Three Weeks of Overload Training Increases Resting Muscle Sympathetic Activity

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Abstract

**Purpose:** Overload training is hypothesized to alter autonomic regulation, though 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 seventeen healthy triathletes and cyclists after 1-week of reduced training (baseline) and following 3-weeks of either regular (CON, n=7) or overload (OL, n=10) training.

**Results:** Following training, the changes (∆) in peak power output (10 ± 10 vs. -12 ± 9 W, \( P < 0.001 \)), maximal heart rate (-2 ± 4 vs. -8 ± 3 beats/min, \( P = 0.006 \)), heart rate variability (standard deviations of normal-to-normal intervals: 27 ± 31 vs. -3 ± 25 ms, \( P = 0.04 \)), and cardiac BRS (7 ± 6 vs. -2 ± 8 ms/mmHg, \( P = 0.02 \)) differed between CON and OL groups. The change in MSNA burst frequency (-2 ± 2 vs. 4 ± 5 bursts/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 following overload training and suggest that increased central sympathetic outflow may be linked with decreased exercise performance.

**Keywords:** MUSCLE SYMPATHETIC NERVE ACTIVITY, ENDURANCE TRAINING, TRAINING, OVERLOAD, OVERTRAINING, AUTONOMIC NERVOUS SYSTEM
Introduction

Endurance athletes often experience periods of under-performance following 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 heart rate recovery following 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). Non-invasive assessments of time- and frequency domain heart rate 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 heart rate variability remains unchanged (11) or is not adequate for monitoring overload training status (8,12). Similarly, measurement of arterial baroreflex control of HR, a reflexive measure of cardiovagal modulation which quantifies the sensitivity (or gain) of changes in HR for a given change in systolic blood pressure (13), has demonstrated either no change (14) or a reduction (15) in cardiac baroreflex sensitivity (BRS) following overload training.
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 has either no impact (2) or reduces (3,7) norepinephrine or epinephrine levels at rest, though 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, muscle). In contrast, microneurographic recordings of muscle sympathetic nerve activity (MSNA) are considered a gold-standard method to examine central sympathetic outflow directed towards 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 impacted by training load was not reported. To our knowledge the effects of overload training on regional peripheral sympathetic outflow has 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 towards skeletal muscle (MSNA) as the primary variable of interest. Given that MSNA is not altered following aerobic exercise training in healthy adults (20), we hypothesized that three weeks of overload training would 1) 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 years [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 sub-elitc cyclists or triathletes who had self-reported 7 ± 4 years of endurance sport training. Inclusion criteria included that subjects be between 18-50 years of age, free from injury, familiar with cycle pacing, and currently following a structured endurance-training program. Subjects were randomized by enrolment 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 PAR-Q+ questionnaire to ensure readiness for physical activity, and abstain from alcohol, drugs, caffeine, and intense exercise for 24 hours prior to 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 one week of reduced training in which exercise volume was decreased by ~50%, to reduce the impact of any prior training-induced fatigue, followed by three weeks of either regular-training control (CON) or overload (OL) training. This protocol was modeled after a three-week 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, heart rate strap, and associated online account (Polar Electro Oy, Kempele, Finland). Exercise performance and autonomic testing took place over two visits separated by 24-48 hours following both the first week of reduced training and three weeks of CON or OL training.

Profile of Mood States

Subjects completed the Profile of Mood States Second Edition (POMS-2) questionnaire online following the reduced training week and again after the three-weeks 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 employs a 4-point rating scale to assess moods of tension-anxiety, depression-dejection, anger-hostility, vigour-activity, fatigue-inertia, confusion-bewilderment, and friendliness. The total mood disturbance is determined by subtractive vigour-activity from the sum of the 5 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, Pennsylvania, USA) in standardized laboratory conditions. A true ramp protocol was used to increase the load on the cycler ergometer, with a slight variation between sexes to ensure the test was of sufficient duration. Men followed a protocol beginning at 100W and increasing 1W every 2s, while women
started similarly at 100W but increased 1W every 3s. 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 above 40 revolutions per minute. HR was monitored (Polar A300) and recorded at the moment of exercise termination. Lactate measures, using standard finger stick sampling, was initiated 60s post-exercise with samples every 30s 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 three-week 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 they did not initiate de-training 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-second Wingate anaerobic tests on an electromagnetically braked cycle ergometer (Racermate Velotron, Seattle, Wa), at a load of 7.5% body weight, with each test separated by 4 minutes of recovery. The second session was a 15 km virtual time trial performed on the same cycle ergometer over a standardised course with undulating terrain. The first two supplemental sessions were completed in the lab under standardised conditions. The final session was a two-hour ride completed on the subject’s own time, and tracked using heart rate monitoring. The ride was prescribed as four blocks, with each
block consisting of ten minutes at a heart rate of 50-60% of VO₂ max followed by 20 minutes at a heart rate of 66-75% of their VO₂ max, based on calculation from their initial VO₂ max at baseline.

**Cardiovascular and Autonomic Testing**

All testing was completed in a light and temperature controlled laboratory. Subjects underwent instrumentation (~1 hr) followed by a 10-minute rest period before continuous measures of heart rate, blood pressure, and MSNA were collected over a subsequent 10-minute baseline for analysis of resting autonomic function. Following the resting collection, a 2 minute 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 following voiding. Heart rate was collected using single-lead electrocardiography (lead II configuration; ADInstruments, Colorado Springs, CO), while beat-to-beat blood pressure was recorded from the right middle finger using photoelectric plethysmography (Finometer MIDI, Finapres Inc, Netherlands). Microneurographic recordings of multi-unit 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 (75,000x), band-pass filtered (0.7-2.0 kHz), rectified, and integrated over a 0.1 second 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 was digitized and stored with LabChart (PowerLab, ADInstruments, Colorado Springs, CO). Heart rate, blood pressure, and the integrated multi-unit 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-minute baseline period. The reactivity to static handgrip was calculated as the change (Δ) from baseline to the second minute of exercise. Measures of heart rate 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 standard deviation of normal R-R intervals (SDNN) and the root mean square of successive R-R interval differences (RMSSD). 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 ms²) 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 interval using established thresholds for changes of 1 mmHg and 6 msec, respectively (23). Systolic blood pressure and R-R intervals were obtained from the same cardiac cycle (i.e. no time lag), as heart rates were below <75 beats/min in all participants (23). BRS was quantified by plotting R-R interval over systolic blood pressure for each identified series with an r value ≥ 0.8, and averaging the slope of all up-sequences and down-sequences.

MSNA was analyzed using a custom LabView (National Instruments, Austin, Texas, USA) semi-automated program (17,22). MSNA was quantified as burst frequency (bursts/min) and burst incidence (bursts/100 heartbeats). Our laboratory has previously demonstrated high inter-test intraclass correlation coefficients (reliability) for resting MSNA burst frequency (r=0.76) and burst incidence (r=0.77) measures separated by one month (17). Spontaneous
sympathetic BRS was calculated by examining the weighted linear regression line between 2 mmHg 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 $\geq 0.5$.

Statistical Analysis

Baseline subject characteristics, and training durations and intensities were compared with unpaired $t$-tests. Two-way repeated measures analyses of variance (ANOVAs) were used to study the changes in training outcomes (total mood disturbance, peak power, maximal heart rate, $\text{VO}_2\text{max}$, peak lactate, and resting hemodynamics, MSNA, cardiac and sympathetic BRS, and HRV) with Bonferroni post-hoc procedures to probe contrast effects when a significant interaction was present. As the primary interest was the group x time interaction, secondary one-way analyses of covariance (ANCOVA) on the pre-post change over time with adjustment for pre-training baseline values were completed to increase statistical power and reduce the impact of chance differences in baseline values, which could impact 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 ($\Delta$) from baseline to the second minute of static handgrip exercise before and after training were similarly examined using two-way repeated measures ANOVAs with Bonferroni post hoc tests. One-way ANCOVAs were also used to assess the changes from baseline to the second minute of static handgrip exercise post-training with the pre-training 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 is reported as mean ± standard deviation. 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, USA).

Results

We recruited twenty-one participants, however, three participants did not complete the study. In the CON group, two subjects were unable to return for post-testing, while in the OL group, we were unable to locate a microneurographic recording site at baseline in one participant. Additionally, one participant in the OL group failed to adhere to the three-week protocol and was excluded from analysis. Therefore, the results are presented for seventeen participants (CON, n=7 [4 male]; OL, n=10 [4 male]). At baseline, participant age (32 ± 11 vs. 36 ± 10 year, \( P = 0.38 \)), 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 years, \( P = 0.45 \)), and VO\(_2\)max (57 ± 10 vs. 53 ± 7 ml kg\(^{-1}\) min\(^{-1}\), \( P = 0.25 \)) were similar between CON and OL groups.

Training Program

Exercise volume was similar between the CON and OL groups during the one week of reduced training (6.1 ± 1.0 vs. 6.4 ± 3.2 hours/week, \( P = 0.84 \)), however, as expected, exercise volume was higher in the OL group during the three weeks of training (8.8 ± 3.0 vs. 12.1 ± 2.6 hours/week, \( P = 0.04 \)). Exercise intensity, represented as time spent in specific heart rate zones divided by overall training time, did not differ between groups during the one week of reduced training or the three weeks 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%, 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 x time interaction was observed for peak power output ($P < 0.001$). Following training, peak power output increased in CON (345 ± 78 vs. 355 ± 77W, $P = 0.02$) and decreased in OL (335 ± 71 vs. 323 ± 71W, $P = 0.001$). Similarly, there was a group x time interaction for maximal HR ($P < 0.006$). Maximal HR was unchanged in CON (182 ± 14 vs. 180 ± 11 beats/min, $P = 0.10$) but decreased in OL (179 ± 11 vs. 172 ± 10 beats/min, $P < 0.001$) following training. Peak lactate and VO$_2_{\text{max}}$ 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.

**Heart Rate 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 OT (All \( P < 0.05 \)). Secondary analysis adjusting for baseline values did not reveal any between-group differences in HR or blood pressure response following training (All \( P > 0.05 \)).

**Autonomic function**

Complete resting heart rate variability results are shown in Table 2. A group x time interaction was observed for SDNN (\( P = 0.04 \)). Following 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 heart rate 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 three-weeks of overload training is shown in Figure 2. Resting group-level MSNA and BRS outcomes are presented in Figure 3. There was a group x time interaction for MSNA burst frequency (\( P = 0.01 \)). Following training, MSNA burst frequency was unchanged in CON (17 ± 3 vs. 15 ± 4 bursts/minute, \( P = 0.33 \)) but increased in OL (20 ± 7 vs. 24 ± 5 bursts/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 x time interaction for MSNA burst incidence (\( P = 0.09 \)). However, as 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 $\Delta 9 \pm 8$ vs. $11 \pm 6$ bursts/minute; OL $\Delta 6 \pm 5$ vs. $7 \pm 5$ bursts/minute) or burst incidence (CON $\Delta 6 \pm 12$ vs. $9 \pm 12$ bursts/100 heartbeats; OL $\Delta 6 \pm 10$ vs. $3 \pm 6$ bursts/100 heartbeats) during static handgrip before and after training; these results were not altered using ANCOVA analyses.

A group x 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. Further, ANCOVA analysis adjusting for baseline values failed to detect a between-group difference in the change in sympathetic BRS following training ($P = 0.12$). There was a group x time interaction for cardiac BRS ($P = 0.02$). Cardiac BRS increased in CON ($23 \pm 11$ vs. $30 \pm 14$ ms/mmHg, $P = 0.02$) but was unchanged in OL ($23 \pm 10$ vs. $22 \pm 6$ ms/mmHg, $P = 0.40$) following 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 ($r = -0.51$, $P = 0.04$; Figure 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 three-week overload training led to reductions in peak power output and maximal HR compared to 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 baroreflex sensitivity, 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 heart rate variability observed following regular CON training. Altogether, this data provides evidence that overload training can perturb the autonomic nervous system at rest and that these neural responses may be related to underperformance.

Heart Rate Variability and Cardiac Baroreflex Sensitivity

The majority of studies probing the neural effects of overload training have utilized non-invasive measures of tonic cardiac autonomic activity through analyses of heart rate 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, lack of a control group), measures of heart rate variability assess changes in sinus node firing which can involve both neural and non-neural mechanisms (25). For example, interpreting changes in heart rate variability with overload training may be difficult due to inter-individual differences in parasympathetic saturation, observed in some athletes (26); changes in intrinsic heart rate control (25); desensitization of beta-adrenergic receptors (27); or stretch of the sinoatrial node (28) through atrial enlargement or expansion of plasma volume.

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 following a 2-week training camp, though 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, following 6-9 weeks of increased training in 9 female athletes; however, only 5 participants were diagnosed as being overtrained and no sub-analysis 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 following OL training; with between-group differences in both measures responses with training. Prospective trials have also shown increased heart rate 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 following regular training provides further evidence that the CON group underwent conventional adaptations to endurance exercise training. Although these responses are not always expected, it is important to consider that our subjects were not elite athletes, allowing them to benefit from consistent 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 following aerobic exercise training in diseased populations (e.g. heart failure, hypertension, sleep apnea) (20). To our knowledge the present study represents the first examination of overload training on MSNA. In agreement with our hypothesis, three weeks of overload training increased modestly the set point of resting MSNA
burst frequency by ~20% without impacting the reflexive responses to static handgrip exercise. Our observations are in line with cross-sectional data that demonstrate higher resting MSNA in healthy middle-aged and older athletes (33,34). However, it should be noted that in these prior studies, athletes demonstrated lower resting heart rate and blood pressure. This raises the possibility that elevated levels of MSNA were secondary to arterial baroreflex-mediated reflex sympathetic activation, in order 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 impact 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 following 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), while 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 following training. All told, these results suggest that inter-individual 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 analyses 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 to 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 weeks apart ensuring a similar within-subject phase of the their menstrual cycle for pre-post testing. Despite unchanged resting blood pressure in both groups following 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). Further, 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-aged 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, non-invasive methods, with mixed results. We demonstrate, using direct microneurographic recordings, that three weeks of overload training increases resting
peripheral sympathetic outflow to skeletal muscle in recreational endurance athletes and blunts training adaptations in heart rate variability and cardiac BRS seen with regular training. An inverse relationship between the changes in MSNA and peak power output suggest that alterations in central sympathetic outflow may be involved in underperformance of athletes suffering from overtraining.

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Disclosures
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 ACSM.
References


Figure Legends

**Figure 1.** Effects of three weeks of regular (CON) or overload (OL) training on peak power output (A), maximal heart rate (B), peak blood lactate (C), and maximal oxygen consumption (D). Mean ± SD. Left side figures represent group x time differences examined using two-way analyses of variance. Right side figures represent the between-group difference in change following training adjusted for baseline values using analyses of covariance. †, \( P < 0.05 \); ††, \( P < 0.001 \); ††††, \( P < 0.0001 \) vs. baseline.

**Figure 2.** Representative microneurographic recording (left side) and spontaneous sympathetic baroreflex sensitivity analysis (right side) before and after three weeks of overload training.

**Figure 3.** Effects of three weeks of regular (CON) or overload (OL) training on muscle sympathetic nerve activity (MSNA) burst frequency (A) and burst incidence (B), and cardiac baroreflex sensitivity (C). Mean ± SD. Left side figures represent group x time differences examined using two-way analyses of variance. Right side figures represent the between-group difference in change following training adjusted for baseline values using analyses of covariance. †, \( P < 0.05 \) vs. baseline; *, \( P < 0.05 \); **, \( P < 0.01 \) vs. CON.

**Figure 4.** Relationship between individual changes in resting muscle sympathetic nerve activity (MSNA) burst frequency and peak power output following three weeks of regular or overload training.
Figure 1

A

- Peak Power (watts)
- Group: p=0.57
- Time: p=0.69
- Interaction: p=0.001

B

- Maximal Heart Rate (bpm)
- Group: p=0.36
- Time: p=0.001
- Interaction: p=0.01

C

- Peak Lactate (mmol/L)
- Group: p=0.45
- Time: p=0.11
- Interaction: p=0.21

D

- Maximal Oxygen Consumption (L/min)
- Group: p=0.71
- Time: p=0.99
- Interaction: p=0.81

- Δ Peak Power (watts)
- p<0.001

- Δ Maximal Heart Rate (bpm)
- p<0.001

- Δ Peak Lactate (mmol/L)
- p=0.17

- Δ Maximal Oxygen Consumption (L/min)
- p=0.68
Figure 2

![Graph showing MSNA Burst Incidence (bursts/100 heartbeats) vs. Diastolic Blood Pressure (mmHg) with data points for Pre and Post conditions. The correlation coefficient for Pre is -0.87 and for Post is -0.93.](image)
Figure 4

The scatter plot shows the relationship between Δ MSNA Burst Frequency (bursts/min) and Δ Peak Power (watts). The correlation coefficient is $r = -0.51$ and the probability value is $P = 0.04$. The data points are differentiated by CON (open circles) and OL (filled circles).
Table 1. Heart rate and blood pressure at rest and in response to static handgrip exercise before and after three weeks of regular (CON) or overload (OL) exercise training.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CON (n=7)</th>
<th></th>
<th>OL (n=10)</th>
<th></th>
<th>Δ CON</th>
<th>Δ OL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>57 ± 6</td>
<td>54 ± 3</td>
<td>54 ± 6</td>
<td>52 ± 5</td>
<td>-3 ± 6</td>
<td>-2 ± 5</td>
<td>0.79</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>102 ± 7</td>
<td>102 ± 6</td>
<td>106 ± 5</td>
<td>105 ± 6</td>
<td>1 ± 6</td>
<td>-1 ± 6</td>
<td>0.50</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>65 ± 9</td>
<td>65 ± 7</td>
<td>69 ± 9</td>
<td>68 ± 4</td>
<td>1 ± 7</td>
<td>-2 ± 8</td>
<td>0.57</td>
</tr>
<tr>
<td>Static Handgrip</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ Heart rate (bpm)</td>
<td>14 ± 8</td>
<td>17 ± 7</td>
<td>7 ± 6</td>
<td>9 ± 4</td>
<td>3 ± 7</td>
<td>2 ± 5</td>
<td>0.64</td>
</tr>
<tr>
<td>Δ Systolic BP (mmHg)</td>
<td>19 ± 9</td>
<td>18 ± 11</td>
<td>14 ± 9</td>
<td>13 ± 7</td>
<td>0 ± 4</td>
<td>-1 ± 10</td>
<td>0.79</td>
</tr>
<tr>
<td>Δ Diastolic BP (mmHg)</td>
<td>14 ± 4</td>
<td>15 ± 5</td>
<td>9 ± 5</td>
<td>10 ± 4</td>
<td>1 ± 3</td>
<td>1 ± 6</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Mean ± SD. BP, blood pressure.
Table 2. Resting heart rate variability before and after three weeks of regular (CON) or overload (OL) exercise training.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CON (n=7)</th>
<th>OL (n=10)</th>
<th>Δ CON</th>
<th>Δ OL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-R interval (ms)</td>
<td>1078 ± 107</td>
<td>1138 ± 65</td>
<td>1172 ± 129</td>
<td>1178 ± 123</td>
<td>59 ± 119</td>
</tr>
<tr>
<td>SDNN (ms)</td>
<td>93 ± 55</td>
<td>120 ± 62*</td>
<td>82 ± 35</td>
<td>79 ± 33</td>
<td>27 ± 31</td>
</tr>
<tr>
<td>RMSSD (ms)</td>
<td>98 ± 86</td>
<td>126 ± 106</td>
<td>72 ± 35</td>
<td>74 ± 34</td>
<td>28 ± 39</td>
</tr>
<tr>
<td>LF (ln ms²)</td>
<td>7.1 ± 0.9</td>
<td>7.6 ± 1</td>
<td>7.2 ± 0.9</td>
<td>7.1 ± 1</td>
<td>0.6 ± 0.7</td>
</tr>
<tr>
<td>LF (nu)</td>
<td>45 ± 24</td>
<td>45 ± 23</td>
<td>51 ± 17</td>
<td>48 ± 17</td>
<td>0 ± 10</td>
</tr>
<tr>
<td>HF (ln ms²)</td>
<td>7.4 ± 1.8</td>
<td>7.9 ± 1.7</td>
<td>7.1 ± 1.2</td>
<td>7.2 ± 1</td>
<td>0.6 ± 0.6</td>
</tr>
<tr>
<td>HF (nu)</td>
<td>55 ± 24</td>
<td>55 ± 23</td>
<td>49 ± 17</td>
<td>52 ± 17</td>
<td>0 ± 10</td>
</tr>
<tr>
<td>LF/HF ratio</td>
<td>1.3 ± 1.4</td>
<td>1.1 ± 1</td>
<td>1.7 ± 2.6</td>
<td>1.3 ± 1.2</td>
<td>-0.5 ± 1.4</td>
</tr>
</tbody>
</table>

SDNN: standard deviation of normal-to-normal R-R intervals; RMSSD: root mean square of the successive differences; LF: low frequency; HF: high frequency; nu: normalized units. *p<0.05 vs. baseline. Mean ± SD.