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ABSTRACT

Purpose: Despite a growing body of literature on the physiological responses to ultramarathon, there is a paucity of data in females. This study assessed the female physiological response to ultramarathon and compared the frequency of perturbations to a group of race- and time-matched males. **Methods**: Data were collected from 53 contestants of an ultramarathon trail race at 2018/19 Ultra Trail du Mont-Blanc (UTMB®). Before and within 2-h of the finish, participants underwent physiological assessments including blood sampling for biomarkers (creatine kinase-MB isoenzyme, CK-MB; cardiac troponin I, cTnI; brain natriuretic peptide, BNP, creatinine, Cr); pulmonary function testing (spirometry, exhaled NO, diffusing capacities, mouth pressures); and transthoracic ultrasound (lung comet tails, cardiac function). Data from eight female finishers (age=36.6±6.9 y; finish time=30:57±11:36 hh:mm) were compared to a group of eight time-matched males (age=40.3±8.3 y; finish time=30:46±10:32 hh:mm). **Results**: Females exhibited significant pre- to post-race increases in BNP (25.8±14.6 vs. 140.9±102.7 pg/mL; p=0.007) and CK-MB (3.3±2.4 vs. 74.6±49.6 IU/L; p=0.005), whereas males exhibited significant pre- to post-race increases in BNP (26.6±17.5 vs. 96.4±51.9 pg/mL; p=0.002), CK-MB (7.2±3.9 vs. 108.8±37.4 IU/L; p=0.002), and Cr (1.06±0.19 vs. 1.23±0.24 mg/dL; p=0.028). Lung function declined in both groups, but males exhibited additional reductions in lung diffusing capacities (DL_{CO}=34.4±5.7 vs. 29.2±6.9 mL/min/mmHg, p=0.004; DL_{NO}=179.1±26.2 vs. 152.8±33.4 mL/min/mmHg, p=0.002) and pulmonary capillary blood volumes (77.4±16.7 vs. 57.3±16.1 mL; p=0.002). Males, but not females, exhibited evidence of mild post-race pulmonary edema. Pooled effect sizes for within-group pre- to post-race changes, for all variables, were generally larger in males versus females (d = 0.86 vs. 0.63). **Conclusions**: Ultramarathon negatively impacts a range of physiological functions but generally evokes more frequent perturbations, with larger effect sizes, in males compared to females with similar race performances. **Key words:** CARDIOVASCULAR, PULMONARY, RESPIRATORY, SEX-DIFFERENCES, ULTRA-ENDURANCE

INTRODUCTION

Ultramarathons are footraces that typically range from ~30 miles (~50 km) to ~150 miles (~240 km) in a single stage and considerably further in multi-stage events. Participation evokes extreme physiological strain on multiple body systems (1), particularly the cardiovascular and respiratory systems (2). For instance, studies show decreased left ventricular function and increased cardiac biomarkers following ultramarathon (3, 4), in addition to lung function derangements of 10–15% with or without evidence of airway obstruction (5). Moreover, while most physiological perturbations are transient and generally recover to baseline within a week, there is the potential for long-term maladaptations and associated health issues (6). For these reasons, there is now a greater emphasis on understanding the acute and chronic physiological and pathophysiological responses to ultramarathon running (1, 2, 6, 7).

Despite the growing body of work, there is a paucity of data in female athletes. A recent review on pulmonary responses to marathon and ultramarathon running collated 15 studies with a cumulative 232 participants of which only 19 (8%) were female (5). This number is considerably below the estimated ~20% of female ultramarathon contestants (8–10) and supports the notion that females may be underrepresented in exercise science research (11). Potential explanations may be a researcher bias that favours males as recruitment participants (12), but also a possible volunteer bias which has males more willing to participate in exercise-related research (13). Nevertheless, anatomical and physiological differences between males and females can influence the exercise response (14–17), and failure to consider these differences may limit the specificity of training programs and negatively impact efforts at promoting competitive longevity.

The issue of sex-based physiological predisposition to ultramarathon has also been a topic of recent discussion (10). Indeed, a number of exceptional, record-breaking performances by female athletes in ultramarathon in recent years has roused speculation that they might be predisposed to success in such events. The male-to-female performance gap in regular endurance sports like marathon is ~10% (18), but studies have calculated the performance gap in ultramarathon to be as low as 4% (19). In some instances, female performances may surpass those of their male counterparts (20). Additionally, in ultramarathon, there are distinct performance predictors for males (e.g., age, BMI, years of running) and females (e.g., weekly running mileage and half-marathon record) (9). Thus, while the question of whether females are physiologically predisposed to ultramarathon has not been directly explored, an ability to better tolerate the physiological stress of racing is likely ergogenic in ultramarathon and may also lead to better long-term health management.

Accordingly, there were two aims of this exploratory study. The first was to provide novel data on the physiological responses of females to an ultramarathon trail race, with specific emphasis on respiratory and cardiopulmonary function. The second was to explore sex differences in the frequency of pre- to post-race physiological perturbations in males and females matched for ultramarathon finish time.

METHODS

Race Characteristics

Data were collected from runners competing in one-of-two races at the annual Ultra Trail du Mont-Blanc (UTMB®) trail running series in 2018 or 2019. The UTMB® (106 miles/171 km, ~10,000

m ascent) and the CCC® (63 miles/101 km, ~6,000 m ascent) are single-stage, mountainous trail races commencing in Chamonix, France and Courmayeur, Italy, respectively. Both races require intermittent bouts of traversal at altitudes ≥ 2,500 m (Fig. 1) and, in the years during which data collection took place, temperature and humidity ranged from -6 to 28°C/35 to 75% (2018) and 6 to 29°C/35 to 70% (2019). Temperature extremes were mediated largely by altitude.

Ethical Approval and Participants

Ethical approval was granted first by the Mayo Clinic Institutional Review Board (IRB# 17-003843) and then by the Comité de Protection des Personnes Sud-Ouest et Outre-Mer 2 (IRB# 2-18-43-2). Thereafter, runners were contacted by the UTMB® organizers who distributed details of the study via electronic recruitment posters. After providing written, informed consent, data were collected from 53 runners of which 10 (19%) were female. One female runner retired early from the race, and another did not return for post-race assessments; thus, eight female finishers remained (CCC®, n=4; UTMB®, n=4;). A subgroup of eight male runners from the same races (CCC®, n=4; UTMB®, n=4;), whose finish times most closely matched the female group mean, were selected as a comparison (Table 1). Runners completed a medical questionnaire and declared that they were free from known cardiorespiratory illnesses. All testing was conducted in accordance with the declaration of Helsinki.

Study Design

Participants attended the laboratory (based near the start/finish line at 1,035 m) in the week preceding the race to complete baseline testing which was organized into three phases (Fig. 2). Initial measures included vital signs (heart rate, systolic and diastolic blood pressure [SBP/DBP],

electrocardiogram [ECG]), basic anthropometry (stature and mass), and venous blood sampling for electrolytes, biomarkers, haemoglobin concentration, and haematocrit. Next, participants completed pulmonary function tests (PFTs) including spirometry, forced oscillation, and exhaled nitric oxide, followed by an assessment of respiratory muscle strength. Lastly, resting lung diffusing capacity was assessed followed by transthoracic ultrasound for cardiac morphology and lung comet tails. All physiological measures were repeated as soon as possible following race completion (mean ± SD, 1 h 41 min ± 54 min).

Blood sampling

Venous blood samples (~8 mL) were collected via venepuncture and analysed using a commercially available, hand-held immunoassay device and cartridges (i-STAT Corporation, New Jersey, USA). Measures included haemoglobin (Hb), haematocrit (Hct), electrolytes (sodium, Na^{2+}; potassium, K^{+}; chloride, Cl^{-}), and biochemical markers relating to cardiac (troponin I, cTnI; brain natriuretic peptide, BNP), renal (creatinine, Cr), and skeletal muscle function (creatine kinase-MB, CK-MB). Plasma volume was calculated from Hct and Hb using the Dill and Costill equation (21).

Pulmonary and respiratory muscle function

Pulmonary volumes (forced expiratory volume in 1-second, FEV_1; forced inspiratory volume in 1-second, FIV_1), capacities (forced vital capacity, FVC; inspiratory capacity, IC), and flows (peak expiratory flow, PEF; forced expiratory flow between 25 and 75% of FVC, FEF_{25-75}) were assessed using a portable spirometer (Breeze Suite 8.5 and CPFS/D USB™, Medgraphics Corporation, Minnesota, USA) during a minimum of three and a maximum of eight forced expiratory

manoeuvres (22). Airway resistance at 5 and 19 Hz (R_5 and R_{19}) were assessed via forced oscillometry (Resmon Pro V3; MGC Diagnostics, Minnesota, USA) during which participants were seated, had the nose occluded, and were asked to maintain tidal breathing while their cheeks were held firmly by an investigator (23). As a marker of airway inflammation, fractional exhaled nitric oxide (Fe_{NO}) was measured using a handheld device (Aerocrine Nixo Vero® 510(k), Solna, Sweden, used in 2018; NObreath; Bedfont, Rochester, UK, used in 2019) (24). Lung diffusing capacity for carbon monoxide (DL_{CO}) and nitric oxide (DL_{NO}) were assessed simultaneously via the single-breath technique using a 4-s breath-hold (Hyp'air Compact system with Exp'air software, version 1.31.05, Medisoft, Dinant, Belgium). Each resting measure was separated by 4 min and performed in duplicate (25). Moreover, DL_{CO} was expressed in absolute terms, expressed relative to alveolar volume (DL_{CO}/VA), and corrected to reference hemoglobin concentrations ($DL_{CO,HbCorr}$) according to the Cotes *et al.* equation (25, 26). Following the assessment of DL_{CO} and DL_{NO}, alveolar-capillary membrane conductance (DM_{CO}) and pulmonary capillary blood volume (V_C) were calculated using equations described by Pavelescu *et al.* (27). Finally, maximum static inspiratory pressure (P_{IMAX}) from residual volume and maximum static expiratory pressure (P_{EMAX}) from total lung capacity (28) were measured using a handheld device (MicroRPM, CareFusion, San Diego, USA). All pulmonary and respiratory muscle function tests were performed in accordance with recommended standards (22–25, 27, 28).

Transthoracic ultrasound

Comet tails. As a measure of extravascular lung water (pulmonary oedema), the number of ultrasound lung comets was determined via transthoracic sonography (Philips CX50 and S5-1 transducer, Philips Healthcare, Netherlands), as previously described (29, 30). Briefly, participants

lay supine while the sonographer sequentially examined 28 intercostal lung fields located at the parasternal, midclavicular, anterior axillary and mid-axillary lines from the second to the fourth intercostal space (left side) and from the second to the fifth intercostal space (right side). A comet was defined as an echogenic, coherent, wedge-shaped signal that originated from the hyperechoic pleural line and extended to the edge of the screen. The presence of an ultrasound lung comet was simultaneously verified by two trained operators. In accordance with Picano *et al.* (31), we employed a semi-quantitative classification for the presence of extravascular lung water, whereby a total lung comet tail count of < 5 was considered "normal"; 5 - 15 was mild extravascular lung water accumulation; 15 - 30 was moderate extravascular lung water accumulation; and > 30 was severe extravascular lung water accumulation (31).

Echocardiography. All images were acquired while the participant was supine and orientated in the left-lateral decubitus position following 10-min rest. Two-dimensional (2-D) and pulsed-wave tissue Doppler echocardiography were performed using ultrasound (Philips CX50 and S5-1 transducer, Philips Healthcare, Netherlands). Images were acquired by an experienced cardiac sonographer in accordance with the guidelines published by the American Society of Echocardiography (32). Echocardiograph data were analysed offline by the same assessor using commercially available software (Q-Lab 13, Philips Healthcare, Netherlands). Measures included cardiac frequency (f_C), stroke volume (SV) determined via the Doppler velocity time integral (DVTI) method, and cardiac output ($\dot{Q}$) determined by the product of f_C and SV (32).

Statistics

Statistical analyses were performed using IBM SPSS Statistics v24 (IBM; Illinois, USA). Normality of distribution was assessed using the Shapiro Wilk test, and data that were not normally distributed were log transformed. Independent samples *t*-tests were used to assess for sex differences in age, race time, velocity, and physiological variables at baseline, with the Welch statistic applied in cases when homogeneity of variance (Levine's test) was violated. Paired samples *t*-tests were used to assess the female (within-group, n=8) pre- to post-race response, the male (within-group, n=8) pre- to post-race response, and the overall pre- to post-race response (n=16). For differences testing, the Benjamini-Hochberg method was used to adjust the *p*-value for the false discovery rate associated with multiple comparisons. The magnitude of the difference between group means was assessed using Cohen's *d* (0.2 = small; 0.5 = medium; 0.8 = large; (33)). Alpha level was 0.05, and descriptive values are reported as mean ± SD (unless stated).

RESULTS

Baseline variables

Participant demographics and race data are shown in Table 1. There was no difference in age between females and males ($p = 0.361$), but males were taller ($p = 0.003$) and heavier ($p = 0.004$). Per study design, there were no between-group differences in average finish time ($p = 0.975$) or running velocity ($p = 0.762$). Baseline physiological variables are shown in Table 2. Males exhibited greater baseline values for SBP, Na^{2+}, Hct, PV, Cr, CK-MB, FVC, FEV_1, PEF, FIV_1, DL_{CO}, $DL_{CO,HbCorr}$, DL_{NO}, V_C, P_{IMAX}, and P_{Emax}. There were no baseline between-group differences in f_C, DBP, K^+, Cl^-, Hb, cTnI, BNP, FEV_1/FVC, FEF_{25-75}, IC, R_5, R_5-R_{19}, Fe_{NO}, DL_{CO}/VA, DM_{CO}, frequency of lung comet tails, SV, or $\dot{Q}$.

Physiological responses to ultramarathon

Participants returned for post-race assessments 1 h 41 min ± 54 min after finishing the event, with no difference between the sexes (1 h 44 min ± 54 min vs. 1 h 38 min ± 57 min, $p = 0.846$, $d = 0.11$). All within-group pre- to post-race data (means, standard deviations, *p*-values, and effect sizes) are shown in the supplemental table (see Supplemental Table, Supplemental Digital Content, Pre- and post-race physiological responses in males and females, http://links.lww.com/MSS/C629).

Vital signs (f_C, SBP, and DBP). Paired-samples *t*-tests revealed a significant overall effect of ultramarathon on f_C ($p = 0.004$, $d = 1.26$) and SBP ($p = 0.010$, $d = 0.88$). There was no overall effect on DBP ($p = 0.290$, $d = 0.45$). The within-group analysis showed that females exhibited significant pre- to post-race increases in f_C, while males exhibited significant pre- to post-race decreases in SBP (see Supplemental Table, Supplemental Digital Content, Pre- and post-race physiological responses in males and females, http://links.lww.com/MSS/C629).

Blood sampling. Paired-samples *t*-tests revealed a significant overall effect of ultramarathon on Hb ($p = 0.032$, $p = 0.77$), Hct ($p = 0.036$, $d = 0.76$), PV ($p = 0.020$, $d = 0.82$), cTn1 ($p = 0.016$, $d = 1.11$), BNP ($p = 0.004$, $d = 1.57$), Cr ($p = 0.028$, $d = 0.39$), and CK-MB ($p = 0.004$, $d = 2.65$). There was no overall effect on Na^{2+} ($p = 0.566$, $d = 0.31$) - with no evidence of hyponatremia in any athlete - and no overall effect on K^+ ($p = 0.236$, $d = 0.77$) or Cl^- ($p = 0.282$, $d = 0.40$). The within-group analysis showed that females exhibited significant pre- to post-race increases in BNP and CK-MB, while males exhibited significant pre- to post-race increases in

BNP, CK-MB, Cr, and PV (Fig. 3; and Supplemental Table, Supplemental Digital Content, Pre- and post-race physiological responses in males and females, http://links.lww.com/MSS/C629).

Pulmonary and respiratory muscle function. Paired-samples *t*-tests revealed a significant overall effect of ultramarathon on FVC ($p = 0.044$, $d = 0.36$), FEV_1 ($p = 0.027$, $d = 0.36$), PEF ($p = 0.016$, $d = 0.37$), IC ($p = 0.004$, $d = 0.95$), Fe_{NO} ($p = 0.004$, $d = 0.72$), DL_{CO} ($p = 0.005$, $d = 0.51$), DL_{NO} ($p = 0.004$, $d = 0.52$), V_C ($p = 0.004$, $d = 0.88$), and P_{IMAX} ($p = 0.010$, $d = 0.56$). There was no overall effect on FEV_1/FVC ($p = 1.000$, $d = 0.11$), FEF_{25-75} ($p = 0.412$, $d = 0.32$), FIV_1 ($p = 0.264$, $d = 0.38$), R_5 ($p = 0.472$, $d = 0.27$), R_5-R_{19} ($p = 0.182$, $d = 0.45$), $DL_{CO,HbCorr}$ ($p = 0.061$, $d = 0.32$), DL_{CO}/VA ($p = 1.000$, $d = 0.08$), DM_{CO} ($p = 0.825$, $d = 0.22$), or P_{EMAX} ($p = 0.096$, $d = 0.38$). The within-group analysis showed that females exhibited significant pre- to post-race decreases in FVC, PEF, IC, Fe_{NO}, and P_{IMAX}, while males exhibited significant pre- to post-race decreases in PEF, IC, Fe_{NO}, DL_{CO}, DL_{NO}, and V_C (Fig. 4; and Supplemental Table, Supplemental Digital Content, Pre- and post-race physiological responses in males and females, http://links.lww.com/MSS/C629).

Transthoracic ultrasound. Paired-samples *t*-tests revealed a significant overall effect of ultramarathon on lung comet tails ($p = 0.004$, $d = 1.31$) and $\dot{Q}$ ($p = 0.020$, $d = 0.75$). There was no overall effect on SV ($p = 0.234$, $d = 0.36$). The within-group analysis showed that females exhibited significant pre- to post-race increases in lung comet tails and $\dot{Q}$, while males exhibited significant pre- to post-race increases in lung comet tails (see Supplemental Table, Supplemental Digital Content, Pre- and post-race physiological responses in males and females, http://links.lww.com/MSS/C629).

DISCUSSION

The aims of this study were to provide novel data on the physiological responses of females to an ultramarathon trail race, and to explore sex differences in the frequency of pre- to post-race physiological perturbations in groups matched for ultramarathon finish time. The main findings were: i) ultramarathon evoked significant increases in skeletal muscle, cardiac, and renal biomarkers, and significant decreases in various aspects of respiratory and cardiopulmonary function; ii) both males and females exhibited biomarker disturbances but with a greater number of perturbations in males; and iii) ultramarathon reduced lung function and increased comet tails in both groups, with additional reductions in diffusing capacities and pulmonary capillary volumes in males. Our data show that ultramarathon negatively impacts a range of physiological functions but generally evokes more frequent perturbations, with larger effect sizes (pooled effect size for all variables, d = 0.86 vs. 0.63) in males compared to females matched for finish time.

In accordance with existing literature (5), ultramarathon resulted in a significant decrease in spirometric indices of lung function; specifically, forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), and peak expiratory flow (PEF) (Fig. 4). The overall decreases in FVC and FEV_1 were driven primarily by females. Wuthrich *et al*. published respiratory data from 23 runners (8 female) who contested the UTMB® in 2012 (35). Congruent with our findings, they also reported significant post-race decreases in FEV_1 and PEF. Airflow during spirometry is a product of the driving pressure of the thoracic muscles offset against the airway resistance (36). Given that we observed no evidence of small airway obstruction post-race, in either group (i.e., no change in FEF_{25-75}, R_5, or R_5-R_{19}), the most likely explanation for the decreases in expiratory flows is a diminished thoracic driving pressure. This may have been attributable to a mild degree of

expiratory muscle fatigue, as proposed by Wuthrich *et al.* (35), and/or a failure to start the FVC manoeuvre from a "true" total lung capacity, as reported by Tiller *et al.* (37). The latter scenario is especially likely given the significantly diminished post-race IC exhibited by both groups.

Females generally have smaller lungs and narrower conducting airways than males (16, 38) and are more likely to exhibit expiratory flow limitation during exercise (39). As such, the larger magnitude of reduction in peak flows in the female athletes was not unexpected. Nevertheless, despite statistically significant decreases in pulmonary function in both groups, follow-up analyses using regression equations from the Global Lung Function Initiative (40) showed that all post-race values of FVC and FEV_1 (with the exception of one male participant, see below) remained within normal limits and were unlikely to pose an acute clinical concern.

The male cohort exhibited a large and significant pre- to post-race decrease in lung diffusing capacities (DL_{CO} = -16%; $DL_{CO,HbCorr}$ = -12%, DL_{NO} = -16%), whereas post-race values in the female group were not significantly different from baseline (Fig. 4). The decreases in DL_{CO} and DL_{NO}, which reflect a reduced capacity for gas transfer from alveoli to the bloodstream, may result from a fall in pulmonary capillary blood volume (V_C) in males, especially given that there was no post-race change in DM_{CO}. There are reports of diminished DL_{CO} and DM_{CO} at altitude without changes in V_C in healthy participants (41). Acute high-intensity exercise has also been shown to reduce DL_{CO} and V_C (42), despite being compensated for, in some cases, by increases in DM_{CO} (43). It is unclear if the reduced capacity for gas transfer in males resulted from ultra-endurance exercise, the intermittent altitude, or a combined effect of both stimuli resulting in a mild post-race pulmonary vascular de-recruitment and an overall null effect on DM_{CO} in males.

Further study in a larger cohort is required to explore this finding and establish whether a pulmonary vascular phenotype in female runners precludes a decline in DL_{CO} and V_C following ultramarathon.

There was an overall increase in lung comet tails following the race, and values were significantly elevated in both females and males. Nevertheless, the male group exhibited considerably larger effect sizes (2.41 vs. 0.96), and all males increased comet tails by >1 versus only 4/8 females. As per Picano *et al.,* (31), post-race comet tails in the range of 5 – 15 indicate "mild" extravascular lung water accumulation, and this threshold was met only by males. By contrast, values in females remained in the "normal" range (i.e., < 5). Although our data somewhat contradict earlier studies showing greater prevalence of interstitial lung oedema in females following marathon (44), there is evidence of pulmonary oedema triggered by both maximal and submaximal (prolonged) exercise, independent of sex and the level of hypoxia (45). As such, there is no reason to think that the present increases in lung comet tails were mediated exclusively by the intermittent altitude experienced during the race. Instead, capillary haemorrhage, increased capillary permeability, and/or pulmonary oedema may result from increased cardiac output and pulmonary vascular pressure during exercise (46). It is worthy of note that the individual male and female athletes who exhibited the greatest increases in lung comet tails also exhibited the largest post-race declines in pulmonary function. In fact, the male individual was the only participant in the cohort to exhibit post-race values for FEV_1 that fell below the lower limit of normal. Although our data confirm earlier observations that there is little relation between the change in oedema score and the change in DM_{CO} or FVC (47), there may yet be an interaction among ultra-endurance exercise, intermittent altitude, and pulmonary oedema which warrants further study.

Relative to baseline, we observed significant overall increases in both BNP and cTnI following the race (Fig. 3). The absolute values were modest and remained within normal limits, as was generally observed in studies of cardiac biomarkers following the Badwater ultramarathon (216 km; (3)) and the Western States Endurance Run (160 km; (4)). Increased cardiac biomarkers are considered to be a common response to endurance exercise and were reported as elevated in endurance athletes without any accompanying signs of persistent cardiac damage (48). Nonetheless, a recent review highlighted the potential for long-term cardiovascular maladaptations with ultra-endurance running (6) such that the prognostic importance of periodic acute increases in biomarkers (particularly cardiac biomarkers) should not be dismissed. Specifically, more research is needed to elucidate the clinical importance of biomarkers that may be repeatedly elevated as a result of frequent ultra-endurance competition.

The observation of smaller and less frequent biomarker disturbances in the female group was unexpected. In fact, only BNP and CK-MB were significantly elevated above baseline in females, whereas males exhibited significant post-race disturbances in BNP, CK-MB, and Cr. Pre-race cTnI assessments were negative ($\leq$ 0.01 ng/mL) in all participants except one male (0.02 ng/mL), and an increase of > 0.01 ng/mL was observed in 5/8 females and 6/8 males, with larger effect sizes in males (0.99 vs 1.18). In marathon runners, Neilan *et al*. (49) reported that the greatest increase in post-race cardiac biomarkers occurred in those athletes training less than 35 miles/wk. Although this would indicate that higher training volumes and better physical condition could be protective in the release of cardiac troponins during and following exercise, George *et al.* found no such relationship in a diverse group of recreational runners (50). Accordingly, the clinical relevance of these modest post-race changes is unclear.

Pre- to post-race SV was 73.0 to 65.2 mL in males (-11.4%; $p = 0.084$, $d = 0.74$) and 63.2 to 61.5 mL in females (-1.4%; $p = 0.744$, $d = 0.11$). Although BNP and cTnI were generally elevated following the race, studies have refuted the notion that these biomarkers reflect cardiomyocyte damage (51). Interestingly, the magnitude of the SV reduction in males was similar to that observed by Scott *et al*. (4) following a 160 km ultramarathon (77 to 64 mL). There are several proposed causes of such post-race decreases, including low-frequency fatigue, the downregulation of cardiac beta-receptors, and decreases in plasma volume (2), although our data exclude this latter mechanism. We can also speculate that the relative post-exercise hypotension observed in males may have influenced cardiac afterload and/or preload.

Following the race, CK-MB concentrations were elevated above normal in both males and females (Fig. 3) and this is considered an indirect marker of muscle damage. Indeed, several ultramarathon studies report significant post-race increases in total creatine kinase (CK) concentrations with values increasing congruent with race distance (52, 53). Some authors consider the muscle damage and metabolic stress associated with ultramarathons to represent a danger to human health (54), causing possible hepatic damage (55), and it may be that there are protective effects of smaller and less frequent CK isoenzyme perturbations following ultra-endurance exercise. We initially speculated that CK-MB concentrations may be associated with peripheral muscle fatigue during ultramarathon; however, previous studies reporting sex differences in peripheral muscle fatigability following short (<60 km) and long (>100 km) distance ultramarathons also showed show no sex differences in post-race CK isoenzyme concentrations when males and females were matched by percent of winning time by sex (56, 57). Accordingly,

any sex differences in peripheral muscle fatigability (14) are likely independent of skeletal muscle damage and/or biomarker levels.

Changes in haematocrit and haemoglobin were used to calculate relative changes in plasma volume. There was a large and significant post-race increase in plasma volume in the male group (21%; $p = 0.043$, $d = 1.36$), whereas the post-race change in females was not significant (7%; $p = 0.143$, $d = 0.61$). The magnitude of the change was almost identical (21 vs. 20%) to that observed by Robach *et al.* in 22 male runners following the UTMB® (58). In that study, the authors speculated that the increase in PV may have resulted from inflammation and an associated interlukin-6-mediated effect on plasma volume expansion. Sex differences in inflammation following ultramarathon have not been comprehensively assessed, but our findings provide some interesting preliminary data that warrant exploration.

Methodological and physiological considerations

The female and male runners in this study were matched for ultramarathon finish time and running velocity (Table 1) because it was deemed that matching the duration of exercise exposure and absolute work rate would be important for comparing the frequency of physiological perturbations. As a result, other aspects of physiological function were unable to be standardized. For example, there will be inherent differences in cardiorespiratory fitness between time-matched females and males, discrepancies that we were unable to quantify. During the race, this may have resulted in the two groups operating at different relative exercise intensities. Other studies comparing physiological functions between male and female ultramarathon runners opted to match groups by relative performance to the first male and the first female of their specific race (57). While this

approach has the advantage that male and female participants would be matched for relative running *ability*, it does not overcome the problem of participants operating at different relative exercise *intensities* and/or metabolic rates. Physiological profiling athletes in future studies would provide clarity in this respect, aid in the interpretation of data, and improve our understanding of the respective male and female ultramarathon performance predictors.

Another consideration is that the remote location of the race necessitated that our extensive laboratory measures were limited to those that could be made using portable/point-of-care devices. More detailed measures of physiological responses (e.g., inflammation, body composition, etc.) would require expensive and fragile equipment to be transported into the field, and this is often impractical. The execution of simulated, lab-based ultramarathon research may be one way of deriving more mechanistic insights in the future. The nature of 'field testing' also made it difficult to perform post-race measurements in a timely fashion because, for instance, the measuring devices could not be situated at the finish line. This required athletes to travel a short distance for their post-race assessments and is a common problem with such studies. Presently, we aimed to retrieve participants for their post-race assessments as soon as possible, with the actual time being 1 h 41 min ± 54 min after finishing the race. Although radiographic findings of mild interstitial oedema have been observed to persist for at least 98 min after endurance exercise (marathon running) (44), comet tails and several of our other measures, including aspects of pulmonary and respiratory muscle function, will have started to recover within a few hours (5). As such, it is possible that there may have been an underestimation of the number and/or magnitude of pre- to post-race physiological changes. Nonetheless, the time in which females and males returned for

post-race assessments was similar, thereby not invalidating a direct comparison of the frequency of between-group perturbations.

Finally, in the present study, we examined sex-specific physiological responses to ultramarathon by comparing the *frequency* of physiological perturbations between males and females. However, although our original data set represents one of the larger samples of its kind among the literature, comprising all female participants from an initial mixed-sex cohort of 53 athletes who contested the event over two years, the relatively small sample size (and the large within-group variance) precluded any direct male-to-female comparisons on the *magnitude* of the response. Based on the data reported herein, a power analysis was performed (G*Power version 3.1.9.6) to determine the sample size that would be required to observe a statistically significant between-group interaction (should one exist) in future studies using a repeated-measures design. Based on an alpha level of 0.05 and a statistical power of 0.8, a total of 32 participants (16 per group) would likely be required where moderate between-group effect sizes are observed (e.g., most biomarker comparisons), although slightly smaller samples sizes would likely be acceptable in the case of larger between-group effects (e.g., diffusing capacity and comet tails). We hope this will inform future research on sex differences in physiological variables in response to ultramarathon.

CONCLUSIONS

Ultramarathon evokes considerable physical stress on multiple body systems, as evidenced by significant pre- to post-race disturbances in numerous aspects of physiological function. In males and females matched for ultramarathon finish time, it was male athletes who exhibited more

frequent perturbations, and with larger effect sizes, most notably in lung diffusing capacities and in biomarkers of skeletal muscle, cardiac, and renal function. These data may inform training prescription and future research on long-term health and injury management in ultramarathon.

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Conflict of Interest

The authors declare no conflict of interest. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of the present study do not constitute endorsement by the American College of Sports Medicine. NBT is supported by a postdoctoral fellowship from the Tobacco-Related Disease Research Program (TRDRP; award no. T31FT1692). GMS is supported by the American Heart Association (AHA#19POST34450022) and a Career Development Award in Cardiovascular Disease Research Honouring Dr. Earl H. Wood from Mayo Clinic.

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FIGURE LEGENDS

Figure 1. Course profiles for the Ultra-Trail du Mont-Blanc (UTMB®; panel A) and the CCC® (panel B). The CCC® begins at 78 km into the UTMB® course (at Courmayeur) and the two races follow a similar, although not identical, route thereafter.

Figure 2. Illustration of testing procedures.

Figure 3. Pre- to post-race changes in haemoglobin (panel A), haematocrit (panel B), troponin I (panel C), brain neuropeptide (panel D), creatinine (panel E), and creatine kinase-MB (panel F) in females (□) and males (■). † = statistically significant overall (n=16) change from baseline; *p* = *p*-value from independent- or paired-samples *t*-test; *d* = Cohen's *d* effect size; *statistically significant within-group (n=8) difference (Benjamini-Hochberg-adjusted p-value). For clarity of presentation, data are presented as mean and standard error of the mean.

Figure 4. Pre- to post-race changes in forced expiratory volume in 1-second (panel A), peak expiratory flow (panel B), inspiratory capacity (panel C), maximum inspiratory pressure (panel D), exhaled NO (panel E), diffusing capacity for CO (panel F), diffusing capacity for NO (panel G), and alveolar capillary volume (panel H) in females (□) and males (■). † = statistically significant overall (n=16) change from baseline; *p* = *p*-value from independent- or paired-samples *t*-test; *d* = Cohen's *d* effect size; *statistically significant within-group (n=8) difference (Benjamini-Hochberg-adjusted p-value). For clarity of presentation, data are presented as mean and standard error of the mean.

SUPPLEMENTAL DIGITAL CONTENT

SDC 1: Supplementary table.docx - Pre- and post-race physiological responses in males and females

Figure 1

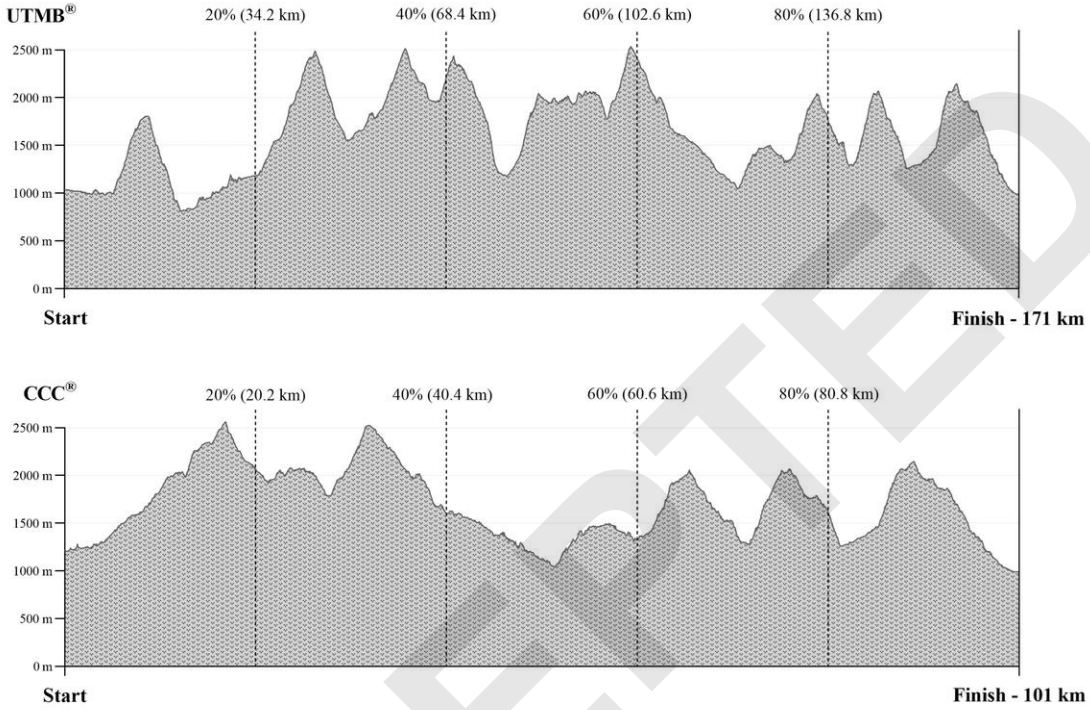

Figure 2

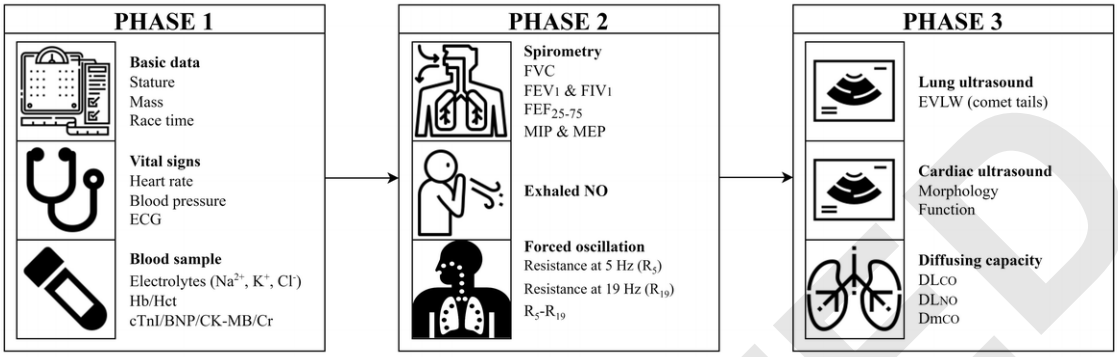

Figure 3

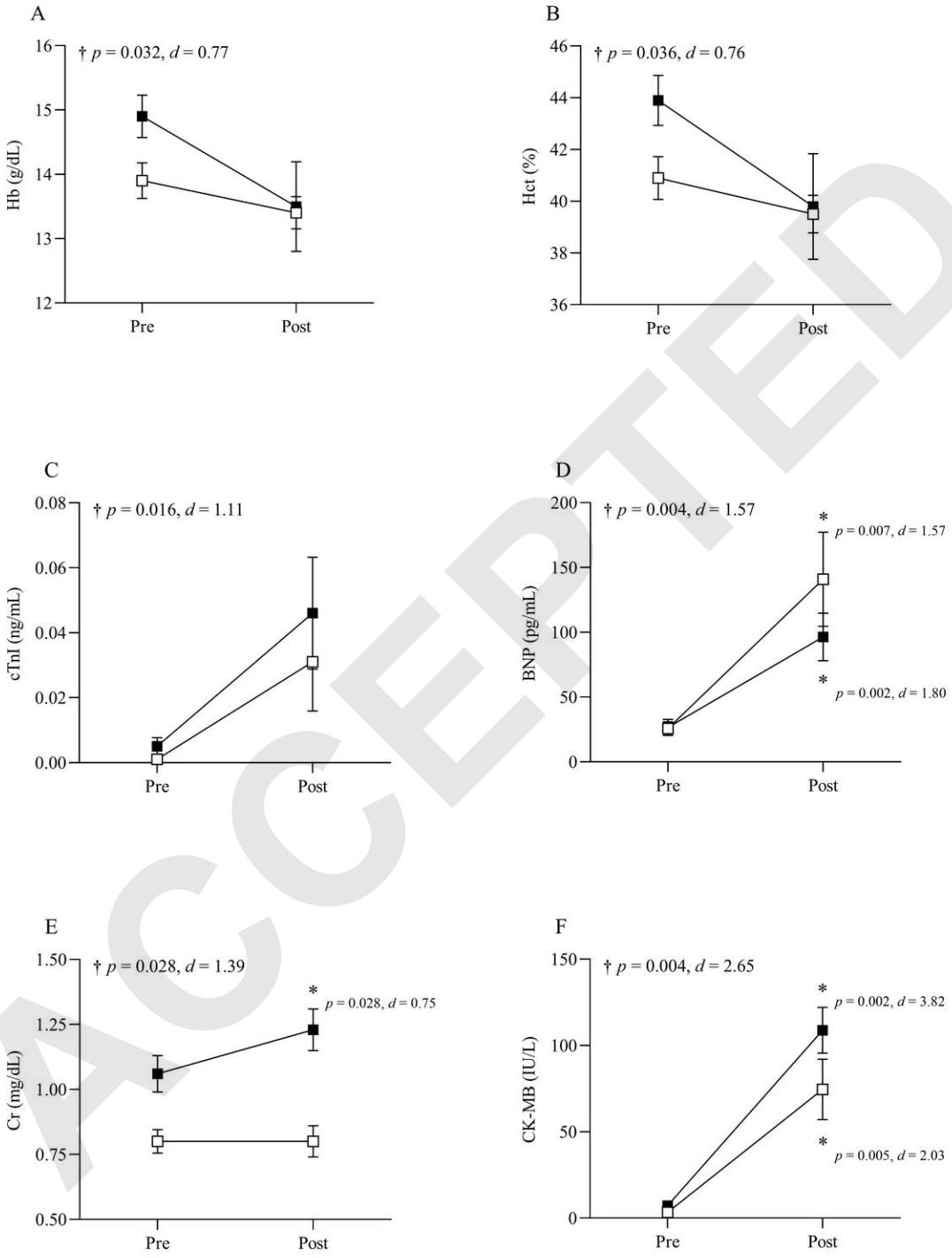

Figure 4

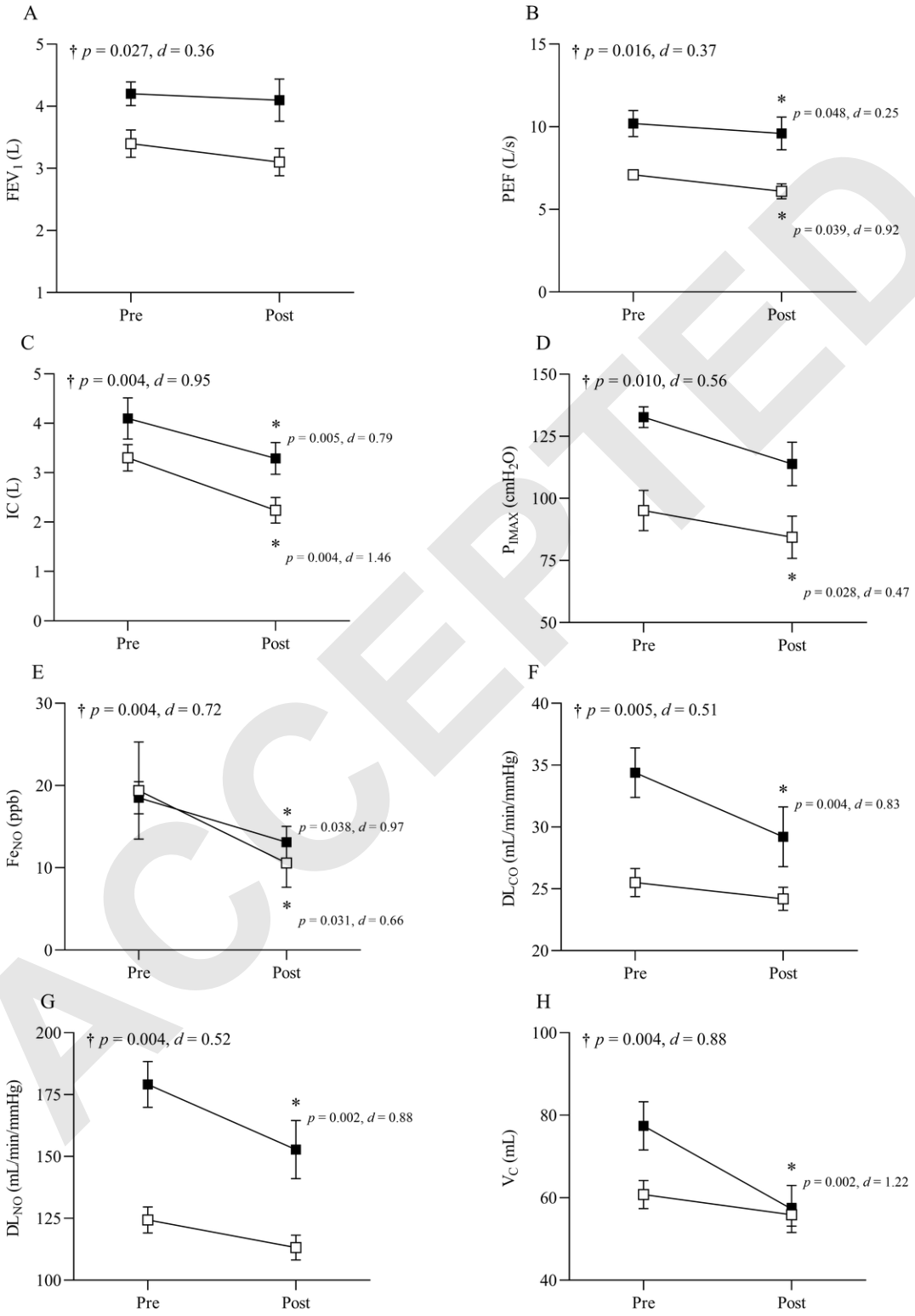

Table 1. Participant demographics and race data.

	Overall (n=16)	Females (n=8)	Males (n=8)	*p*	*d*
Age (y)	38.4 ± 7.6	36.6 ± 6.9	40.3 ± 8.3	0.361	0.48
Stature (cm)	171.3 ± 6.3	167.1 ± 5.3	175.5 ± 4.0	0.003*	1.79
Mass (kg)	63.9 ± 9.0	56.9 ± 6.1	71.0 ± 4.6	0.004*	2.58
Finish time (h:min)	30:52 ± 10:42	30:57 ± 11:36	30:46 ± 10:32	0.975	0.02
UTMB®	39:56 ± 06:42	40:24 ± 06:49	39:28 ± 07:34	0.860	0.12
CCC®	21:48 ± 03:33	21:30 ± 05:24	22:05 ± 00:19	0.837	0.13
Velocity (m/s)	1.2 ± 0.2	1.2 ± 0.3	1.2 ± 0.1	0.762	0.00
UTMB®	1.1 ± 0.1	1.1 ± 0.0	1.1 ± 0.1	0.425	0.00
CCC®	1.3 ± 0.2	1.4 ± 0.4	1.3 ± 0.0	0.615	0.35

Mean ± SD; *p* = independent-samples *t*-test; *d* = Cohen's *d* effect size.

Table 2. Baseline physiological comparisons.

	Females (n=8)			Males (n=8)			*P*	*d*
Vital Signs								
f_C (beats/min)	57	±	7	50	±	9	0.129	0.81
SBP (mmHg)	107	±	7	122	±	11	0.011*	1.69
DBP (mmHg)	73	±	8	76	±	7	0.303	0.66
Blood Sampling								
Na^{2+} (mmol/L)	138.4	±	1.3	141.0	±	1.5	0.008*	1.87
K^+ (mmol/L)	4.0	±	0.4	3.9	±	0.3	0.775	0.30
Cl^- (mmol/L)	103.5	±	3.3	104.0	±	2.1	0.943	0.19
Hb (g/dL)	13.9	±	0.8	14.9	±	0.9	0.057	1.12
Hct (%)	40.9	±	2.4	43.9	±	2.7	0.045*	1.18
PV (L)	2.7	±	0.2	3.1	±	0.1	0.004*	2.53
cTnI (ng/mL)	0.001	±	0.004	0.005	±	0.008	0.233	0.68
BNP (pg/mL)	25.8	±	14.6	26.6	±	17.5	0.971	0.05
Cr (mg/dL)	0.8	±	0.1	1.1	±	0.2	0.012*	1.79
CK-MB (IU/L)	3.3	±	2.4	7.2	±	3.9	0.039*	1.25
Pulmonary Function								
FVC (L)	4.3	±	0.6	5.4	±	0.7	0.010*	1.67
FEV_1 (L)	3.4	±	0.6	4.2	±	0.5	0.028*	1.40
FEV_1/FVC	79.9	±	7.1	78.9	±	6.4	0.801	0.14
PEF (L/s)	7.1	±	0.8	10.2	±	2.2	0.012*	2.05
FEF_{25-75} (L)	3.3	±	1.1	3.9	±	0.9	0.496	0.61
IC (L)	3.3	±	0.8	4.1	±	1.2	0.117	0.81
FIV_1 (L)	2.5	±	0.7	4.2	±	0.8	0.004*	2.22
R_5 (cmH_2O/L/s)	3.2	±	1.2	2.0	±	0.4	0.128	1.43
R_5-R_{19} (cmH_2O/L/s)	-0.24	±	0.27	0.00	±	0.20	0.232	1.05
Fe_{NO} (ppb)	19.4	±	16.7	18.5	±	5.6	0.619	0.08
DL_{CO} (mL/min/mmHg)	25.5	±	3.2	34.4	±	5.7	0.008*	2.00
$DL_{CO,HbCorr}$ (mL/min/mmHg/g/dL)	25.1	±	3.2	34.2	±	5.7	0.008*	1.96
DL_{CO}/VA (mL/min/mmHg/L)	4.9	±	0.6	4.7	±	1.0	1.000	0.16
DL_{NO} (mL/min/mmHg)	124.4	±	15.0	179.1	±	26.2	0.001*	2.66
DM_{CO} (mL/min/mmHg)	118.4	±	18.3	338.5	±	447.5	0.108	0.94
V_C (mL)	60.8	±	9.7	77.4	±	16.7	0.039*	1.26
P_{IMAX} (cmH_2O)	95.1	±	22.8	132.7	±	11.7	0.020*	2.17
P_{EMAX} (cmH_2O)	117.1	±	22.8	202.5	±	28.9	0.004*	3.31
Transthoracic Ultrasound								
Lung comet Tails (n)	0.8	±	1.4	2.4	±	2.2	0.081	0.91
SV (mL)	63.2	±	14.2	73.0	±	11.9	0.209	0.75
$\dot{Q}$ (L/min)	3.6	±	0.8	3.6	±	0.7	0.787	0.13

Mean ± SD. f_C = cardiac frequency (heart rate); SBP = systolic blood pressure; DBP = diastolic blood pressure; Na^{2+} = sodium concentration; K^+ = potassium concentration; Cl^- = chloride concentration; Hb = haemoglobin concentration; Hct = haematocrit; PV = plasma volume; cTnI = cardiac troponin-1; BNP = brain natriuretic peptide; Cr = creatinine; CK-MB = creatine kinase; FVC = forced vital capacity; FEV_1 = forced expiratory volume in 1-second; PEF = peak expiratory flow; FEF_{25-75} = forced expiratory flow between 25 and 75% of FVC; IC = inspiratory capacity; FIV_1 = forced inspiratory volume in 1-second; R_5 = airway resistance at 5 Hz; R_5-R_{19} = airway resistance at 5 Hz minus resistance at 19 Hz (small airways); Fe_{NO} = exhaled nitric oxide; DL_{CO} = diffusing capacity of the lung for carbon monoxide; $DL_{CO,HbCorr}$ = diffusing capacity of the lung for carbon monoxide corrected to reference haemoglobin concentrations; DL_{CO}/VA = diffusing capacity of the lung for carbon monoxide relative to alveolar volume; DL_{NO} = diffusing capacity of the lung for nitric oxide; DM_{CO} = diffusing capacity of the pulmonary membrane for carbon monoxide; V_C = pulmonary capillary blood volume; P_{IMAX} = maximum inspiratory pressure; P_{EMAX} = maximum expiratory pressure; SV = stroke volume; $\dot{Q}$ = cardiac output. *p* = *p*-value from independent-samples *t*-test; *d* = Cohen's *d* effect size; *statistically significant between-group difference (Benjamini-Hochberg-adjusted *p*-value).

Supplementary table. Pre- and post-race physiological responses in males and females.

	Females (n=8)				Males (n=8)			
	Pre-race	**Post-race**	***P***	***d***	**Pre-race**	**Post-race**	***P***	***d***
Body mass & vital signs								
Mass (kg) †	56.9 ± 6.1	55.8 ± 5.9	0.027*	0.17	71.0 ± 4.6	69.4 ± 5.2	0.027*	0.32
f_C (beats/min) †	57 ± 7	73 ± 15	0.012*	1.43	50 ± 9	62 ± 7	0.053	1.43
SBP (mmHg) †	107 ± 6	105 ± 10	0.500	0.24	122 ± 11	106 ± 10	0.008*	1.53
DBP (mmHg)	72 ± 8	71 ± 12	0.781	0.12	77 ± 8	71 ± 7	0.344	0.80
Blood Sampling								
Na^{2+} (mmol/L)	138.4 ± 1.3	137.6 ± 1.9	0.490	0.46	141.0 ± 1.5	140.2 ± 2.2	0.580	0.33
K^+ (mmol/L)	4.0 ± 0.4	3.3 ± 0.8	0.122	1.20	3.9 ± 0.3	3.9 ± 0.4	0.690	0.19
Cl^- (mmol/L)	103.5 ± 3.3	103.5 ± 2.5	0.984	0.00	104.0 ± 2.1	106.1 ± 2.1	0.256	1.00
Hb (g/dL) †	13.9 ± 0.8	13.4 ± 0.7	0.164	0.66	14.9 ± 0.9	13.5 ± 2.0	0.052	0.90
Hct (%) †	40.9 ± 2.4	39.5 ± 2.1	0.196	0.62	43.9 ± 2.7	39.8 ± 5.8	0.052	0.91
PV (L) †	2.7 ± 0.2	2.9 ± 0.4	0.143	0.61	3.1 ± 0.1	3.7 ± 0.6	0.043*	1.36
cTnI (ng/mL) †	0.001 ± 0.004	0.031 ± 0.043	0.117	0.99	0.005 ± 0.008	0.046 ± 0.049	0.060	1.18
BNP (pg/mL) †	25.8 ± 14.6	140.9 ± 102.7	0.007*	1.57	26.6 ± 17.5	96.4 ± 51.9	0.002*	1.80
Cr (mg/dL) †	0.8 ± 0.1	0.8 ± 0.2	0.504	0.24	1.1 ± 0.2	1.2 ± 0.2	0.028*	0.75
CK-MB (IU/L) †	3.3 ± 2.4	74.6 ± 49.6	0.005*	2.03	7.2 ± 3.9	108.8 ± 37.4	0.002*	3.82
Pulmonary Function								
FVC (L) †	4.3 ± 0.6	3.8 ± 0.6	0.008*	0.79	5.4 ± 0.7	5.3 ± 0.8	0.636	0.14
FEV_1 (L) †	3.4 ± 0.6	3.1 ± 0.6	0.052	0.54	4.2 ± 0.5	4.1 ± 0.9	0.337	0.24
FEV_1/FVC	79.9 ± 7.1	80.8 ± 5.3	0.800	0.14	78.9 ± 6.4	76.2 ± 10.1	1.000	0.33
PEF (L/s) †	7.1 ± 0.8	6.1 ± 1.3	0.039*	0.92	10.2 ± 2.2	9.6 ± 2.6	0.048*	0.25
FEF_{25-75} (L)	3.3 ± 1.1	3.0 ± 1.0	0.333	0.29	3.9 ± 0.9	3.6 ± 1.2	0.292	0.31
IC (L) †	3.3 ± 0.8	2.2 ± 0.7	0.004*	1.46	4.1 ± 1.2	3.3 ± 0.9	0.005*	0.79
FIV_1 (L)	2.5 ± 0.7	2.4 ± 0.5	0.607	0.19	4.2 ± 0.8	3.8 ± 0.6	0.200	0.58
R_5 (cmH_2O/L/s)	3.2 ± 1.2	3.6 ± 1.7	0.455	0.28	2.0 ± 0.4	2.2 ± 0.7	0.325	0.46
R_5-R_{19} (cmH_2O/L/s)	-0.24 ± 0.27	-0.08 ± 0.23	0.213	0.66	0.00 ± 0.20	0.05 ± 0.17	0.368	0.26
Fe_{NO} (ppb) †	19.4 ± 16.7	10.6 ± 8.4	0.031*	0.66	18.5 ± 5.6	13.1 ± 5.5	0.038*	0.97
DL_{CO} (mL/min/mmHg) †	25.5 ± 3.2	24.2 ± 2.5	0.328	0.45	34.4 ± 5.7	29.2 ± 6.9	0.004*	0.83

$DL_{CO,HbCorr}$ (mL/min/mmHg/g/dL)	25.1 ± 3.2	24.3 ± 2.4	0.550	0.30	34.2 ± 5.7	30.5 ± 7.8	0.090	0.54
DL_{CO}/VA (mL/min/mmHg/L)	4.9 ± 0.6	4.9 ± 0.7	0.981	0.00	4.7 ± 1.0	4.6 ± 1.4	1.000	0.11
DL_{NO} (mL/min/mmHg) †	124.4 ± 15.0	113.2 ± 13.3	0.064	0.79	179.1 ± 26.2	152.8 ± 33.4	0.002*	0.88
DM_{CO} (mL/min/mmHg)	118.4 ± 18.3	105.0 ± 12.6	0.106	0.86	338.5 ± 447.5	239.0 ± 87.4	0.924	0.31
V_C (mL) †	60.8 ± 9.7	55.9 ± 7.3	0.179	0.57	77.4 ± 16.7	57.3 ± 16.1	0.002*	1.22
P_{IMAX} (cmH_2O) †	95.1 ± 22.8	84.4 ± 22.4	0.028*	0.47	132.7 ± 11.7	113.9 ± 23.4	0.071	1.02
P_{EMAX} (cmH_2O)	117.1 ± 22.8	105.6 ± 20.7	0.147	0.53	202.5 ± 28.9	174.1 ± 54.3	0.193	0.65
Transthoracic Ultrasound								
Lung comet Tails (n) †	0.8 ± 1.4	2.9 ± 2.8	0.048*	0.96	2.4 ± 2.2	8.3 ± 2.7	0.006*	2.41
SV (mL)	63.2 ± 14.2	61.5 ± 17.4	0.744	0.11	73.0 ± 11.9	65.2 ± 9.1	0.084	0.74
$\dot{Q}$ (L/min) †	3.6 ± 0.8	4.4 ± 1.2	0.048*	0.80	3.6 ± 0.7	4.0 ± 0.5	0.177	0.70

Mean ± SD. f_C = cardiac frequency (heart rate); SBP = systolic blood pressure; DBP = diastolic blood pressure; Na^{2+} = sodium concentration; K^+ = potassium concentration; Cl^- = chloride concentration; Hb = haemoglobin concentration; Hct = haematocrit; PV = plasma volume; cTnI = cardiac troponin-1; BNP = brain natriuretic peptide; Cr = creatinine; CK-MB = creatine kinase; FVC = forced vital capacity; FEV_1 = forced expiratory volume in 1-second; PEF = peak expiratory flow; FEF_{25-75} = forced expiratory flow between 25 and 75% of FVC; IC = inspiratory capacity; FIV_1 = forced inspiratory volume in 1-second; R_5 = airway resistance at 5 Hz; R_5-R_{19} = airway resistance at 5 Hz minus resistance at 19 Hz (small airways); Fe_{NO} = exhaled nitric oxide; DL_{CO} = diffusing capacity of the lung for carbon monoxide; $DL_{CO,HbCorr}$ = diffusing capacity of the lung for carbon monoxide corrected to reference haemoglobin concentrations; DL_{CO}/VA = diffusing capacity of the lung for carbon monoxide relative to alveolar volume; DL_{NO} = diffusing capacity of the lung for nitric oxide; DM_{CO} = diffusing capacity of the pulmonary membrane for carbon monoxide; V_C = pulmonary capillary blood volume; P_{IMAX} = maximum inspiratory pressure; P_{EMAX} = maximum expiratory pressure; SV = stroke volume; $\dot{Q}$ = cardiac output. † = statistically significant overall (n=16) change from baseline; *p* = *p*-value from paired-samples *t*-test; *d* = Cohen's d effect size; *statistically significant within-group (n=8) change from baseline (Benjamini-Hochberg-adjusted *p*-value).