that underpin these relationships require further scrutiny, these studies do provide an insight into lung function and its potential predictive power on endurance running performance.

Finally, a pertinent question is whether the observed changes in pulmonary function were clinically meaningful. Given that the majority of values remained within the predicted range (i.e., above the lower-limit of normal), it is reasonable to suppose that - with adequate rest between stimuli - the respiratory systems of trained runners are sufficiently robust to recover from multiple,

consecutive days of endurance exercise, providing that athletes begin the race with a healthy baseline function. Although speculative, the same responses in individuals with underdeveloped baseline parameters or a pre-existing respiratory disorder (e.g., asthma), may result in manifestations of clinical significance.

In conclusion, we present novel data to suggest that the expiratory muscles are prone to acute contractile fatigue during ultramarathon stage-racing; however, we found limited evidence of a cumulative baseline-drift in respiratory muscle strength. Moreover, relatively well-maintained pulmonary and perceptual responses throughout the challenge suggest that the respiratory systems of trained runners are sufficiently robust to recover from multiple, consecutive days of endurance exercise. Nevertheless, acute fatigue of the expiratory muscles, combined with that of the locomotor muscles during marathon/ultramarathon, might impact on exercise performance and expose the individual to an increased risk of running-related injury. Further studies should aim to assess the pulmonary and respiratory muscle response to stage-races of a greater ventilatory demand and/or duration.

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REFERENCES

NHLBI Workshop summary. Respiratory muscle fatigue. Report of the Respiratory Muscle Fatigue Workshop Group. (1990) Am Rev Respir Dis 142:474-480. doi:10.1164/ajrccm/142.2.474 [doi]

Abraham KA, Feingold H, Fuller DD, Jenkins M, Mateika JH, Fregosi RF (2002) Respiratory-related activation of human abdominal muscles during exercise. J Physiol 541:653-663. doi:PHY_13462 [pii]

American Thoracic Society/European Respiratory Society (2002) ATS/ERS Statement on respiratory muscle testing. Am J Respir Crit Care Med 166:518-624. doi:10.1164/rccm.166.4.518 [doi]

Babcock MA, Pegelow DF, Harms CA, Dempsey JA (2002) Effects of respiratory muscle unloading on exercise-induced diaphragm fatigue. J Appl Physiol (1985) 93:201-206. doi:10.1152/japplphysiol.00612.2001 [doi]

Barkley RA (2001) The executive functions and self-regulation: an evolutionary neuropsychological perspective. Neuropsychol Rev 11:1-29

Bonini M, Braido F, Baiardini I et al (2009) AQUA: Allergy Questionnaire for Athletes. Development and validation. Med Sci Sports Exerc 41:1034-1041. doi:10.1249/MSS.0b013e318193c663 [doi]

Celli B, Criner G, Rassulo J (1988) Ventilatory muscle recruitment during unsupported arm exercise in normal subjects. J Appl Physiol (1985) 64:1936-1941. doi:10.1152/jappl.1988.64.5.1936 [doi]

Chevrolet JC, Tschopp JM, Blanc Y, Rochat T, Junod AF (1993) Alterations in inspiratory and leg muscle force and recovery pattern after a marathon. Med Sci Sports Exerc 25:501-507

Cohen J (1977) S*tatistical Power Analysis For the Behavioral Sciences.* Routledge. Routledge, New York

Dempsey JA, Amann M, Romer LM, Miller JD (2008) Respiratory system determinants of peripheral fatigue and endurance performance. Med Sci Sports Exerc 40:457-461. doi:10.1249/MSS.0b013e31815f8957 [doi]

Dimitriadis Z, Kapreli E, Konstantinidou I, Oldham J, Strimpakos N (2011) Test/retest reliability of maximum mouth pressure measurements with the MicroRPM in healthy volunteers. Respir Care 56:776-782. doi:10.4187/respcare.00783 [doi]

Evans JA, Whitelaw WA (2009) The assessment of maximal respiratory mouth pressures in adults. Respir Care 54:1348-1359

Freedman S (1991) Exercise as a bronchodilator. Clinical Science 83:383-9

Gordon B, Levine SA, Wilmaers A (1924) Observations on a group of **marathon** runners with special reference to the circulation. Archives of Internal Medicine (Chic.) 33:425

Guenette JA, Romer LM, Querido JS et al (2010) Sex differences in exercise-induced diaphragmatic fatigue in endurance-trained athletes. J Appl Physiol (1985) 109:35-46. doi:10.1152/japplphysiol.01341.2009 [doi]

Harms CA, Wetter TJ, McClaran SR et al (1998) Effects of respiratory muscle work on cardiac output and its distribution during maximal exercise. J Appl Physiol (1985) 85:609-618. doi:10.1152/jappl.1998.85.2.609 [doi]

Haverkamp HC, Metelits M, Hartnett J, Olsson K, Coast JR (2001) Pulmonary function subsequent to expiratory muscle fatigue in healthy humans. Int J Sports Med 22:498-503. doi:10.1055/s-2001-17612 [doi]

Hodges PW, Eriksson AE, Shirley D, Gandevia SC (2005) Intra-abdominal pressure increases stiffness of the lumbar spine. J Biomech 38:1873-1880. doi:S0021-9290(04)00410-5 [pii]

Hodges PW, Cresswell AG, Daggfeldt K, Thorstensson A (2001) In vivo measurement of the effect of intra-abdominal pressure on the human spine. J Biomech 34:347-353. doi:S0021929000002062 [pii]

Hodges PW, Heijnen I, Gandevia SC (2001) Postural activity of the diaphragm is reduced in humans when respiratory demand increases. J Physiol 537:999-1008. doi:PHY_12648 [pii]

Hodges PW, Gandevia SC (2000) Activation of the human diaphragm during a repetitive postural task. J Physiol 522 Pt 1:165-175. doi:PHY_9644 [pii]

Janssens L, Brumagne S, McConnell AK et al (2013) The assessment of inspiratory muscle fatigue in healthy individuals: a systematic review. Respir Med 107:331-346. doi:10.1016/j.rmed.2012.11.019 [doi]

Johnson BD, Babcock MA, Suman OE, Dempsey JA (1993) Exercise-induced diaphragmatic fatigue in healthy humans. J Physiol 460:385-405

Jones DA (1996) High-and low-frequency fatigue revisited. Acta Physiol Scand 156:265-270. doi:10.1046/j.1365-201X.1996.192000.x [doi]

Ker JA, Schultz CM (1996) Respiratory muscle fatigue after an ultra-marathon measured as inspiratory task failure. Int J Sports Med 17:493-496. doi:10.1055/s-2007-972884 [doi]

Loke J, Mahler DA, Virgulto JA (1982) Respiratory muscle fatigue after marathon running. J Appl Physiol Respir Environ Exerc Physiol 52:821-824

Lomax ME, McConnell AK (2003) Inspiratory muscle fatigue in swimmers after a single 200 m swim. J Sports Sci 21:659-664. doi:10.1080/0264041031000101999 [doi]

Mahler DA, Horowitz MB (1994) Perception of breathlessness during exercise in patients with respiratory disease. Med Sci Sports Exerc 26:1078-1081

Maron MB, Hamilton LH, Maksud MG (1979) Alterations in pulmonary function consequent to competitive marathon running. Med Sci Sports 11:244-249

Mathur S, Sheel AW, Road JD, Reid WD (2010) Delayed onset muscle soreness after inspiratory threshold loading in healthy adults. Cardiopulm Phys Ther J 21:5-12

Miller MR, Hankinson J, Brusasco V et al (2005) Standardisation of spirometry. Eur Respir J 26:319-338. doi:26/2/319 [pii]

Miller MR, Pincock AC, Oates GD, Wilkinson R, Skene-Smith H (1990) Upper airway obstruction due to goitre: detection, prevalence and results of surgical management. Q J Med 74:177-188

Millet GY, Lepers R (2004) Alterations of neuromuscular function after prolonged running, cycling and skiing exercises. Sports Med 34:105-116. doi:3424 [pii]

Millet GP, Millet GY (2012) Ultramarathon is an outstanding model for the study of adaptive responses to extreme load and stress. BMC Med 10:77-7015. doi:10.1186/1741-7015-10-77 [doi]

Peters EM, Bateman ED (1983) Ultramarathon running and upper respiratory tract infections. An epidemiological survey. S Afr Med J 64:582-584

Polkey MI, Green M, Moxham J (1995) Measurement of respiratory muscle strength. Thorax 50:1131-1135

Robson-Ansley P, Howatson G, Tallent J et al (2012) Prevalence of allergy and upper respiratory tract symptoms in runners of the London marathon. Med Sci Sports Exerc 44:999-1004. doi:10.1249/MSS.0b013e318243253d [doi]

Romer LM, Polkey MI (2008) Exercise-induced respiratory muscle fatigue: implications for performance. J Appl Physiol (1985) 104:879-888. doi:01157.2007 [pii]

Ross E, Middleton N, Shave R, George K, Mcconnell A (2008) Changes in respiratory muscle and lung function following marathon running in man. J Sports Sci 26:1295-1301. doi:10.1080/02640410802104904 [doi]

Salinero JJ, Soriano ML, Ruiz-Vicente D et al (2016) Respiratory function is associated to marathon race time. J Sports Med Phys Fitness 56:1433-1438. doi:R40Y9999N00A16020202 [pii]

Taylor BJ, How SC, Romer LM (2006) Exercise-induced abdominal muscle fatigue in healthy humans. J Appl Physiol (1985) 100:1554-1562. doi:01389.2005 [pii]

Teixeira RN, Mendes FA, Martins MA, Mickleborough TD, Carvalho CR (2014) AQUA(c) as predictor of allergy in elite marathon runners. World Allergy Organ J 7:7-4551-7-7. doi:10.1186/1939-4551-7-7 [doi]

Tiller NB, Campbell IG, Romer LM (2017) Influence of upper-body exercise on the fatigability of human respiratory muscles. Med Sci Sports Exerc 49:1461-1472. doi:10.1249/MSS.0000000000001251 [doi]

Vernillo G, Rinaldo N, Giorgi A et al (2015) Changes in lung function during an extreme mountain ultramarathon. Scand J Med Sci Sports 25:e374-80. doi:10.1111/sms.12325 [doi]

Volianitis S, McConnell AK, Koutedakis Y, McNaughton L, Backx K, Jones DA (2001) Inspiratory muscle training improves rowing performance. Med Sci Sports Exerc 33:803-809

Warren GL, Cureton KJ, Sparling PB (1989) Does lung function limit performance in a 24-hour ultramarathon? Respir Physiol 78:253-263

Wen AS, Woo MS, Keens TG (1997) How many maneuvers are required to measure maximal inspiratory pressure accurately. Chest 111:802-807. doi:S0012-3692(15)46838-2 [pii]

Wuthrich TU, Marty J, Kerherve H, Millet GY, Verges S, Spengler CM (2015) Aspects of respiratory muscle fatigue in a mountain ultramarathon race. Med Sci Sports Exerc 47:519-527. doi:10.1249/MSS.0000000000000449 [doi]

FIGURE/TABLE CAPTIONS

Figure 1. Individual and group-mean marathon times throughout the 10-day challenge.

Figure 2. Maximum static expiratory (panel A) and inspiratory (panel B) mouth-pressure, before and after marathons on day 1, 4, 7 and 10. *significantly different versus pre-challenge baseline, $p < 0.05$.

Figure 3. Forced vital capacity (panel A), forced expiratory volume in 1 second (panel B), and peak expiratory flow (panel C), before and after marathons on day 1, 4, 7 and 10. *significantly different versus pre-challenge baseline, $p < 0.05$; †significantly different versus pre-marathon, $p < 0.05$.

Table 1. Within- and between-day reliability of respiratory measures.

Table 2. Perceptual responses before and after marathons on day 1, 4, 7, and 10.

Figure 1

Click here to access/download;Figure;Fig 1.tif

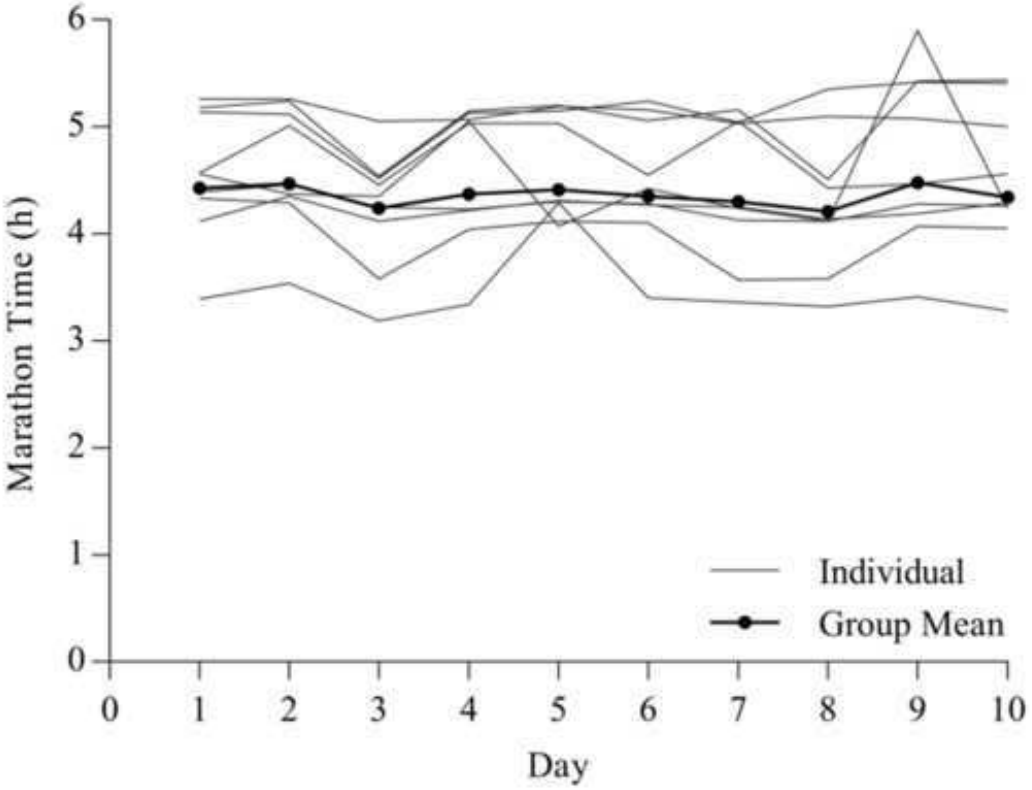

Figure 2

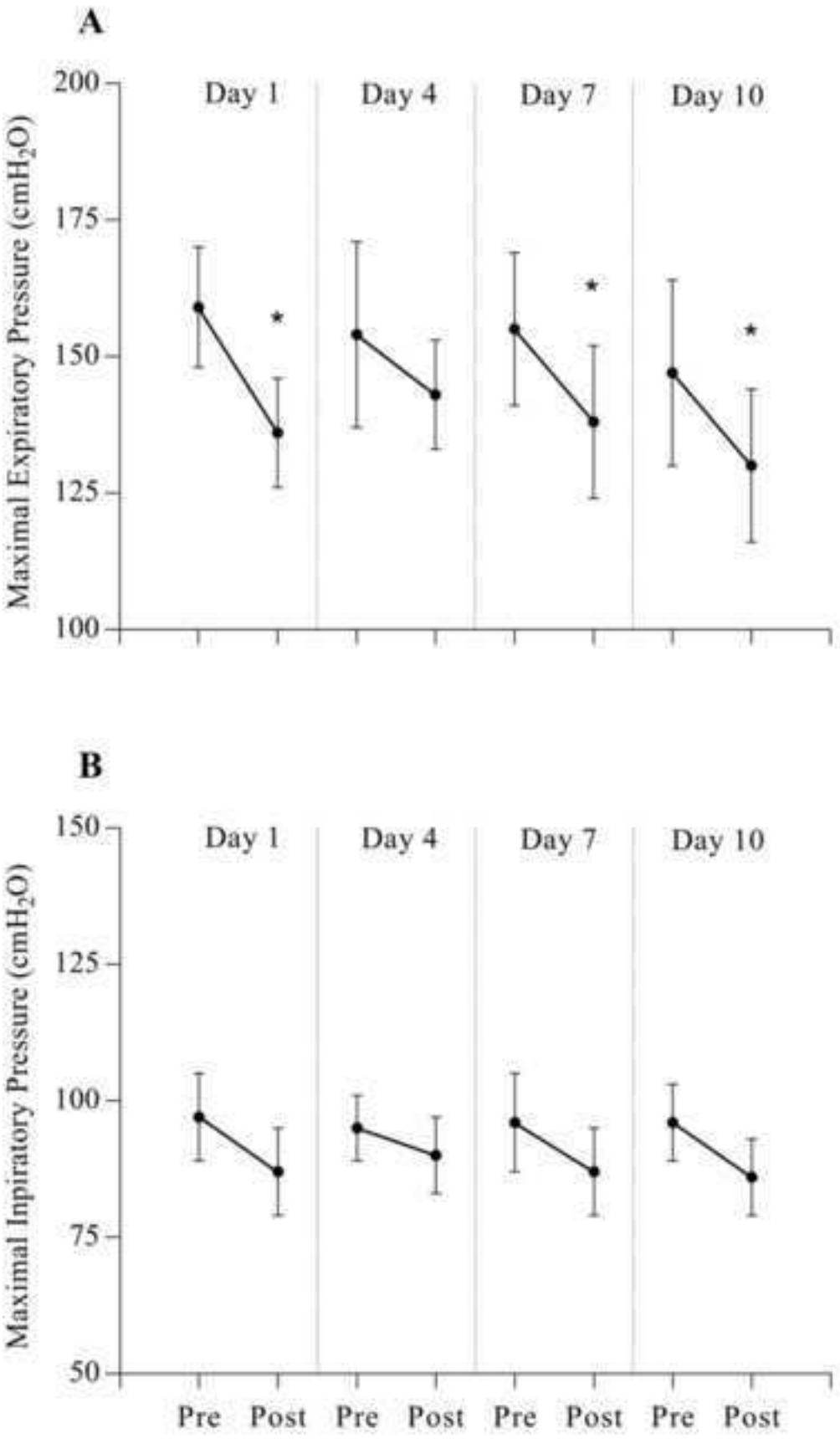

Figure 3

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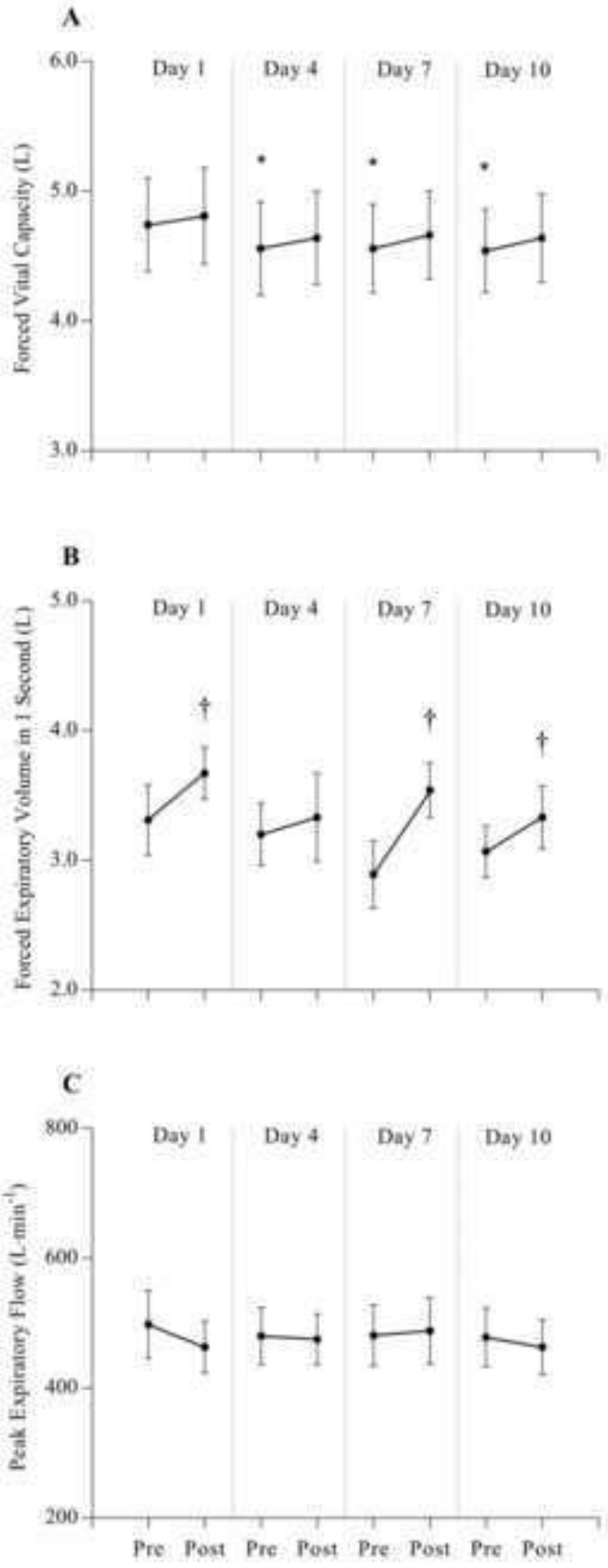

Table 1

Table 1. Within- and between-day reliability of respiratory measures.

	Trial 1	Trial 2	Trial 3	CV (%)	SEM	ICC
FVC (L)	5.07 ± 0.75	5.02 ± 0.76	5.06 ± 0.74	0.7	0.075	0.999 (0.996-1.000)
FEV_1 (L)	3.89 ± 0.71	3.84 ± 0.79	3.78 ± 0.69	2.6	0.103	0.994 (0.975-0.999)
FEV_1/FVC	0.77 ± 0.05	0.76 ± 0.06	0.75 ± 0.03	2.5	0.016	0.943 (0.760-0.991)
PEF ($L \cdot min^{-1}$)	607 ± 96	612 ± 135	615 ± 102	4.6	31.4	0.963 (0.842-0.994)
MIP (cmH_2O)	124 ± 30	126 ± 32	124 ± 30	4.0	6.16	0.988 (0.950-0.998)
MEP (cmH_2O)	200 ± 53	194 ± 51	193 ± 51	2.9	7.32	0.996 (0.983-0.999)

FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 second; PEF, peak expiratory flow; MIP, maximal static inspiratory pressure; MEP, maximal static expiratory pressure; CV, coefficient of variation; SEM, standard error of measurement; ICC, intra-class correlation coefficient. Data are means ± SD.

Table 2

Table 2. Perceptual responses before and after marathons on day 1, 4, 7, and 10.

	Day 1		Day 4		Day 7		Day 10	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
MIP_{VAS} (mm)	2.4 ± 4.3	0.3 ± 0.7	0.2 ± 0.4	0.4 ± 1.0	0.1 ± 0.3	0.2 ± 0.4	0.2 ± 0.4	0.3 ± 1.0
MEP_{VAS} (mm)	0.2 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.7	0.0 ± 0.0	0.1 ± 0.3	0.4 ± 1.0	0.3 ± 0.7
Dyspnea (CR10)	0.0 ± 0.0	1.7 ± 0.9	0.1 ± 0.3	2.3 ± 0.7	0.2 ± 0.4	2.0 ± 1.3	0.3 ± 1.0	2.0 ± 1.2
URTI (VAS):								
Cough (mm)	1.2 ± 2.4	0.7 ± 0.9	2.1 ± 5.6	2.8 ± 6.9	3.9 ± 10.6	2.0 ± 5.3	1.3 ± 2.6	3.3 ± 5.3
Wheeze (mm)	1.4 ± 4.3	0.9 ± 1.7	1.2 ± 2.6	3.1 ± 5.9	0.8 ± 2.0	0.6 ± 1.7	0.7 ± 2.0	2.1 ± 4.4
Chest (mm)	0.2 ± 0.7	1.8 ± 4.0	1.6 ± 3.1	5.9 ± 8.5	3.9 ± 7.6	3.3 ± 6.4	3.7 ± 8.4	3.4 ± 7.2
Mucus (mm)	9.2 ± 17.2	10.1 ± 17.2	3.3 ± 5.4	11.6 ± 18.0	9.1 ± 14.6	13.0 ± 24.0	13.0 ± 24.4	13.7 ± 22.1

MIP, maximal static inspiratory pressure; MEP, maximal static expiratory pressure; VAS, visual analogue scale; URTI, upper-respiratory tract infection; Cough, current experience of cough; Wheeze, current experience of wheeze; Chest, current experience of chest-tightness; Mucus, current experience of mucus secretions.