Mechanisms of orthostatic intolerance following very prolonged exercise

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^1School of Physical Education and ^2Department of Physiology, University of Otago, Dunedin, New Zealand; ^3Department of Chemical Pathology, St. George’s Healthcare National Health Service Trust, Tooting, London; and ^4Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, United Kingdom

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Lucas SJ, Cotter JD, Murrell C, Wilson L, Anson JG, Gaze D, George KP, Ainslie PN. Mechanisms of orthostatic intolerance following very prolonged exercise. J Appl Physiol 105: 213–225, 2008. First published May 15, 2008; doi:10.1152/japplphysiol.00175.2008.—Nine men completed a 24-h exercise trial, with physiological testing sessions before (T1, ~0630), during (T2, ~1640; T3, ~0045; T4, ~0630), and 48-h afterwards (T5, ~0650). Participants cycled and ran/trekced continuously between test sessions. A 24-h sedentary control trial was undertaken in crossover order. Within testing sessions, participants lay supine and then stood for 6 min, while heart rate variability (spectrum analysis of ECG), middle cerebral artery perfusion velocity (MCAv), mean arterial pressure (MAP, Finometer), and end-tidal Pco2 (PetCO2) were measured, and venous blood was sampled for cardiac troponin I. During the exercise trial: 1) two, six, and four participants were orthostatically intolerant at T2, T3, and T4, respectively; 2) changes in heart rate variability were only observed at T2; 3) supine MAP (baseline = 81 ± 6 mmHg) was lower (P < 0.05) by 14% at T3 and 8% at T4, whereas standing MAP (75 ± 7 mmHg) was lower by 16% at T2, 37% at T3, and 15% at T4; 4) PetCO2 was reduced (P < 0.05) at all times while supine (~3–4 Torr) and standing (~4–5 Torr) during exercise trial; 5) standing MCAv was reduced (P < 0.05) by 23% at T3 and 30% at T4 during the exercise trial; 6) changes in MCAv with standing always correlated (P < 0.01) with changes in PetCO2 (r = 0.78–0.93), but only with changes in MAP at T1, T2, and T3 (P < 0.05; r = 0.62–0.84); and 7) only two individuals showed minor elevations in cardiac troponin I. Recovery was complete within 48 h. During prolonged exercise, postural-induced hypotension and hypacapnia exacerbate cerebral hypoperfusion and facilitate syncope. Syncope; hypotension; cerebrovascular function

INCREASED SYMPATHETIC ACTIVITY and reduced vagal activity following prolonged exercise have been observed in athletes competing in events ranging from 4 to >100 h (2, 4, 16, 29). Despite elevations in sympathetic activity, low arterial blood pressure (BP) (hypotension) is also observed during recovery (16, 29). Such changes in reflex autonomic mechanisms affect hemodynamic responses to orthostasis. Indeed, loss of consciousness (syncope) following prolonged endurance exercise (marathon and ultramarathon races) has been reported (16, 19). The role of the autonomic nervous system combined with cardio- and cerebrovascular measures and their integrated function during orthostasis have been examined only after ~4 h of exercise (29). No studies have explored the related changes in autonomic and hemodynamic function across more prolonged exercise, where the volume of exercise and cardiovascular stress may exacerbate the risk of syncope.

The maintenance of cerebral blood flow (CBF) during physiological challenges such as hypotension and orthostasis is of critical importance to maintaining brain function and avoiding cerebral hypoxia and syncope (58). Mean arterial BP (MAP), cardiac output (Q), and hypacapnia are important determinants of cerebral perfusion (58). On standing, MAP initially falls due to a drop in total peripheral vascular resistance (TPR) and stroke volume (SV) (51). Consequently, there is an immediate drop in parasympathetic activity (vagal withdrawal), and sympathetic activity increases to elevate heart rate (HR) and constrict arterioles and veins (to increase TPR, venous return, and Q) to restore MAP. The pronounced hypotension during standing after prolonged exercise seems to be due to an inadequate compensatory mechanism, due to failure of either arteriolar constriction (17) or venous constriction (14, 21, 55), or both, to increase Q. Impairment in myocardial contractility following prolonged exercise (2) may also contribute to decreased Q, although this linkage has yet to be established. Any impairment in the maintenance of Q can compromise cerebral perfusion (29, 33, 34) and ultimately lead to syncope, if perfusion is sufficiently compromised.

It is also possible that postural-induced hyperventilation and related hypacapnia may exacerbate cerebral vasoconstriction and potentially “trigger” syncope (42). It is unknown, however, how very prolonged exercise may collectively influence these cardiorespiratory and cerebrovascular responses to orthostatic stress. Therefore, the primary aim of this study was to measure hemodynamic (MAP, HR, Q, and CBF velocity), respiratory [end-tidal Pco2 (PetCO2) and P02 (PETO2)], and autonomic function during orthostatic stress before, during, and after 24 h of exercise. In addition, studies have demonstrated the appearance of biomarkers of cardiomyocyte damage in the systemic circulation after prolonged exercise, indicating that strenuous physical exertion may result in myocardial injury (30, 47, 57). Thus a secondary aim was to assess circulating levels of cardiac troponin I (cTnI), a highly specific cardiomyocyte biomarker, during and after a 24-h trial of endurance exercise and investigate a potential association with an impaired maintenance of Q. It was hypothesized that, compared with a time-matched control trial of no exercise; 1) increased sympathetic activity and reduced vagal activity would be observed during supine rest, but elevations in sympathetic activity during an orthostatic challenge would be progressively reduced across the 24-h exercise trial; 2) hypotension would be observed at all testing points, and exaggerated during postural change, during the 24-h exercise trial; 3) CBF velocity would

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be maintained while resting supine, but, in relation to posture-induced hypotension and hypocapnia, would be reduced to a greater extent during an orthostatic challenge across the 24-h exercise trial, coinciding with greater occurrences of syncope; and 4) cTnI release would be limited, likely due to the prolonged duration and relatively low intensity of exercise and thus would not be associated with the postexercise control of Q.

METHODS

Participants. Nine (mean ± SD) healthy male endurance athletes (age, 30 ± 8 yr; mass, 77.2 ± 8.5 kg; running peak O2 consumption, 61.6 ± 4.7 ml·kg⁻¹·min⁻¹) volunteered for this study, which was approved by the University of Otago Ethics Committee. Participants were recruited on the basis that they had previously competed in at least one ultraendurance event of 24 h or longer. Participants were informed of the experimental procedures and possible risks involved in the study before their written, informed consent was obtained. None of the participants was taking any medications, all were nonsmokers, and none had any history of cardiovascular, cerebrovascular, or respiratory disease.

Experimental design. A crossover design was used. Participants completed a 24-h exercise and physiological testing trial and a 24-h sedentary control and physiological testing trial, separated by at least 14 days. With one exception, participants exercised in pairs, and the order of exercise and control was pseudorandomized so that, within each pair, it was reversed. One participant exercised with a training partner who was not part of the testing. For the exercise trial, participants reported to the laboratory at 0538 ± 11 min, and baseline testing began at 0630 ± 32 min. Repeat tests occurred three times across the 24-h trial (T2, 1637 ± 37 min; T3, 0046 ± 40 min; T4, 0633 ± 28 min), and 48 h following completion of exercise (T5, 0648 ± 36 min, n = 7). Exercise was cycling (mountain biking; T1–T2 and T3–T4; 122 ± 8 and 50 ± 9 km, respectively) and running/trekking (T2–T3; 33 ± 10 km) predominantly on off-road terrain within close proximity to the laboratory. Participants were not permitted to sleep during the exercise trial. The purposes of using mixed mode and terrain exercise with fitness-matched pairs were as follows: 1) to maximize participants’ motivation and ability to maintain their exercise intensity and thus cardiovascular stress across 24 h; and 2) to simulate the demands of adventure racing, which typically involves competitions of at least this duration. For the control trial, testing session start times for T1, T2, T3, and T4 were 0635 ± (46 min), 1508 ± (247 min), 2252 ± (44 min), and 0630 ± (47 min), respectively. Participants’ start time for experimental testing was determined by the participants’ exercise trial pair order, i.e., the first participant in each pair started testing ~45 min before the second participant for those sessions. Participants completed normal daily sedentary activities across the control protocol, except for 10 min of fast walking and 6 min of jogging within each testing session (completed after measures reported here), and sleep was permitted between testing sessions T3 and T4. Alcohol and caffeine were prohibited in the 12 h before testing sessions and throughout each of the control and exercise protocols.

Measurements and protocol. All tests and measures at testing sessions T1, control T4, and T5 were undertaken in a rested (>12 h) and fasted (>6 h) state. For testing sessions T2, T3, and exercise T4, participants were instructed not to consume foods or macronutrient-containing fluids from >1 h before arriving back at the laboratory. On arrival at the laboratory for each session, participants lay supine for 15 min before a venous blood sample was drawn for analysis of a biomarker of cardiomyocyte damage (cTnI) and plasma volume shifts (12).

Exercise intensity across the 24-h trials of exercise and control was measured at 1-min intervals from telemetry of the R-wave-to-R-wave interval (RR1) (Polar S810i monitors, Polar, Finland). Exercise intensity was calculated as percentage of HR range [%HR range = (HR – HRmin)/(HRmax – HRmin) × 100], where HRmax was the lowest HR recorded across the control trial, and HRmax was determined using an incremental treadmill test to maximum exertion, conducted at completion of the control trial.

Measurements of CBF velocity, arterial BP, and HR. During each session, blood flow velocity in the middle cerebral artery (MCAv), arterial BP, PetCO2, PetO2, and electrocardiography (ECG) were recorded continuously. Right MCAv was measured using a 2-MHz pulsed Doppler ultrasound system (DWL Doppler, Sterling, VA). Beat-to-beat BP was measured by finger photoplethysmography (Finometer, TPD Biomedical Instruments). From the BP waveform, HR, SV, and Q were calculated using the model flow method, which incorporates sex, age, height, and mass data (BeatScope 1.0 software, TNO, TPD Biomedical Instruments). This method provides a reliable estimate of changes in Q at rest and during exercise in healthy young human adults (52). Although photoplethysmographic measurements correlate well with intra-arterial measurements during experimental manipulations of arterial pressure (38), the absolute values can sometimes be inaccurate; therefore, BP data were normalized to a 3-min baseline preceding the stand, and systolic and diastolic values are generally expressed as change from this baseline. Similarly, as used in other studies (29), MCAv was also expressed as the percent change from this baseline to enable the same relative comparison to the changes in MAP, and to reduce interindividual variability that is unrelated to the experimental manipulation (i.e., subtle changes in the angle of insonation of the Doppler probe) (16, 29). In an attempt to minimize the test-to-test variance, the same settings (i.e., depth and gain) of the Doppler probe were used across all trials for each participant. In our laboratory, the day-to-day reproducibility (coefficient of variation) of MCAv is 4.5%; this variation is consistent across the CO2 ranges used in this study (11). Participants breathed through a one-way non-rebreathing valve (Hans-Rudolph 2700). The fractional concentrations of O2 and CO2 were measured continuously (model CD-3A, AEI Technologies, Pittsburgh, PA), and converted to end-tidal partial pressures. HR was recorded using three-lead ECG via a BioAmp (Model ML132, ADInstruments, Colorado Springs, CO). The beat-to-beat assessment of HR and BP was used for HR variability (HRV) and baroreceptor sensitivity (BRS) analysis (described below). All data were sampled at 200 Hz throughout each session using an analog-digital converter (Powerlab/16SP ML795; ADInstruments) interfaced with a computer and displayed in real time. Data were later analyzed using commercially available software (Chart version 5.4.2, ADInstruments). TPR was calculated as MAP/Q.

Orthostatic tolerance test. Following at least 25 min of supine rest, participants quickly (within ~3 s) assumed a standing posture, which they maintained for 6 min. MCAv, BP, HR, and ECG were monitored continuously, as above. This 6-min stand was truncated if participants showed signs of syncope (e.g., continuous decline in diastolic BP, wanted to sit down, dizziness, nausea), whereupon the subject was returned to a supine position.

HRV and BRS. Power spectral analysis of the beat-to-beat variability of HR was obtained by the autoregressive method. Specific characteristics of the power spectrum of HRV were used as an index of sympathetic and parasympathetic control of the cardiovascular system (53). Two frequency bands were considered: LF (0.05–0.15 Hz) and high frequency (HF, 0.15–0.30 Hz). Power of the RRI spectra (LF-RRI and HF-RRI, respectively) was calculated from the integration of the autospectra. While the limitations of spectral analysis of HRV should be emphasized (27, 39, 40), the method provides an index of the dynamic, frequency-dependent changes in HR, which reflects autonomic modulation of sinus node activity (1, 36). HF power of RRI variability appears to be modulated predominantly by respiration-induced changes in vagal activity, whereas LF power is modulated by both vagal and sympathetic activity (1, 36, 53). HF and LF values at each specific frequency range were also normalized by dividing the total spectral power (36) to...
minimize the effect of the changes in total power on the LF and HF components. This data acquisition and processing strategy conforms to consensus panel recommendations for the assessment of cardiovascular variability (53).

While supine, participants were asked to control their respiratory frequency (controlled breathing) at a fixed rate of 12 breaths/min (0.2 Hz). This controlled rate was used across all testing sessions, since breathing frequency alone can affect HRV, independent of changes in arterial $PCO_2$ (43). After a 3-min adjustment period, 3 min of steady-state data were recorded for this controlled respiratory period. Participants were then instructed to “breathe normally” (normal breathing), and, after venous blood sampling and a 3-min adjustment period, 3 min of uncontrolled (normal) respiration data were collected. Steady-state data from both the controlled and normal respiration protocols were used for spectral HRV and BRS analysis.

Time-domain analysis of spontaneous BRS data was obtained from the Finometer BP waveform using the cross-correlation method PRVXBRS (60). The systolic BP (SBP) and interbeat interval (IBI) time series were interpolated and resampled at 1 Hz. In 10-s windows, the correlation and regression slopes between SBP and IBI were computed. Delays of 0- to 5-s increments in IBI were computed, and the delay with the highest positive coefficient of correlation was selected; the optimal delay ($\tau$) was stored. The slope between SBP and IBI was then recorded as a BRS estimate, if the correlation was significant at $P = 0.01$. In addition to the 3 min of steady-state data collection during controlled and normal breathing, HRV and BRS data were also averaged over the final 2 min of upright standing.

cTnl analysis. Whole venous blood (5 ml) was collected in serum-gel vacutainer tubes and allowed to clot. After centrifugation, serum was aliquoted, frozen, and stored at $-80^\circ$C for later analysis. The quantitative assessment of cTnl was made using the Tnl-Ultra ADVIA Centaur assay (Siemens Medical Solutions Diagnostics, Surrey, UK). The Tnl-Ultra assay is a three-site sandwich immunoassay using direct chemilumimetric technology. The assay coefficient of variation was 20% (functional sensitivity) at 0.015 g/l and 10% at 0.03 g/l. The calibration range was 0.02–50 g/l. The upper reference limit of normal (99th centile) of an apparently healthy population was 0.04 g/l.

Data analysis. A repeated-measures ANOVA was used to test significance between and within conditions for each dependent variable (SPSS 15.0, SPSS). Pairwise comparisons (Bonferroni corrected) were used to examine significant interactions and/or main effects. A priori comparison to baseline (T1) was conducted as planned comparison, regardless of ANOVA outcome (23).

Absolute and percent (%) change from baseline (supine normal breathing) were calculated using the periods defined above. Because of a marked and prevalent orthostatic intolerance during the exercise trial (T2–T4), the final 2-min period of standing data often preceded the 4- to 6-min period. If syncope occurred within 2.5 min at standing, data involving the transient initial hypotension upon standing were excluded. Data were also averaged in 30-s blocks over the 6-min stand and compared with baseline. Within- (compared to baseline) and between-condition (compared to matched control) differences were assessed with paired t-tests of the area under the curve (as described by Ref. 44). Syncopeal symptomatic participants were time matched across all trials. In addition, Pearson correlations were done between the relative change in MCAv and MAP, as well as with absolute $PETCO_2$, and absolute change in $PETCO_2$. Significance for all tests was established at an $\alpha$-level of $P < 0.05$, and data are expressed as means ± SD.

RESULTS

Exercise intensity and duration. The start time for all participants for the first field-exercise stage was 0900 (±18 min). The first mountain biking stage lasted 7 h 2 min (±12 min), with HR averaging 65% [95% confidence interval (CI): 62–69%] of HR range (Fig. 1A). The jogging/trekking stage began at 1855 (±34 min) and took 5 h 11 min (±32 min) to complete, with HR averaging 48% (43–52%) of HR range. Start time for the final exercise stage was 0239 (±32 min) and was 3 h 11 min in duration, with HR averaging 45% (40–51%) of HR range. Participants had no sleep during the exercise trial (verified by HR profiles). Recorded ambient temperature during the exercise trials was between 4.2 and 18.1°C. HRs (Fig. 1B) averaged 20% (95% CI: 17–24%) of HR range for the first control sedentary period, 18% (15–21%) of HR range for the second sedentary period, and 9% (8–10%) of HR range for the final sedentary period. The HR was lower during all three sedentary periods than in their corresponding exercise periods (all $P < 0.01$). Participants slept 4 h 57 min (±43 min) between testing sessions T3 and T4 during the control trial.

Orthostatic tolerance. During the exercise trial, the 6-min orthostatic challenge (active standing) could not be completed by one participant at T1, two participants at T2, six participants at T3, and four participants at the T4 testing session due to presyncopal symptoms. The mean time of the failed orthostatic challenges was 189 ± 86 s (range: 72–326 s). All seven participants who undertook the 48-h postexercise testing (T5) completed the orthostatic challenge in that session. Typical symptoms were light-headedness and/or dizziness/nausea. All participants during the 24-h control trial (with one exception at T1) completed the orthostatic challenge at all sessions with no reports of light-headedness and/or dizziness.

Cardiorespiratory function: supine. HR was significantly elevated and SV reduced at exercise testing sessions T2, T3, and T4 compared with T1 (all $P < 0.05$, Table 1), whereas, compared with matched controls, all HR were elevated but SV was significantly ($P < 0.05$) lower only at T2 (Table 1). MAP during the exercise trial was reduced ($P < 0.05$) by 14% at T3.
and 8% at T4 compared with T1, while T3 was also reduced (by 12%) compared with the matched-control MAP. During the control trial, MAP was reduced compared with baseline only at T3 (from 84 to 79 mmHg, P < 0.05). During the exercise trial, PETCO₂ was reduced at T2, T3, and T4 compared with T1 (Fig. 2), as well as compared with the control trial (condition * time interaction, P < 0.01). Despite these changes, MCAv in the supine posture was maintained across the exercise 24-h trial (time effect, P = 0.23; Table 1).

Cardiorespiratory function: postural challenge. A representative trace from one participant (Fig. 3) illustrates the general hypotension, lowered MCAv, and differential alterations in SV and HR occurring across the exercise trial. In all tests during the 24-h trials (exercise and control T1–T4), there was an increase in HR and reductions in SV, MCAv (P < 0.05; Table 1), and PETCO₂ from supine to “steady-state” standing (Fig. 2).

During the 24-h exercise trial, standing MAP was reduced by 16% at T2, 37% at T3, and 15% at T4, whereas standing MAP during the control was stable (P > 0.05) across the 24 h. During the exercise trial, PETCO₂ was lower (P < 0.05) compared with baseline at all times while standing (−4.5 Torr), whereas standing PETCO₂ was stable across the control trial (P > 0.05; Fig. 2). With standing, MCAv was reduced (P < 0.05) by 23% at T3 and 30% at T4 during the exercise trial (Table 1), but was stable during the control (P > 0.05). Upon standing, Q was transiently elevated at exercise T1 and for all control sessions (T1–T4), whereas such an elevation was absent throughout the exercise trial (T2–T4; Figs. 4D and 5D).

### Table 1. Steady-state cardiovascular and cerebrovascular measures during supine and standing for testing sessions across the 24-h exercise trial

<table>
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<tr>
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<th>T1</th>
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<th>T2</th>
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<td>Supine</td>
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<td>HR, beats/min</td>
<td>53 ± 8</td>
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<td>73 ± 10‡</td>
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<td>73 ± 8‡</td>
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<td>98 ± 10тип</td>
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<td>MAP, mmHg</td>
<td>81 ± 6</td>
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<td>76 ± 11</td>
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<td>SBP, mmHg</td>
<td>118 ± 9</td>
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<td>105 ± 14‡</td>
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<td>110 ± 17</td>
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<td>88 ± 22тип</td>
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<td>DBP, mmHg</td>
<td>62 ± 5</td>
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<td>61 ± 5</td>
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<td>59 ± 9</td>
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<td>50 ± 11тип</td>
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<td>SV, ml</td>
<td>113 ± 16</td>
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<td>80 ± 12‡</td>
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<td>94 ± 16тип</td>
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<td>62 ± 20тип</td>
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<td>Q, l/min</td>
<td>5.9 ± 1.3</td>
<td>5.8 ± 1.2</td>
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<td>6.8 ± 1.3*</td>
<td>5.9 ± 1.8</td>
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<td>TPR, mmHg⁻¹·min⁻¹</td>
<td>14.0 ± 2.4</td>
<td>13.2 ± 1.8</td>
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<td>11.6 ± 3.3*</td>
<td>11.0 ± 1.8*</td>
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<td>MCAv, cm/s</td>
<td>59.1 ± 10.8</td>
<td>51.3 ± 10.4‡</td>
<td>62.0 ± 9.3</td>
<td>44.7 ± 6.9тип</td>
<td>59.3 ± 5.3</td>
<td>39.5 ± 7.9тип</td>
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<td>SMCAv, cm/s</td>
<td>100.0 ± 16.5</td>
<td>84.7 ± 15.9‡</td>
<td>103.3 ± 12.8</td>
<td>77.4 ± 9.2тип</td>
<td>98.6 ± 10.0</td>
<td>74.1 ± 8.5тип</td>
<td>94.2 ± 10.0</td>
<td>64.8 ± 16.3тип</td>
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<td>DMCAv, cm/s</td>
<td>38.7 ± 8.3</td>
<td>34.6 ± 7.9‡</td>
<td>41.4 ± 8.3</td>
<td>28.3 ± 7.1тип</td>
<td>39.7 ± 4.6</td>
<td>22.1 ± 9.2тип</td>
<td>38.2 ± 8.3</td>
<td>21.3 ± 13.0тип</td>
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Values are means ± SD based on 9 participants. Supine data were averaged over the 3 min immediately before the stand. Standing data were averaged over the last 2 min of a 6-min stand. See METHODS for definitions of T1–T4. Different compared with *T1, †matched-control session, and ‡preceding supine: P < 0.05.
This transient elevation in \( \dot{Q} \) upon standing, as well as MCAv during the stand, were restored within 48 h (T5) of recovery from the completion of the exercise trial. The shift to standing also transiently lowered TPR and MAP (Figs. 4 and 5). TPR was restored to the preceding supine level in all but control testing sessions T2 and T3 (\( P < 0.01 \)).

Figure 6 shows that relative changes in MCAv with standing always correlated (\( P < 0.01 \)) with absolute changes in PETCO\(_2\) (Fig. 6A) during the exercise trial (\( r = 0.78-0.94 \) at each sampling time) and the control (\( r = 0.86-0.94 \)), but only correlated (\( P < 0.05 \)) with relative changes in MAP (Fig. 6B) at T1 (\( r = 0.62 \)), T2 (\( r = 0.84 \)), and T3 (\( r = 0.64 \)) during the exercise trial and at T2 (\( r = 0.89 \)) during the control. Thus lower resting and postural-associated reductions in PETCO\(_2\) and MAP were associated with lower standing MCAv during the exercise trial.

HRV and BRS. There were no three- or two-way interactions (all \( P > 0.05 \)) for normalized LF or HF RRI of the HRV analysis. Main effects of protocol were observed for both LF and HF (\( P < 0.01 \)). Pairwise comparisons revealed that supine controlled and normal breathing protocols were not different (LF, \( P = 0.81 \); HF, \( P = 0.64 \)), while both were different from standing (\( P < 0.01 \); Table 2). During standing, HF decreased, whereas LF and LF-to-HF ratio increased compared with supine at all exercise and control testing sessions, reflecting the increased sympathetic activity and reduced vagal activity during orthostasis (\( P < 0.01 \); Table 2). During supine rest, LF was increased and HF was decreased (\( P < 0.05 \)) in the exercise trial only at T2 compared with baseline and the matched-control session (Table 2). During standing, the LF were not different (\( P > 0.05 \)) across the exercise trial, whereas HF at exercise T2 decreased (\( P < 0.05 \)) compared with baseline. All supine and standing measures of LF and HF across the control trial were not different (\( P > 0.05 \)), indicating no discernable circadian effect. Changes in LF and HF during the exercise trial were also reflected in the LF-to-HF ratio, with only supine and standing LF-to-HF ratios at exercise T2 being different from baseline (both ratios elevated; \( P < 0.05 \), Table 2).

Postural changes (stand minus supine) for measures of autonomic function are presented in Fig. 7. These data further illustrate that the major changes in autonomic function occurred at exercise T2. The postural change in sympathetic...
activity was reduced at exercise T2, since standing LF was stable, while supine LF was elevated (Table 2). BRS to the postural change was reduced, on average, across the exercise trial, although only significantly at T2 compared with baseline (Fig. 7).

**Cardiac damage (cTnI).** During the control trial, all participants had undetectable levels of cTnI (≤0.02 µg/l) at all tests. Two participants had raised cTnI during exercise. In one participant, this was a cTnI of 0.02 µg/l at T4 (exercise completion). In a second individual, cTnI of 0.12, 0.09, and 0.07 µg/l were reported at T2, T3, and T4, respectively. In both of these individuals, cTnI was undetectable at T5. Consequently, exercise-induced elevations in cTnI were not associated with changes in cardiovascular or cerebrovascular performance or control within the current cohort. Even at a case study level, there was no pattern of change in cardiovascular or cerebrovascular control specific to the two athletes with detectable cTnI, either across the exercise trial or at testing sessions when cTnI was elevated.

**DISCUSSION**

Three major findings were evident in this study of autonomic, cardiovascular, and cerebrovascular function during and following a 24-h trial of exercise and physiological testing compared with a sedentary control trial. First, major changes in autonomic function and baroreflex control during orthostasis were only evident after the first (and highest exercise intensity) stage of the exercise trial. Second, hypotension and hypocapnia occurred while supine and were further exaggerated during postural change, during all testing sessions within the exercise trial, but reductions in cerebral perfusion only occurred during the stand. Third, postural-induced hypocapnia and hypotension during the exercise trial were strongly related to cerebral hypoperfusion, potentially facilitating the greater occurrence of syncope. Collectively, during prolonged exercise, marked postural-induced hypotension and hypocapnia further exacerbate cerebral hypoperfusion, facilitating the onset of syncope. With the exception following the highest exercise intensity (T2),
these changes seem to occur independently of major changes in HRV and BRS, or the occasional presence of biomarkers of cardiomyocyte damage.

Changes in resting autonomic and cardiorespiratory function during prolonged exercise. The postexercise hypotension (14 and 8% lower MAP at T3 and T4 compared with baseline, respectively) during supine rest observed in the present study is consistent with other reports (16, 29). Similar to these previous observations, the general hypotension and reduction in TPR occurred, despite changes in autonomic function (i.e., increased LF and decreased HF) and Q, potentially indicating additional influence from hormonal factors or the technological limitations of using HRV to estimate "sympathetic-vagal" balance (27, 39, 40). The differences in exercise intensity during each stage of the exercise trial may account for some of the differences in autonomic function at each of the exercise trial testing sessions, especially since the observations in the present study were consistent with those of others (29) only when duration and exercise intensity were most similar, i.e., after the ~7 h of the highest exercise intensity (~65% of HR range) of the first stage. Thus the intensity may be more important than the mode or volume of endurance exercise, although it is also acknowledged that a possible time dependency or differences in exercise mode (cycling vs. running) cannot be ruled out.

A lowered TPR response after prolonged exercise can be expected (16), due in part to metabolic vasodilation in previously active muscle, from local mediators such as heat, nitric oxide, and CO₂. Thus, carry-over effects of exercise before testing (e.g., increased body temperature and metabolism), and reductions in BRS (see below) are likely to account for some of the resting hypotension during the exercise trial. Interestingly, the degrees of postexercise hypotension were similar at T2 (after ~7 h) and at T4 (after 24 h) to one another and to that

Fig. 5. Relative exercise (left) and control (right) postural changes from baseline (steady-state preceding supine) in MAP (A), MCAv (B), HR (C), Q (D), and TPR (E). Syncopal symptomatic participants were time matched across all trials (as above); each data point is an average of the preceding 30 s. AUC different from *T1 and †matched-control session: P < 0.05.
observed following a 4- to 5-h mountain marathon (29). After ~15 h (at T3; 0046 ± 40 min) of exercise, supine resting BP was at its lowest, coinciding with the greatest number of syncopal episodes upon standing (6 of 9 participants). BP is known to have a circadian rhythm, peaking midmorning and lowest at 0300 (28), which was also observed in the present control data. Interestingly, this circadian effect may have been maintained across the exercise trial, since the hypotension at exercise T3 was greater than that at T2 and T4 (Table 1); therefore, it seems reasonable to speculate that circadian rhythm for BP is independent of exercise effects. However, consideration for the different exercise mode preceding that testing session needs to be taken into account; specifically, the greater muscle mass recruited during the trekking stage may have resulted in greater release of metabolic mediators of vasodilation, perhaps explaining some of the lower MAP compared with that following the cycling stages.

Hyperventilation-induced hypocapnia was present during the exercise testing sessions compared with baseline and matched-control sessions (Fig. 2). Interestingly, while hypocapnia was present during supine rest, MCAv was unchanged (P > 0.05). Normally, MCAv decreases 2–4%/mmHg decrease in CO2 (20); thus the maintained MCAv in the presence of hypocapnia indicates an alteration in the normal reactivity of MCAv to PETCO2, and perhaps the existence of other modulating factors (e.g., local metabolic factors, including brain temperature). The mechanism causing hyperventilation while resting supine is unclear, but could be influenced from the
Changes in autonomic and cardiorespiratory function during an orthostatic stress across prolonged exercise. Cerebral perfusion during the orthostatic challenge was impaired during the prolonged exercise trial, as evidenced through decreased MCAv and the occurrence of syncope. The reduced cerebral perfusion was related to hypotension, but, interestingly, not primarily as a consequence of an attenuated TPR response during orthostasis (Figs. 4E and 5E). As discussed above, an altered TPR response after prolonged exercise can be expected due, in part, to metabolic vasodilation. Some data indicate that orthostatic hypotension results from inadequate compensatory responses of the venous system (14, 21, 55), while other data point more to an underlying arterial vascular resistance deficit (17, 49, 56). Additional to these central and metabolic vasodilatory factors, Gratze and colleagues (16) suggested that their observed lowering of TPR in athletes during an orthostatic challenge (stand) at the conclusion of an ironman event may also be due to an inability to further stimulate an already maximally stimulated sympathetically mediated vasmotor response. Furthermore, the transduction of sympathetic activity into vascular resistance has been shown to be altered after 60 min of dynamic exercise, most likely due to an ineffective transduction of sympathetic outflow at arterial smooth muscle (17). This finding indicates the possibility that postexercise hypotension may also be due to a desensitization to sympathetic activation after chronic stimulation.

In the present study, the observed lower TPR at supine and steady-state standing is indicative of a metabolic vasodilatory response (and potentially also a lowered adrenergic constrictor sensitivity), thus certainly contributing to the overall hypotensive state, as seen during supine rest. However, the transient drop in TPR upon standing for all sessions during the exercise trial recovered to the preceding supine value, similar to baseline and matched-control sessions. Therefore, the impaired cerebral perfusion during orthostasis, via hypotension, is more likely a consequence of an impaired venous system response, i.e., inadequate compensatory increases in Q during the stand. This appeared to be most obvious during the initial 30 s of the stand, when the normally present transient increase in Q was attenuated during the exercise trial (Figs. 4D and 5D), consistent with observations following a mountain marathon (29). Furthermore, at exercise T3, Q was significantly reduced across the whole stand period, coinciding with the lowest preceding supine BP and greatest number of syncpe episodes. Likely candidates for the inadequate Q compensatory response are as follows; reduced SV, since lower SV was observed during the stand across the exercise trial testing sessions compared with baseline and matched-control; impaired venous response, or both. The lower SV is unlikely to be related to reduced filling time, as HRs were still low (<100 beats/min). Postexercise orthostatic hypotension has also been attributed to, in part, a drop in SV as a result of myocardial dysfunction (2, 13). Evidence from the cTnI data would seemingly rule out cardiomyocyte damage and any associated left ventricular dysfunction, in the genesis of hypotension in the present study. The limited elevation of cTnI after ultradurance activity supports a previous meta-analytic conclusion and likely reflects the low intensity of exercise (48). Even in the two participants with elevated cTnI, no noticeable pattern of change in cardio-

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Values are means ± SD, based on 9 participants. Controlled breathing was at a rate of 12 breaths/min. LF, low frequency; HF, high frequency; LF/HF, LF/HF ratio; BPM, beats per minute; MCAv, mean cerebral artery velocity; BP, blood pressure; HR, heart rate; TPR, total peripheral resistance; cTnI, cardiac troponin I; Q, cardiac output; SV, stroke volume.
vascular or cerebrovascular control was observed between or within participants.

Influence of respiratory instability. An important, novel finding from the present study was the relation between the degree of hyperventilatory-induced hypocapnia with both hypotension and reduced MCAv. In animal studies, the related physiological consequences of hyperventilation result in a persistently positive intrathoracic pressure, thereby decreasing cardiac preload (10) and Q˙ (6) and impeding right ventricular function (54). Increased tidal volume is also known to adversely affect Q˙ (22). In the present study, it seems reasonable to speculate that elevations in the mean intrathoracic pressures caused by excessive ventilation might reduce venous blood flow back to the heart (right side), thus contributing to the reduced SV, because of insufficient time to allow for the development of negative intrathoracic pressure. Moreover, the related hypocapnic-induced cerebral vasoconstriction will further compound the presyncopal symptoms. In support of this, patients with orthostatic intolerance exhibit an excessive decrease in PETCO₂ during orthostatic stress; these symptoms of cerebral hypoperfusion and the decline in MCAv can be reversed with CO₂ rebreathing (31). The precise sequence by which hyperventilatory-induced cerebral vasoconstriction and hypotension interrelate and lead cerebral hypoperfusion and ultimately syncope is unknown.

Changes in autonomic function. Changes in BRS on postural change (i.e., less of a decrease; Fig. 7) were only significantly affected at exercise T2, similar to the other autonomic function measures. Studies on BRS report lowered (7, 16, 29), unchanged (50), or enhanced BRS (8) following exercise. Differences in intensity and duration of the exercise and the methodological assessment of BRS may explain the discrep-
ancy between the studies (29). Within the present study, the higher intensity of the first stage compared with that of the second and third stages may explain the differences across the exercise trial. As with the HRV analysis observations, BRS at T2 was affected similarly to that following a mountain marathon (29), when the intensity and duration of exercise during the exercise trial were most similar to that of the marathon. It is important to recognize that using BRS alone provides only the operating point values of the baroreflex stimulus-response curve, so the present data do not indicate whether the maximal gain was unchanged (34). Further research is needed to clarify the effect of exercise intensity and duration on BRS activity.

Influence of hypohydration. Hypohydration during exercise may compromise orthostatic tolerance (5). In the present study, reduced MCAv and orthostatic tolerance at exercise T2, T3, and T4 appear not to be due to hypohydration, despite the drop in body mass, since 1) plasma volume was greater at exercise T3 (95% CI: 0–8%) and T4 (2–12%); and 2) urine-specific gravity was not different (P > 0.05) across the exercise trial (baseline, 1.017), and a 2–3% decrease in body mass with unchanged extracellular fluid volume would be expected from glycogenolysis and release/loss of the previously glycogen-bound water (27a). These observations have some limitations, such as the fact that changes in plasma volume and urine-specific gravity are indirect measures of hydration status, circulating red cell volume is raised slightly by splenic release following sympathetic nervous system activation, and urine-specific gravity shows some delay in reflecting hydration status. Even so, it seems unlikely that a lack of blood volume per se could account for the observed hemodynamic responses.

Methodological considerations should be noted. First, the practical value of BRS from spontaneous BP and HRV analysis as an insight into autonomic function has been discussed extensively in the literature (27, 39, 40). These reports indicate that the use of the variability methods, while valid from a theoretical perspective, requires more clinical application for validation. The relationship between spectral components of cardiovascular variables and direct measures of muscle sympathetic nerve activity in humans has been shown to correlate closely over a range of arterial pressure changes (37), except when sympathetic activity is reduced (45). In relation to the available techniques, however, it has been reported that spontaneous and pharmacologically determined BRS are complementary (41) and that the BRS methods may contribute to more in-depth examination of the baroreflex circulatory control (59).

Second, similar to other studies of the hemodynamic effects of endurance exercise (9, 16, 29), the stand test was used because of its practical and physiological relevance to the problems that occur following prolonged exercise (i.e., the inability to maintain an upright posture). A more severe orthostatic stress test (e.g., lower body negative pressure; tilt) may produce greater hemodynamic changes than induced by the active stand used here; however, both active standing and passive head-up tilt have been reported to provoke comparable changes in spontaneous baroreflex and related hemodynamic variables (3). It is unknown if this relation changes following prolonged exercise. While it was ensured that there were no major muscle contractions at rest or during the stand, it is acknowledged that some leg muscles are required to maintain posture and that it is not possible (and seldom relevant) to remove this action during a stand. Some of our findings are based on Q calculations derived indirectly from estimates of the arterial pulse waveform to calculate SV. It should be noted that this arterial pulse pressure reflects both changes in SV and arterial compliance, and that the long duration of exercise and the orthostatic challenge are likely to alter arterial compliance, thereby making accurate calculation of SV problematic. Although the absolute drop in SV seems to compare with previous reports using Doppler ultrasound after 4 h of continuous rowing (18), there are no direct measurements of SV or Q to confirm our findings. To partly circumvent this issue, we caution the use of absolute measurements of Q and present the relative change of these key variables from baseline (see Fig. 5).

Doppler ultrasound was used to measure flow velocity, rather than blood flow, in the middle cerebral artery. Nevertheless, MCAv appears to be a valid index of CBF (15, 46). No cardiac ultrasound scans were performed to assess cardiac function; therefore, exercise-induced cardiac dysfunction could occur independent of cardiomyocyte damage via other mechanisms, such as β-adrenocortreceptor downregulation (18, 35). Finally, direct (i.e., serial) comparison between the different mode (i.e., cycling or running) and intensity of exercise observed in this setting is problematic. The purpose of using mixed-mode exercise was to maximize the work completed, but a consequence is that we cannot differentiate our findings from the carry-over effects of the previous exercise episode from that of the hemodynamic changes induced by the different exercise conditions (i.e., mode and intensity) and related engagement of different degrees of muscle mass. The extent to which orthostatic tolerance might be worsened by different modes and intensity of exercise warrants future study.

In summary, during prolonged exercise, marked postural-induced hypotension and hypopnoea further exacerbate cerebral hypoperfusion, facilitating the onset of syncope. It seems possible that, following exercise, the regularization of breathing, via appropriate training, together with validated techniques, such as lower body muscle tensing (24–26), may reduce cardiorespiratory instability, representing a simple non-pharmacological means capable of offsetting eminent syncope.

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ORTHOSTATIC STRESS RESPONSE ACROSS 24-H EXERCISE TRIAL


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