Three Weeks of Overload Training Increases Resting Muscle Sympathetic Activity

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1The Human Performance and Health Research Laboratory, Department of Human Health and Nutritional Sciences, University of Guelph, Guelph, ON, CANADA; 2Department of Human Health and Nutritional Sciences, University of Guelph, Guelph, ON, CANADA; and 3Toronto General Research Institute, Toronto General Hospital, Toronto, ON, CANADA

ABSTRACT

COATES, A. M., A. V. INCOCNITO, J. D. SEED, C. J. DOHERTY, P. J. MILLAR, and J. F. BURR. Three Weeks of Overload Training Increases Resting Muscle Sympathetic Activity. Med. Sci. Sports Exerc., Vol. 50, No. 5, pp. 928–937, 2018. Purpose: Overload training is hypothesized to alter autonomic regulation, although interpretations using indirect measures of heart rate variability are conflicting. The aim of the present study was to examine the effects of overload training on muscle sympathetic nerve activity (MSNA), a direct measure of central sympathetic outflow, in recreational endurance athletes. Methods: Measurements of heart rate variability, cardiac baroreflex sensitivity (BRS), MSNA (microneurography), and sympathetic BRS were obtained in 17 healthy triathletes and cyclists after 1 wk of reduced training (baseline) and again after 3 wk of either regular (n = 7) or overload (n = 10) training. Results: After training, the changes (Δ) in peak power output (10 ± 10 vs −12 ± 9 W, P < 0.001), maximal heart rate (−2 ± 4 vs −8 ± 3 bpm, P = 0.006), heart rate variability (SD of normal-to-normal intervals, 27 ± 31 vs −3 ± 25 ms; P = 0.04), and cardiac BRS (7 ± 6 vs −2 ± 8 ms/mg Hg−1, P = 0.02) differed between the control and overload groups. The change in MSNA burst frequency (−2 ± 2 vs 4 ± 5 bursts per minute, P = 0.02) differed between groups. Across all participants, the changes in resting MSNA and peak power output were correlated negatively (r = −0.51, P = 0.04). No between-group differences in resting heart rate or blood pressure were observed (all P > 0.05). Conclusions: Overload training increased MSNA and attenuated increases in cardiac BRS and heart rate variability observed with regular training. These results support neural adaptations after overload training and suggest that increased central sympathetic outflow may be linked with decreased exercise performance. Key Words: MUSCLE SYMPATHETIC NERVE ACTIVITY, ENDURANCE TRAINING, TRAINING, OVERLOAD, OVERTRAINING, AUTONOMIC NERVOUS SYSTEM

Endurance athletes often experience periods of underperformance after intensified training, a phenomenon termed functional overreaching or overtraining syndrome depending on recovery duration and severity of physiological perturbations (1,2). These conditions are defined by decreases in power output and time trial performance (1), but are also accompanied by other physiological disturbances, including decreased submaximal and maximal heart rate (HR) during exercise, disturbed mood states, a rightward shift in the blood lactate curve (2,3), an increase in HR recovery after exercise (4), and decreased cardiac output and stroke volume during exercise (2). Unfortunately, the mechanisms responsible for these responses and whether they exert a causal relationship to the observed decrements in performance remain unclear. Given the consistently observed changes in exercising HR, it has been hypothesized that overload training may alter the regulation of the autonomic nervous system (1,5,6).

Neural mechanisms for blunting submaximal and maximal HR could arise from either decreased cardiac sympathetic activity or heightened parasympathetic activity (5,7). However, whether overload training produces changes in cardiac autonomic modulation is unclear (5,6,8,9). Noninvasive assessments of time- and frequency-domain HR variability measures related to resting tonic cardiac parasympathetic activity have been shown to both increase (5,9) and decrease (6,10). Other studies have demonstrated that HR variability remains unchanged (11) or is not adequate for monitoring overload training status (8,12).
who had self-reported recruitment from local clubs, were subelite cyclists or triathletes in the study. This sample represented a subset of subjects recruited (OL; n = 12) in a block-randomized format.

A number of studies have also measured plasma or urinary catecholamine concentrations to estimate sympathetic outflow (2,3,7). These results suggest that overload training either has no effect (2) or reduces (3,7) norepinephrine or epinephrine levels at rest, although more consistent reductions in catecholamine levels are found during exercise (2). Plasma or urinary measurements of norepinephrine provide little insight into which target organ may have altered sympathetic outflow (e.g., heart, kidneys, and muscle). In contrast, microneurographic recordings of muscle sympathetic nerve activity (MSNA) are considered a gold-standard method to examine central sympathetic outflow directed toward skeletal muscle vasculature (16) and exhibit excellent short- and long-term reproducibility at rest (16,17) and in response to acute stress (18). Prior cross-sectional work has shown an inverse relationship between resting MSNA and treadmill running time to exhaustion in a homogenous group of young male club-level runners (19); however, whether this association was affected by training load was not reported. To our knowledge, the effects of overload training on regional peripheral sympathetic outflow have not been studied.

Therefore, the aim of this study was to characterize the effects of overload training on autonomic function in recreational endurance athletes, with sympathetic outflow directed toward skeletal muscle (MSNA) as the primary variable of interest. Given that MSNA is not altered after aerobic exercise training in healthy adults (20), we hypothesized 1) that 3 wk of overload training would increase direct measures of resting central sympathetic outflow (MSNA) and 2) that the change in MSNA would relate inversely to the decrement in exercise performance.

METHODS

Participants. Twenty-one healthy men and women (11 male; 36 ± 10 yr (mean ± SD)) volunteered to participate in the study. This sample represented a subset of subjects recruited in a larger (n = 33) study on overload training. All subjects, recruited from local clubs, were subelite cyclists or triathletes who had self-reported 7 ± 4 yr of endurance sport training. Inclusion criteria included that subjects be between 18 and 50 yr of age, free from injury, familiar with cycle pacing, and currently following a structured endurance-training program. Subjects were randomized by enrollment order to either the regularly training control group (CON; n = 9) or the overload training group (OL; n = 12) in a block-randomized format.

All subjects were required to complete a Physical Activity Readiness Questionnaire (PAR-Q+) questionnaire to ensure readiness for physical activity and abstain from alcohol, drugs, caffeine, and intense exercise for 24 h before laboratory testing. Subject diets were not restricted, but they were asked to consume the same meal at a similar time of day before both testing visits. All participants provided written informed consent in accordance with the declaration of Helsinki, and this study was approved by the University of Guelph Research Ethics Board.

Experimental protocol. The experimental protocol consisted of 1 wk of reduced training in which exercise volume was decreased by ~50%, to reduce the effect of any prior training-induced fatigue, followed by 3 wk of either CON or OL training. This protocol was modeled after a 3-wk overreaching protocol used previously (2). To track exercise duration and intensity over the duration of the study, all subjects were provided a Polar A300 watch, HR strap, and associated online account (Polar Electro Oy, Kempele, Finland). Exercise performance and autonomic testing took place over two visits separated by 24–48 h after both the first week of reduced training and 3 wk of CON or OL training.

Profile of mood states. Subjects completed the Profile of Mood States Second Edition (POMS-2) questionnaire online after the reduced training week and again after the 3 wk of training. The POMS-2 is a 65-item questionnaire in which total mood disturbance is shown to increase in a dose–response manner with increased training stimulus (1,21). It uses a 4-point rating scale to assess moods of tension–anxiety, depression–dejection, anger–hostility, vigor–activity, fatigue–inertia, confusion–bewilderment, and friendliness. The total mood disturbance is determined by subtractive vigor–activity from the sum of the five negative mood scales and is reported in arbitrary units (au). A constant of 100 was added to the total mood disturbance to account for negative values (21).

Exercise testing. Subjects performed a maximal incremental exercise test on an electromagnetically braked cycle ergometer (Racermate Velotron, Seattle, WA). Oxygen consumption was measured using a metabolic cart with a mixing chamber (Moxus; AEI Technologies, Pittsburgh, PA) in standardized laboratory conditions. A true ramp protocol was used to increase the load on the cycle ergometer, with a slight variation between sexes to ensure that the test was of sufficient duration. Men followed a protocol beginning at 100 W and increasing 1 W every 2 s, whereas women started similarly at 100 W but increased 1 W every 3 s. Subjects were blinded from their power output (or duration) during the exercise test. The test was terminated and peak watts recorded when subjects could no longer maintain a seated or standing cadence greater than 40 rpm. HR was monitored (Polar A300) and recorded at the moment of exercise termination. Lactate measures, using standard finger stick sampling, was initiated 60 s after exercise with samples every 30 s until values declined (Lactate Plus; Nova Biomedical, Waltham, MA). Maximal blood lactate concentration was recorded as the highest observed value.

Training protocols. Subjects in the CON group were instructed to continue with their regular training schedule for the 3-wk duration of the training block, but not to increase their load above what was prescribed by either their coach, online training program, or self-planned program. The maintenance of training in the control was important to ensure that they did not initiate detraining by ceasing to follow their normal program.
The OL training protocol consisted of three supplementary high-load cycle training sessions per week in addition to the subjects’ normal training schedule. The first weekly session was a high-intensity interval workout consisting of four 30-s Wingate anaerobic tests on an electromagnetically braked cycle ergometer (Racermate Velotron), at a load of 7.5% body weight, with each test separated by 4 min of recovery. The second session was a 15-km virtual time trial performed on the same cycle ergometer over a standardized course with undulating terrain. The first two supplemental sessions were completed in the laboratory under standardized conditions. The final session was a 2-h ride completed on the subject’s own time and tracked using HR monitoring. The ride was prescribed as four blocks, with each block consisting of 10 min at an HR of 50% to 60% of maximal oxygen consumption (V\textsubscript{O2max}) followed by 20 min at an HR of 66%–75% of their V\textsubscript{O2max}, on the basis of calculation from their initial V\textsubscript{O2max} at baseline.

**Cardiovascular and autonomic testing.** All tests were completed in a light- and temperature-controlled laboratory. Subjects underwent instrumentation (~1 h) followed by a 10-min rest period before continuous measures of HR, blood pressure, and MSNA were collected over a subsequent 10-min baseline for analysis of resting autonomic function. After the resting collection, a 2-min static handgrip contraction at 30% of maximal voluntary contraction was completed in the left hand (Model 78010, Hand Dynamometer; Lafayette Instrument, Lafayette, LA). All recordings were collected in the supine position after voiding. HR was collected using single-lead electrocardiography (lead II configuration; ADInstruments, Colorado Springs, CO), whereas beat-to-beat blood pressure was recorded from the right middle finger using photoelectric plethysmography (Finometer MIDI; Finapres Inc, Enschede, the Netherlands). Microneurographic recordings of multiunit MSNA were obtained from the right fibular nerve using a low-impedance 2-MΩ tungsten microelectrode (Frederick Haer, Brunswick, ME), as described previously (17,22). A ground electrode was placed 1–3 cm away from the recording electrode. The neural signal was amplified (75,000×), band-pass filtered (0.7–20 kHz), rectified, and integrated over a 0.1-s time constant to obtain the mean voltage neurogram (Nerve Traffic Analyzer, Model 662C-4; Absolute Design and Manufacturing Services, Salon, IA). Confirmation of muscle sympathetic activity was made by testing signal responsiveness to unexpected noise and end-expiratory breath holds. All continuous data were digitized and stored with LabChart (PowerLab; ADInstruments). HR, blood pressure, and the integrated multiunit MSNA signal were recorded at a sampling frequency of 1000 Hz.

**Data analysis.** All resting cardiovascular variables and measures of autonomic function were assessed from the 10-min baseline period. The reactivity to static handgrip was calculated as the change (Δ) from baseline to the second minute of exercise. Measures of HR variability were determined using Kubios HRV Analysis Software 2.2 ( Biosignal Analysis and Medical Imaging Group, Department of Applied Physics, University of Eastern Finland, Kuopio, Finland). Time-domain variables included the SD of normal R-R intervals (SDNN) and the root mean square of successive R-R interval differences. Frequency-domain variables included low-frequency (LF) power (0.04–0.15 Hz), high-frequency (HF) power (0.15–0.4 Hz), and the LF/HF ratio. The power spectra of the LF and HF bands are presented in natural logarithm transforms (ln ms\(^2\)) and normalized units (nu). Spontaneous cardiac BRS was quantified at rest using the sequence technique (23). We identified sequences of three or more consecutive increases or decreases in systolic blood pressure and R-R intervals using established thresholds for changes of 1 mm Hg and 6 ms, respectively (23). Systolic blood pressure and R-R intervals were obtained from the same cardiac cycle (i.e., no time lag), because HR values were less than 75 bpm in all participants (23). BRS was quantified by plotting R-R interval over systolic blood pressure for each identified series with an r value of ≥0.8, and averaging the slope of all up-sequences and down-sequences.

MSNA was analyzed using a custom LabView (National Instruments, Austin, TX) semiautomated program (17,22). MSNA was quantified as burst frequency (bursts per min) and burst incidence (bursts per 100 heartbeats). Our laboratory has previously demonstrated high intertest intraclass correlation coefficients (reliability) for resting MSNA burst frequency (r = 0.76) and burst incidence (r = 0.77) measures separated by 1 month (17). Spontaneous sympathetic BRS was calculated by examining the weighted linear regression line between 2-mm Hg bins of diastolic blood pressure and MSNA burst occurrence (17). The slope of the line was taken as sympathetic BRS if the regression possessed an r value of ≥0.5.

**Statistical analysis.** Baseline subject characteristics, and training durations and intensities were compared using unpaired t-tests. Two-way repeated-measures ANOVA was used to study the changes in training outcomes (total muscle disturbance, peak power, maximal HR, \textsubscript{V}O\textsubscript{2}max, peak lactate, resting hemodynamics, MSNA, cardiac and sympathetic BRS, and HRV) with Bonferroni post hoc procedures to probe contrast effects when a significant interaction was present. Because the primary interest was the group–time interaction, secondary one-way ANCOVA on the pre–post change over time with adjustment for pretraining baseline values was completed to increase statistical power and reduce the effect of chance differences in baseline values, which could affect the magnitude of responses due to regression to the mean (24). This secondary analysis examines the responses over time if both groups had the same baseline value and reduces the between-subject variability (24). The hemodynamic and neural changes (Δ) from baseline to the second minute of static handgrip exercise before and after training were similarly examined using two-way repeated-measures ANOVA with Bonferroni post hoc tests. One-way ANCOVA was also used to assess the changes from baseline to the second minute of static handgrip exercise after training, with the pretraining change scores used as the covariate. Linear regression analysis was used to examine the...
relationship between changes in resting MSNA burst frequency and peak power output. Data are reported as mean ± SD. Significance was set a priori at $P < 0.05$. Statistical analysis was executed using Statistical Package for the Social Science (SPSS, version 24; IBM, Chicago, IL).

RESULTS

We recruited 21 participants; however, three participants did not complete the study. In the CON group, two subjects were unable to return for posttesting, whereas in the OL group,

FIGURE 1—Effects of 3 wk of CON or OL training on peak power output (A), maximal HR (B), peak blood lactate (C), and $\dot{V}O_2$max (D). Mean ± SD. Left-side figures represent group–time differences examined using two-way ANOVA. Right-side figures represent the between-group difference in change after training adjusted for baseline values using ANCOVA. †$P < 0.05$; ††$P < 0.001$; †††$P < 0.0001$ vs baseline.
we were unable to locate a microneurographic recording site at baseline in one participant. In addition, one participant in the OL group failed to adhere to the 3-wk protocol and was excluded from analysis. Therefore, the results are presented for 17 participants (CON, n = 7 (4 male); OL, n = 10 (4 male)). At baseline, participant age (32 ± 11 vs 36 ± 10 yr, P = 0.38), body weight (69 ± 5 vs 73 ± 11 kg, P = 0.48), body mass index (24 ± 1 vs 24 ± 3 kg m⁻², P = 0.55), training duration (6.4 ± 4.0 vs 7.9 ± 4.0 yr, P = 0.45), and VO₂max (57 ± 10 vs 53 ± 7 mL·kg⁻¹·min⁻¹, P = 0.25) were similar between the CON and OL groups.

Training program. Exercise volume was similar between the CON and OL groups during the 1 wk of reduced training (6.1 ± 1.0 vs 6.4 ± 3.2 h·wk⁻¹, P = 0.84); however, as expected, exercise volume was higher in the OL group during the 3 wk of training (8.8 ± 3.0 vs 12.1 ± 2.6 h·wk⁻¹, P = 0.04). Exercise intensity, represented as time spent in specific HR zones divided by overall training time, did not differ between groups during the 1 wk of reduced training or the 3 wk of training periods (all P > 0.05). To characterize the reduced training week, time spent in zone 1 (<60% of max), zone 2 (60%–80% of max), and zone 3 (>80% of max) was 18% ± 12%, 53% ± 10%, and 29% ± 15%, respectively, for CON and 7% ± 3%, 45% ± 16%, and 48% ± 17%, respectively, for OL. During the training period, average time spent in zone 1, zone 2, and zone 3 was 13% ± 5%, 58% ± 12%, and 30% ± 15%, respectively, for CON and 11% ± 3%, 58% ± 7%, and 32% ± 8%, respectively, for OL.

Mood state. Total mood disturbance scores (POMS-2) increased after the reduced training week (P = 0.002) in both groups in concordance with the increased training volumes (CON: 89 ± 9 vs 103 ± 17 au; OL: 87 ± 20 vs 111 ± 23 au), but no group or interaction effects were observed (both P > 0.05).

Maximal exercise performance. Exercise testing outcomes are presented in Figure 1. A group–time interaction was observed for peak power output (P < 0.001). After training, peak power output increased in CON (345 ± 78 vs 355 ± 77 W, P = 0.02) and decreased in OL (335 ± 71 vs 323 ± 71 W, P = 0.001). Similarly, there was a group–time interaction for maximal HR (P < 0.001). Maximal HR was unchanged in CON (182 ± 14 vs 180 ± 11 bpm, P = 0.10) but decreased in OL (179 ± 11 vs 172 ± 10 bpm, P < 0.001) after training. Peak lactate and VO₂max did not demonstrate any time, group, or interaction effects (all P > 0.05). Secondary analysis adjusting for baseline values did not alter any of these results.

HR and blood pressure. The effects of exercise training on resting HR and blood pressure and their response to static handgrip exercise are presented in Table 1. There were no time, group, or interaction effects observed for resting HR and blood pressure (all P > 0.05); ANCOVA analyses adjusting for baseline values did not alter these results. In response to static handgrip exercise, there were no time or interaction effects for the change in HR or blood pressure (all P > 0.05). HR, diastolic blood pressure, and mean arterial pressure demonstrated a group effect, such that responses in CON were greater than those in OT (all P < 0.05). Secondary analysis adjusting for baseline values did not reveal any between-group differences in HR or blood pressure response after training (all P > 0.05).

Autonomic function. Complete resting HR variability results are shown in Table 2. A group–time interaction was

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TABLE 1. HR and blood pressure at rest and in response to static handgrip exercise before and after 3 wk of CON or OL exercise training.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CON (n = 7)</th>
<th>OL (n = 10)</th>
<th>ΔCON</th>
<th>ΔOL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td></td>
</tr>
<tr>
<td>Rest HR, bpm</td>
<td>57 ± 6</td>
<td>64 ± 6</td>
<td>-3 ± 6</td>
<td>-2 ± 5</td>
<td>0.79</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>102 ± 7</td>
<td>106 ± 5</td>
<td>4 ± 1</td>
<td>1 ± 1</td>
<td>0.50</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>65 ± 9</td>
<td>69 ± 9</td>
<td>4 ± 0</td>
<td>1 ± 7</td>
<td>0.57</td>
</tr>
<tr>
<td>Static handgrip HR, bpm</td>
<td>14 ± 8</td>
<td>7 ± 6</td>
<td>7 ± 4</td>
<td>3 ± 7</td>
<td>2 ± 5</td>
</tr>
<tr>
<td>Static handgrip Diastolic BP, mm Hg</td>
<td>19 ± 9</td>
<td>14 ± 9</td>
<td>5 ± 0</td>
<td>0 ± 4</td>
<td>1 ± 0</td>
</tr>
</tbody>
</table>

| Table 2. Resting HR variability before and after 3 wk of CON or OL exercise training.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CON (n = 7)</th>
<th>OL (n = 10)</th>
<th>ΔCON</th>
<th>ΔOL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td></td>
</tr>
<tr>
<td>R-R interval, ms</td>
<td>1078 ± 107</td>
<td>1172 ± 129</td>
<td>59 ± 119</td>
<td>6 ± 91</td>
<td>0.31</td>
</tr>
<tr>
<td>SDNN, ms</td>
<td>93 ± 55</td>
<td>82 ± 35</td>
<td>27 ± 31</td>
<td>-3 ± 25</td>
<td>0.04</td>
</tr>
<tr>
<td>RMSSD, ms</td>
<td>98 ± 86</td>
<td>72 ± 35</td>
<td>28 ± 39</td>
<td>2 ± 28</td>
<td>0.13</td>
</tr>
<tr>
<td>LF, In ms²</td>
<td>7.1 ± 0.9</td>
<td>7.2 ± 0.9</td>
<td>0.6 ± 0.7</td>
<td>-0.1 ± 0.7</td>
<td>0.09</td>
</tr>
<tr>
<td>HF, In ms²</td>
<td>7.4 ± 1.8</td>
<td>7.1 ± 1.2</td>
<td>0.6 ± 0.6</td>
<td>0.1 ± 0.6</td>
<td>0.13</td>
</tr>
<tr>
<td>LF/HF ratio</td>
<td>1.3 ± 1.4</td>
<td>1.7 ± 2.6</td>
<td>-0.5 ± 1.4</td>
<td>-0.1 ± 0.5</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD. Boldface indicates P < 0.05. RMSSD, root mean square of the successive differences. *P < 0.05 vs baseline.
observed for SDNN ($P = 0.04$). After training, SDNN increased in CON ($P = 0.02$) but was unchanged in OL ($P = 0.71$). There were no time, group, or interaction effects detected for any of the other time- and frequency-domain measures of HR variability (all $P > 0.05$). Analysis adjusting for baseline values did not alter any of these results.

A representative MSNA tracing and sympathetic BRS analysis before and after 3 wk of overload training is shown in Figure 2. Resting group-level MSNA and BRS outcomes are presented in Figure 3. There was a group–time interaction for MSNA burst frequency ($P = 0.01$). After training, MSNA burst frequency was unchanged in CON (17 ± 3 vs 15 ± 4 bursts per minute, $P = 0.33$) but increased in OL (20 ± 7 vs 24 ± 5 bursts per minute, $P = 0.006$). MSNA burst frequency demonstrated a group effect ($P = 0.02$), and ANCOVA analysis adjusting for baseline values increased the strength of evidence for a between-group difference ($P = 0.005$). There was a trend for a group–time interaction for MSNA burst incidence ($P = 0.09$). However, because MSNA burst incidence demonstrated a group effect ($P = 0.02$), secondary ANCOVA analysis adjusting for baseline values revealed a significant between-group difference in the change in MSNA burst incidence with training ($P = 0.02$). In contrast, there were no time, group, or interaction effects observed for the change in MSNA burst frequency (CON: Δ9 ± 8 vs 11 ± 6 bursts per minute; OL: Δ6 ± 5 vs 7 ± 5 bursts per minute) or burst incidence (CON: Δ6 ± 12 vs 9 ± 12 bursts per 100 heartbeats; OL: Δ6 ± 10 vs 3 ± 6 bursts per 100 heartbeats) during static handgrip before and after training; these results were not altered using ANCOVA analyses.

A group–time interaction was detected for resting sensitivity of the sympathetic baroreflex ($P = 0.047$); however, post hoc analysis did not identify any within-group or between-group differences. Furthermore, ANCOVA analysis adjusting for baseline values failed to detect a between-group difference in the change in sympathetic BRS after training ($P = 0.12$). There was a group–time interaction for cardiac BRS ($P = 0.02$). Cardiac BRS increased in CON (23 ± 11 vs 30 ± 14 ms·mm Hg$^{-1}$, $P = 0.02$) but was unchanged in OL (23 ± 10 vs 22 ± 6 ms·mm Hg$^{-1}$, $P = 0.40$) after training; these results were not altered by ANCOVA analysis.

Examining the study cohort as a continuous variable, training-mediated changes in resting MSNA burst frequency were negatively associated with alterations in peak power output ($r = -0.51$, $P = 0.04$; Fig. 4).

### DISCUSSION

The purpose of this study was to characterize comprehensively the effects of overload training on autonomic function in healthy recreational endurance athletes. In agreement with prior studies (2,4), our model of 3-wk overload training led to reductions in peak power output and maximal HR compared with regular training controls. The primary novel observation was that overload training, but not regular training, increased the resting set point for central sympathetic outflow to skeletal muscle (MSNA) without altering the reflexive response to static handgrip exercise. The increase in resting MSNA observed in the OL group was independent of any detectable change in sympathetic BRS, arguing against a peripheral afferent mechanism for sympathoexcitation. Across the entire cohort, the changes in MSNA burst frequency correlated with those in peak power output, suggesting that neurogenic vasoconstriction may contribute to the decrements in exercise performance. Overload training also blunted increases in cardiac BRS and HR variability observed after regular CON training. Altogether, these data provide evidence that overload training can perturb the autonomic nervous system at rest and that these neural responses may be related to underperformance.

**HR variability and cardiac BRS.** Most studies probing the neural effects of overload training have used noninvasive measures of tonic cardiac autonomic activity through analyses of HR variability (5,6,9–11,15). However, these prior studies demonstrated considerable between-study variability with overload training reporting decreases (6,10,15), increases (5,9), or no change (11) in cardiac parasympathetic
modulation. In addition to concerns about study design (e.g., small sample sizes and lack of a control group), measures of HR variability assess changes in sinus node firing which can involve both neural and nonneural mechanisms (25). For example, interpreting changes in HR variability with overload training may be difficult because of interindividual differences in parasympathetic saturation, observed in some athletes (26); changes in intrinsic HR control (25); desensitization of beta-adrenergic receptors (27); or stretch of the sinoatrial node (28) through atrial enlargement or expansion of plasma volume.

FIGURE 3—Effects of 3 wk of CON or OL training on MSNA burst frequency (A) and burst incidence (B), and cardiac BRS (C). Mean ± SD. Left-side figures represent group–time differences examined using two-way ANOVA. Right-side figures represent the between-group difference in change after training adjusted for baseline values using ANCOVA. †P < 0.05 vs baseline; *P < 0.05; **P < 0.01 vs CON.
To date, only two studies have assessed the reflex control of cardiac parasympathetic modulation using measures of cardiac BRS (14,15). Baumert et al. (15) reported that spontaneous cardiac BRS decreased in 10 athletes after a 2-wk training camp, although this study lacked a control group for comparison. In contrast, Uusitalo et al. (14) observed no change in cardiac BRS, assessed pharmacologically in response to phenylephrine administration, after 6–9 wk of increased training in nine female athletes; however, only five participants were diagnosed as being overtrained and no subanalysis of cardiac BRS was performed in this group.

In our cohort, SDNN, a time-domain measure of parasympathetic modulation (29), and cardiac BRS were both increased in the regular training CON group but unchanged after OL training, with between-group differences in both measures responses with training. Prospective trials have also shown increased HR variability and cardiac BRS with endurance training (30), but results are not consistent between individual studies with training protocols of varying types and intensities and with differing subject fitness levels (14,31,32). The observation that peak power output increased after regular training provides further evidence that the CON group underwent conventional adaptations to endurance exercise training. Although these responses were not expected, it is important to consider that our subjects were not elite athletes, allowing them to benefit from participation in a monitored training program. These results highlight further the critical need for randomized control groups within overtraining studies.

Peripheral sympathetic outflow. Aerobic exercise training does not alter resting MSNA in healthy adults despite increasing cardiorespiratory fitness (20). These results are in contrast to reductions in central sympathetic outflow typically observed after aerobic exercise training in diseased populations (e.g., heart failure, hypertension, and sleep apnea) (20). To our knowledge, the present study represents the first examination of overload training on MSNA. In agreement with our hypothesis, 3 wk of overload training increased modestly the set point of resting MSNA burst frequency by ~20% without affecting the reflexive responses to static handgrip exercise. Our observations are in line with cross-sectional data that demonstrate higher resting MSNA in healthy middle-age and older athletes (33,34). However, it should be noted that in these prior studies, athletes demonstrated lower resting HR and blood pressure. This raises the possibility that elevated levels of MSNA were secondary to arterial baroreflex-mediated reflex sympathetic activation, to maintain appropriate perfusion pressure. In the present series, no such differences were observed between groups before or after the training protocols. Arguing further against a peripheral afferent mechanism, overload training had no effect on the sensitivity of arterial baroreflex control of MSNA. Although speculative, overtraining may be associated with increases in oxidative stress (35), which are linked to increased central sympathetic activation (36). The mechanisms responsible for increased peripheral sympathetic outflow after overload training warrant further study.

It is important to consider that sympathetic outflow can be regulated differentially to distinct target organs such that changes in MSNA may not reflect alterations in cardiac sympathetic activity (37). However, peripheral sympathetic outflow is involved in the regulation of skeletal muscle blood flow as a result of the critical importance of maintaining perfusion pressure (38). Prior studies have shown that resting MSNA is inversely associated with exercise tolerance in young healthy male athletes (19), whereas measurements of MSNA during dynamic exercise exhibit a similar negative correlation with peak oxygen consumption in older healthy and diseased adults (22). From our cohort, the change in MSNA was inversely correlated with changes in peak power output after training. All told, these results suggest that interindividual differences in central sympathetic outflow may contribute to variability in maximal exercise performance.

Limitations. We acknowledge several considerations for the application of our findings. The modest sample size may increase the chance of a type II error. To increase our statistical power (24), we completed secondary ANCOVA adjusting for baseline values. The small sample size precluded investigation into whether autonomic responses to overload training differed by sex. It has been demonstrated previously that women have lower resting MSNA compared with men and lower autonomic support of blood pressure (39). We did not study women at one particular phase of their menstrual cycle. However, although fluctuations in sex hormones can alter resting autonomic function (39), we tested participants ~4 wk apart ensuring a similar within-subject phase of the their menstrual cycle for pre–post testing. Despite unchanged resting blood pressure in both groups after training, we did not assess hydration status in this study, which could influence MSNA (40). We assessed cardiac and sympathetic BRS using spontaneous techniques, and our results may differ from those obtained using external perturbations (e.g., modified Oxford technique) to evoke a larger range of pressure inputs (13). Furthermore, the inclusion of daily-waking weekly averages for HRV may have provided a
more thorough assessment of cardiac autonomic balance, rather than singular resting measurements (5). Finally, our sample consisted of young to middle-age recreational endurance athletes and may not be generalizable to other populations including older or elite athletes.

CONCLUSIONS

The effects of overload training on the autonomic nervous system have been largely investigated using indirect, noninvasive methods, with mixed results. We demonstrate, using direct microneurographic recordings, that 3 wk of overload training increases resting peripheral sympathetic outflow to skeletal muscle in recreational endurance athletes and blunts training adaptations in HR variability and cardiac BRS seen with regular training. An inverse relationship between the changes in MSNA and peak power output suggests that alterations in central sympathetic outflow may be involved in underperformance of athletes suffering from overtraining.

Alexandra M. Coates, Anthony V. Incognito, and Jamie F. Burr are co-first and senior authors.

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The authors have no conflicts of interest to declare. 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.

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